SYNTHESIS AND ESTIMATION OF COPPER NANOPARTICLES AS AGROCHEMICALS AGAINST *PHYTOPHTHORA* SPP.

TỔNG HỢP VÀ ĐÁNH GIÁ HOẠT TÍNH KHÁNG NẤM *PHYTOPHTHORA* SPP. CÁC HẠT NANO ĐỒNG

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ABSTRACT

Nanoparticles have been extensively in recent years. The effective applications of nanoparticles depend on their sizes. Copper nanoparticles (CuNPs) are among metal nanoparticles which have been reported by many researchers. In this work, CNPs have been synthesized from copper sulfate via chemical reduction method in the presence of trisodium citrate dispersant and polyvinylpyrrolidone (PVP) as protecting agents. The colloidal CuNPs were characterized by using UV–Visible spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD) techniques. By using a certain amount of materials which has been described in literatures, the size of CuNPs obtained within a range of 4-20 nm in diameter. The colloidal solution of CuNPs was investigated the potential against Phytophthora spp. which cause economically crop diseases. Under in vitro test conditions, the inhibition of Phytophthora spp. mycelia growth at three concentrations of CuNPs (10, 20, 30 ppm) after 48 hours are 90.18%, 91.87% and 100%, respectively. These results provided a simple and economical method to develop the CuNPs-based-fungicide.

Keywords: *Copper nanoparticles; antifungal activity; Phytophthora spp.; protecting agents; chemical reduction.*

TÓM TẮT

Các hạt nano đã được nghiên cứu nhiều trong những năm gần đây. Hiệu quả ứng dụng của các hạt nano phụ thuộc vào kích thước của chúng. Các hạt nano đồng là trong số các hạt nano kim loại đã được nghiên cứu. Trong nghiên cứu này, các hạt nano đồng được tổng hợp bằng phương pháp khử hóa học trong sự hiện diện của các tác chất bảo vệ như trinatri citrate và polyvinylpyrrolidone. Đặc điểm hạt nano đồng được xác định bằng phổ tử ngoại-khả kiến (UV-Vis), chụp ảnh dưới kính hiển vi điện tử truyền qua (TEM) và nhiễu xạ tia X (XRD). Bằng việc sử dụng các lượng chất nhất định cho quá trình tổng hợp, đường kính của các hạt nano đồng thu được trong khoảng 4-20 nm. Hoạt tính kháng nấm của các hạt nano đồng được thử nghiệm đối với nấm Phytophthora spp. Thử nghiệm in vitro cho thấy, chế phẩm nano đồng tại các nồng độ 10, 20 và 30 ppm đã ức chế 90.18%, 91.87% và 100% sự phát triển của tơ nấm Phytophthora spp. sau 48 giờ. Kết quả này là cơ sở để phát triển chế phẩm diệt nấm đơn giản, kinh tế dựa trên các hạt nano đồng.

Từ khóa: *Hạt nano đồng; hoạt tính kháng nấm; Phytophthora spp.; tác chất bảo vệ; khử khóa học.*

1. INTRODUCTION

In recent years, nanoparticles have been extensively studied due to their unusual chemical and physical properties [1, 2]. The effective applications of the nanoparticles generally depend on their size, shape and protecting agents which could be controlled by the preparation conditions [3]. A number of different approaches to prepare metal nanoparticles such as Cu, Ag, Pt, Au have been reported. Some of these methods include photoreduction, chemical reduction using reducing agents in association with protecting agents [4-6].

Interestingly, the nanoparticles strongly exhibited the antifugal and antimicrobial activities [5, 6]. Among them, copper nanoparticles (CuNPs) have much attention. CuNPs showed a significant antifungal activity against various plant pathogenic fungi such as *Phytophthora*, *Corticium salmonicolor* [7]. *Phytophthora* is a genus of plant-damaging Oomycete whose member species are capable of causing enormous economic losses on crops. The genus *Phytophthora* approximately includes one hundred species [8]. *Phytophthora* spp. cause diseases such as blight, stem rots, fruit rots…. Worldwide crop losses due to *Phytophthora* diseases are estimated to be multibillion dollars [9]. Synthetic chemicals are currently used for inhibiting this fungal growth. However, *Phytophthora* spp. are known to be able to develop the resistance to chemicals rapidly [10]. Thus, the discovery of new alternatives with lower risk of resistance plays a major role for controlling the pathogens as *Phytophthora* spp.

As mentioned, CuNPs showed a significant antifungal activity against *Phytophthora*. In addition, the cost to produce CuNPs is much cheaper than the others such as silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs). However, the studies on antifungal activity of CuNPs have not yet received much attention in Vietnam. The low cost to prepare the CuNPs is an advantage to use them in agriculture as agrochemicals. In this study, CuNPs were prepared by chemical reduction method in the presence of the sodium citrate dispersant and polyvinylalcol (PVP) as protecting agents. UV–Visible spectroscopy, transmission electron microscopy (TEM) and X-ray diffraction (XRD) techniques were used to characterize CuNPs. The antifungal activity against the growth of *Phytophthora* spp. mycelia was estimated under *in vitro* conditions on Potato Dextrose Agar (PDA) medium.

2. MATERIALS AND METHOD

2.1 Materials

Copper (II) sulfate ($CuSO_4$, 99.0%), glycerol ($C_3H_8O_3$, 99.0%), polyvinylpyrrolidone (M_w 58.000 g/mol), trisodium citrate dihydrate ($HOC(COONa)(CH_2COONa)_2.2H_2O$, 99.0%), hydrazin dihydrate ($N_2H_4.2H_2O$, 50%) were purchased from Acros Organics. All reagents were used without further purification. *Phytophthora* spp. were supplied by Laboratory Applications in Microbiology, Institute of Tropical Biology, Vietnam Academy of Science and Technology, Linh Trung, Thu Duc, Ho Chi Minh City.

2.2 Synthesis of CuNPs

The mixture including PVP (0.2 g, 5 wt% to copper salt), $CuSO_4$ (10.0 mg) and $HOC(COONa)(CH_2COONa)_2.2H_2O$ (9.2 mg) was dissolved in 30 mL glycerol. The mixture was heated and stirred at 110^{o}C for 5 minutes. The Cu^{2+} ions in the reaction mixture were then reduced to copper metal by the introduction of a solution of hydrazine hydrate (100 µL 0.1 M). As thermal reduction proceeded, the blue solution turned to red, indicating the formation of the CuNPs for 10 minutes. The colloidal CuNPs were characterized by UV–Vis, transmission electron microscopy (TEM), and X-ray diffraction (XRD).

2.3 Product characterization

UV–Vis absorption spectrum of the CuNPs solution was measured by Jasco V670 (Jasco Analytical Instrument), TEM images were measured by JEM-1400 version (JEM-1400, JEOL). The samples for TEM measurement were prepared by dropping CuNPs solution onto a carbon-coated copper grid. The histogram of the particle-size distribution and the average diameter were obtained by measuring particles. The XRD result was characterised using D8 advanced Bragg X-ray (D8 Advance, Brucker) with Cu

Kα radiation. For sample handling, glass slide was used as a substrate for measurement. Leaned substrate was covered with the colloidal CuNPs solution and dried in air.

2.4 Determination of antifungal activity

The antifungal activity against *Phytophthora* spp. was estimated by using the *in vitro* plate dilution method. The colloidal CuNPs with various concentrations (10, 20, 30 ppm) in glycerol were mixed with melting PDA medium to obtain a 15 mL total volume in Petri dishes. The control dishes contained glycerol, PVP, copper sulfate without colloidal CuNPs. Petri plates were wrapped with parafilm and incubated at room temperature. The diameters of the colony growth of the control and CuNP samples were observed after 24 and 48 hours. Each treatment for each concentration of CuNPs was replicated three times. The inhibition of the growth of the mycelia was estimated by measuring the colony diameter and calculated by formula: growth inhibition (%) = (d_1 − d_2/d_1) × 100, where d_1 and d_2 are colony diameters of the control and CuNPs contained samples, respectively.

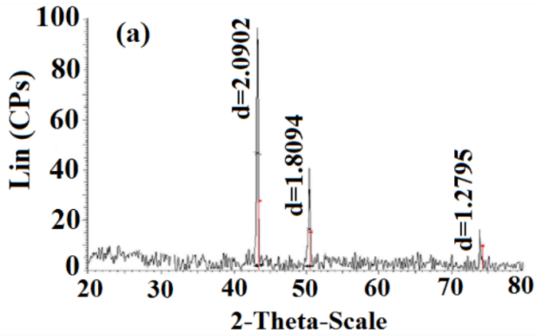
3. RESULTS AND DISCUSSION

3.1 Characterization of CuNPs

The formation of CuNPs is confirmed by the powder X-ray diffraction (XRD). Figure 1a showing the peak positions with high crystallinity at 43.2^o, 50.4^o, and 74.0^o in XRD pattern are consistent with metallic copper. These peaks correspond to the typical face-centered cubic of copper with miller indices at (111), (200), and (220) which are in good agreement with the literature value [5, 11, 12]. Figure 1b depicts the absorption spectrum of the CuNPs. The spectrum displays only an absorption peak at around 570nm. This peak can be assigned to the absorption of CuNPs. This phenomenon was a result of the surface plasmon resonance that occurs with copper nanoparticles [13,14]. The result also indicates that copper oxides (Cu_2O and CuO) were not formed in the synthetic process.

3.2 Microstructure of CuNPs

Figure 2 reveals the TEM microstructure of the CuNPs in the the presence of citrate dispersant and PVP capping agent. The size of CuNPs is in a range from 4 to 20nm in diameter with a molar ratio of citrate to copper salt optimized at 0.5. Due to capping effect, polyvinylpyrrolidone (PVP) has been extensively used as a capping polymer to protect colloidal solution containing metallic nanoparticles [5, 6].



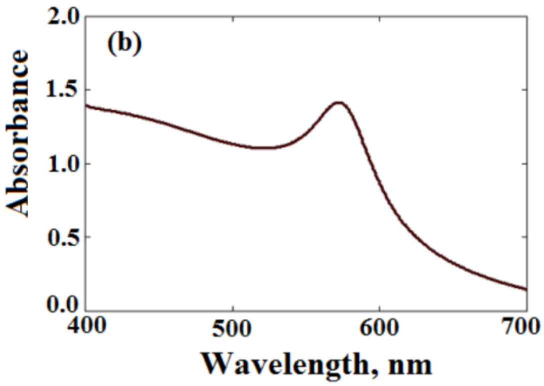


Figure 1. *X-Ray diffractogram (a) and UV-Vis spectrum (b) of CuNPs.*

In this work, a weight percentage (5 wt%) of PVP to copper salt was added to the reaction mixture. However, the bulky polymer is ineffective to coat all surfaces of the metallic nanoparticles. This results in an outgrowth in size of particles due to their collision. To prevent this disadvantage, a small molecular protecting agent like trisodium citrate could be used. A certain amount of trisodium citrate molecules is adsorbed on the surface of metallic nanoparticles. As a consequence, the aggregation of nano particles due to their collision was significantly reduced.

Furthermore, it has been hard to prepare these small and uniform-sized metallic nanoparticles in the sole presence of capping polymers or citrate dispersant [6].

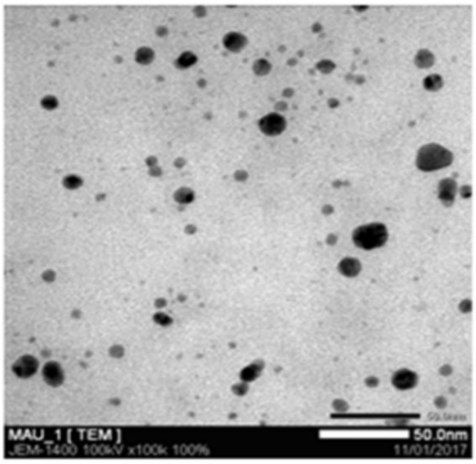


Figure 2. *TEM images of CuNPs prepared in the presence of citrate dispersant and PVP capping agent at* Cu^{2+}*/citrate ratio of 0.5.*

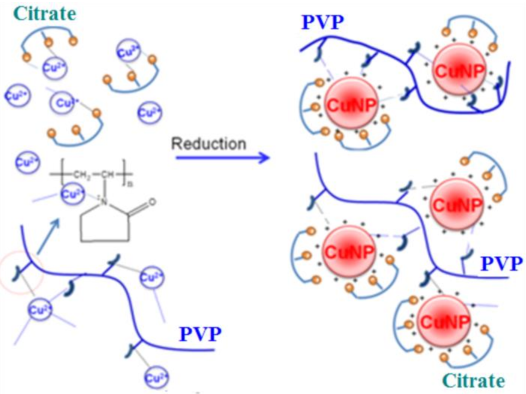


Figure 3. *A demonstration of the synergistic effect of citrate dispersant and PVP capping polymer on controlling size growth of CuNPs. Left figure depicts the formation of complexes of copper ions and citrate or PVP. The synergistic effect of citrate and PVP is given in the right figure.*

The synergistic effect of citrate dispersant and capping polymer has been expected to control size growth of CuNPs as demonstrated in Figure 3. Citrate and PVP work as size controller and polymeric capping agents, because they hinder the nuclei from aggregation through negative charge and polar groups, which strongly absorb the CuNPs on the surface via electrostatic interactions coordination bonds [15, 16].

3.3 CuNPs inhibit *Phytopthora* spp. *in vitro*

The potential of the colloidal CuNP solutions at various concentrations (10, 20, 30 ppm) was estimated against *Phytophthora* spp. Figure 4 showed the antifungal ability against *Phytophthora* spp. After 48 hours of the incubation, the highest antifungal activity was observed at the CuNP concentration of 30 ppm (100%). At lower concentrations of 10 ppm and 20 ppm, CuNPs were less effective with 90.18% and 91.87% of fungal growth inhibition, respectively.

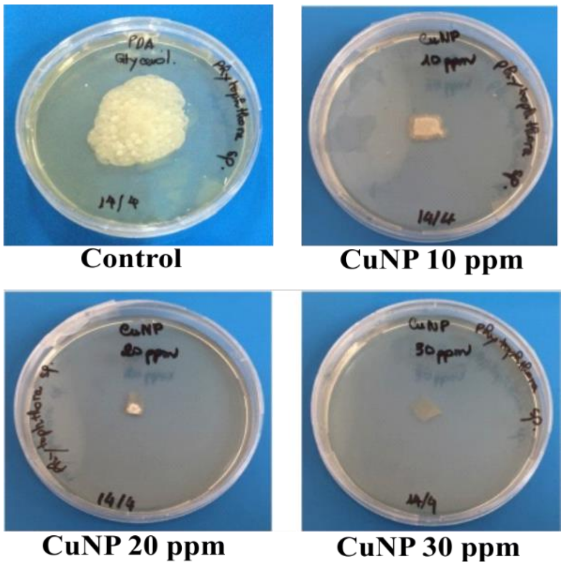


Figure 4*. The fungal growth inhibition of CuNPs at various concentrations against Phytophthora spp. after 48 hours of incubation. CuNPs were not added to control*

Nanoparticles can be currently used as alternatives to chemical pesticides. Most of CuNP studies have focused on antibacterial activities and to a lesser extent on antifungal activities. Under *in vivo* condition, the chromosomal DNA degradation in *E. coli* started within 30 minutes of treatment with CuNPs, and more degradation occured with the increasing of the nano particle exposure time. The mechanism of antibacterial activity of CuNPs in *E. coli* cells has been proposed. The copper ions (Cu^{2+}) attributed to be the main effector for DNA degradation, the nascent ions were generated from the oxidation of metallic CuNPs when they were in the vicinity of agents, namely cells, biomolecules or medium components [17]. To the best of our knowledge no study has been reported to explore the mechanism of the growth inhibition of CuNPs on *Phytophthora* spp..

4. CONCLUSION

CuNPs ranging from 4 to 20 nm in diameter were prepared via chemical reduction method under the presence of citrate dispersant and PVP capping polymer. Purity and stability of the CuNPs were revealed by UV–Vis spectroscopy, TEM, and X-ray diffraction (XRD) techniques. The CuNPs having the size of 4 nm were estimated the inhibition of the fungal growth and exhibited a high potency of the antifungal against *Phytophthora* spp. under *in vitro* treatments. The result showed a complete inhibition of the *Phytophthora* spp. mycelia growth at 30 ppm. This result demonstrated that CuNPs not only used as alternatives to chemical pesticides against *Phytophthora* spp. without any phytotoxicity but also can be applied as a novel antifungal agent in agriculture to control the plant pathogenic fungi.

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