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*Research Article*

# Silver Core-Shell Nanoclusters Exhibiting Strong Growth Inhibition of Plant-Pathogenic Fungi

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We introduced a novel method to prepare silver core-shell nanoclusters (NCs) in which 3,4-dihydroxyphenyl acetic-conjugated oligochitosan (DHPAC) reduced silver salt and subsequently protected the produced nanosilver via mussel adhesion mechanism. Results indicated that the degree of conjugation was 14 dihydroxyphenyl acetamide moieties over 100 glucosamine units of oligochitosan. We used chitosan-catechol derivative to prepare the well-defined silver core-shell NCs and applied UV-visible spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD) techniques to characterize the NCs. The core-shell NCs exhibited strong growth inhibition of plant-pathogenic fungi such as *Phytophthora capsici, Phytophthora nicotianae*, and *Phytophthora colocasiae*. These positive results may offer great potential to produce silver core-DHPAC shell NCs for several biomedical applications.

## 1. Introduction

Usage of metallic nanoparticles (NPs) for biomedical and industrial applications has recently had much attention due to several novel properties, such as optical, catalytic, and antimicrobial properties, compared to their bulk metallic forms [1–3]. Several kinds of metallic nanoparticles like silver, copper, and gold exhibit activity against some microbes, pathogenic fungi, and microorganisms [4–8]. Among them, silver NPs also had strong activity against various plant-pathogenic fungi, such as *Phytophthora* and *Corticium* fungi [9–12]. This is the reason why industrial and agricultural fields have studied silver nanoparticles (AgNPs) for their antibacterial or fungicide applications.

In tropical countries, *Phytophthora* has caused much damage to agriculture, especially economic plants. The *Phytophthora* (*P.*) fungi caused serious damage to durian tree (*P. palmivora*), black pepper/chili/tomato plant (*P. capsici*), tobacco, citrus (*P. nicotianae*), and taro (*P. colocasiae*) which resulted in dead plants and significant decrease in productivity [13, 14]. The plant-pathogenic fungi are controlled mainly by treatment with chemical fungicides, such as fosetyl-aluminium, metalaxyl and potassium phosphonate; however, results showed that these fungicides exhibited a low economic efficacy due to high cost, toxicity to humans, and environment pollution, when used in large scale. For these reasons, finding an environment-friendly antimicrobial agent against pathogenic fungi is important in the sustainable agriculture development. This is why AgNPs-based antimicrobial agents for agricultural field have recently received much attention, especially when the AgNPs can be prepared by simple methods and without using toxic reducing agents [10, 12].

In this study, we introduced a simple method to prepare silver (Ag) core-shell nanoclusters (NCs) in which DHPAC reduced silver salt and subsequently protected the produced AgNPs via its surface adhesion with 3,4-dihydroxyphenyl acetamide moieties (demonstrated in Figure 1). This is

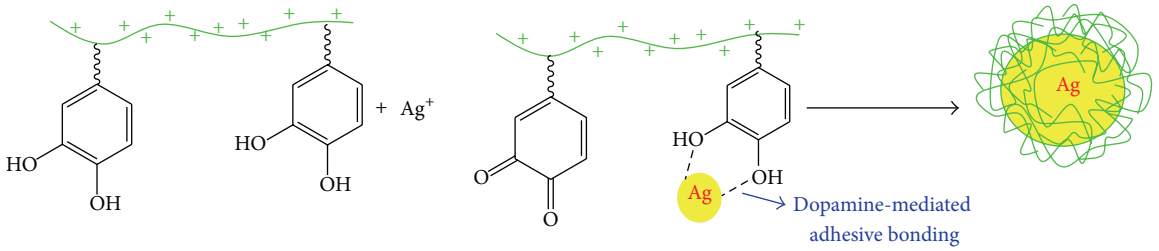
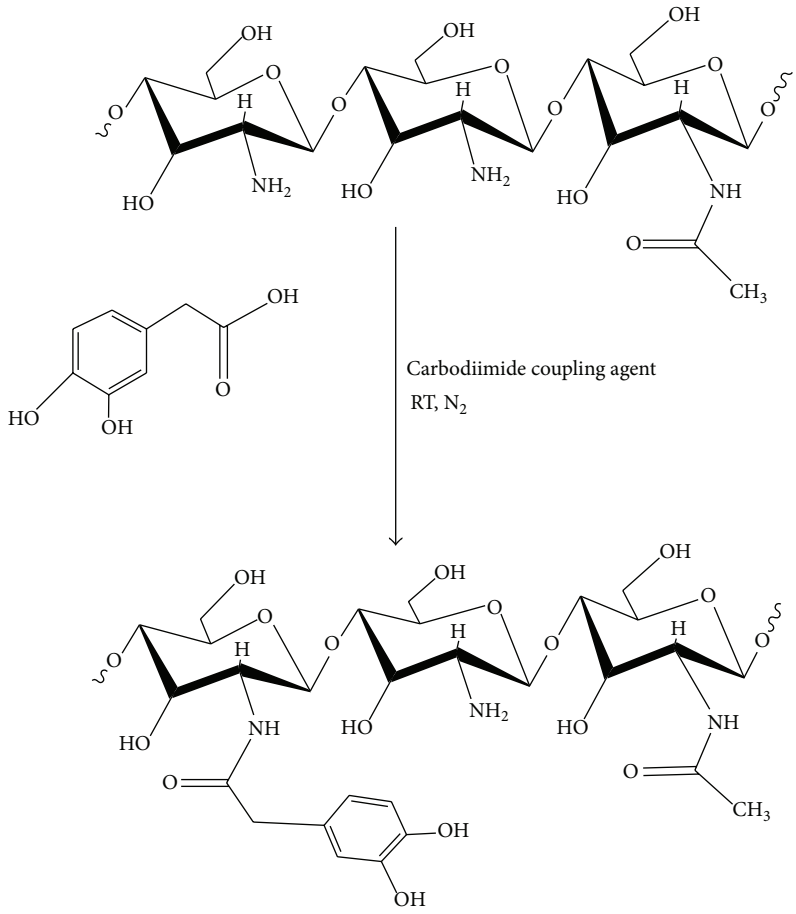



Figure 1: Preparation of Ag core-DHPAC shell via mussel adhesion mechanism.




Scheme 1: Synthetic route of DHPAC.

a biomimetic adhesion mechanism like mussel adhesive proteins [15, 16]. The Ag core-shell NCs were characterized by UV-Vis spectroscopy, TEM, and XRD techniques. Their activities against plant-pathogenic fungi were evaluated on *P. capsici*, *P. nicotianae*, and *P. colocasiae*.

## 2. Experimental

*2.1. Materials.* Silver nitrate ($AgNO_3$) and 3,4-dihydroxyphenyl acetic acid (DHPA) were purchased from Acros Organics (Belgium). Oligochitosan (45,000 D, 85% deacetylation) was prepared in the Department of Materials and Pharmaceutical Chemistry (Vietnam). We obtained 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) from Sigma Aldrich (United States). *P. capsici*, *P. nicotianae*, and *P. colocasiae* were isolated in the School of Agriculture and Forestry, Hue University (Vietnam).

*2.2. Synthesis of DHPAC.* The DHPAC was prepared from oligochitosan and 3,4-dihydroxyphenyl acetic acid in the presence of carbodiimide coupling reagent as shown in Scheme 1.

In summary, we added one gram of oligochitosan (6 mmol glucosamine) and 192 mg of DHPA (1.2 mmol) to

50 mL distilled water under stirring and then added 290 mg of EDC (1.4 mmol) to the mixture. The reaction mixture was stirred under nitrogen atmosphere at room temperature overnight and dialyzed with membrane with molecular weight cutoff of 6000–8000 afterward. After two days of dialysis, the polymer solution was lyophilized using a freeze-drying machine to obtain DHPAC. The degree of DHPA substitution was estimated by $^1$H NMR spectrum.

*2.3. Preparation of Ag Core-DHPAC Shell NCs.* Silver core-DHPAC shell NCs were prepared by mixing 50 mL DHPAC solution (1%) containing around 1600 ppm of DHPA (calculated according to $^1$H NMR) and 50 mL $AgNO_3$ solution containing 800 ppm of silver ion and then stirred for 30 minutes at room temperature. The Ag core-DHPAC shell NCs could be formed (as demonstrated in Figure 1) and characterized using UV-Vis, TEM, and XRD. For control sample, DHPA was the reducing agent without DHPAC. The experiment was conducted in the same manner with preparation of Ag core-shell NCs.

*2.4. Characterization of Ag Core-DHPAC Shell NCs.* UV-Vis absorption spectrum of the colloidal solutions was measured using Jasco V670. TEM images were collected using a JEM-1400 instrument (JEM-1400, JEOL) which was operated at an accelerating voltage of 100 kV. Samples for TEM measurement were prepared by dropping AgNPs solution onto a carbon-coated copper grid. The colloidal solution-covered grids were allowed to dry for several hours. The XRD result was characterized using D8 advanced Bragg X-ray with Cu K$\alpha$ radiation. For sample handling, glass slide was used as a substrate for measurement. Cleaned substrate was covered with the colloidal NCs and dried in air.

*2.5. Studies on Growth Inhibition Ability of Plant-Pathogenic Fungi.* The antifungal activity against pathogen was evaluated by using the *in vitro* plate dilution method. The colloidal NPs were mixed with 25 mL of melting potato dextrose agar (PDA) medium and then were poured into Petri dishes with final concentrations of 3, 6, and 9 ppm. The control dishes contained distilled water. The fungus was transferred equally onto the center point of the prepared Petri dishes and incubated at room temperature for six days. The growth inhibition of the fungus was evaluated by measuring the diameter of colony growth and calculated with the following formula: growth inhibition (%) = $((d_1 - d_2)/d_1) \times 100$, where $d_1$ and $d_2$ are diameters of the colony of control and NCs-containing samples, respectively. All experiments were performed in triplicate.

## 3. Results and Discussion

*3.1. Characterization of DHPAC.* Structure of DHPA-conjugated oligochitosan was well determined by the $^1$H NMR spectrum. Figure 2 showed three aromatic protons of the conjugated DHPA with chemical shift ($\delta_H$) at 6.78, 6.34, and 6.73 ppm. Some typical glucosamine protons of

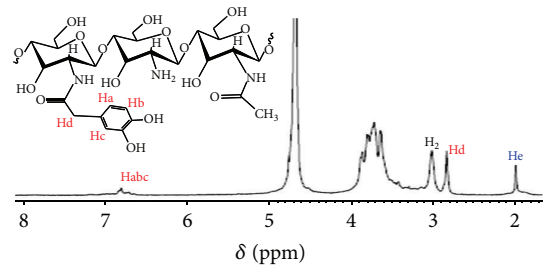



Figure 2: $^1$H NMR spectra of chitosan-catechol derivative.

oligochitosan appear at 2 ppm (acetyl protons), 3.02 ppm ($H_2$ proton), and 3.5–4 ppm ($H_{1-6}$ protons). These results confirm that the DHPA was grafted to the chitosan effectively. According to integral values of DHPA aromatic protons and methyl protons of chitosan, the degree of DHPA substitution (DS) was calculated as 14 DHPA/100 glucosamine units.

*3.2. Preparation of Ag Core-DHPAC Shell NCs.* Preparation of AgNPs using natural extracts containing polyphenol compounds or catechol-functionalized polymers has recently been reported that is regarded as a green method [17–19]. In our study, DHPA moieties were utilized to reduce $Ag^+$ into a metallic form and then protected the produced AgNPs. After mixing DHPAC solution and $AgNO_3$ solution, the color of the mixed solution immediately changed. After shaking for 15 minutes, UV-Vis absorbance of the solution performed two UV-Vis absorption peaks at wavelength ranging from 270 to 320 nm and 375 to 430 nm that was ascribed to absorbance of the DHPA moiety/oxidized DHPA product and surface plasmon resonance of AgNPs, respectively (as shown in Figure 3). The result indicated formation of the AgNPs.

Figure 4 showed XRD diffractogram of the Ag core-DHPAC shell NCs and the crystalline phase of metallic silver at 38.0, 44.2, 64.4, 77.6, and 81.6°. These peaks corresponded to the typical face centered cubic structure of Ag with miller indices at (111), (200), (220), (311), and (222), respectively [4, 20].

Figure 5 showed clearly that the core-shell NCs were formed in which Ag cores had the size distribution of 26 ± 9 nm and polymer shell layer of 18 ± 8 nm of thickness. These values were estimated by mean value of 50 selected nanoparticles. The experiment preparing only silver nanoparticle without shell had also been conducted to compare difference in morphology of AgNPs but the reaction mixture showed precipitation after $AgNO_3$ and DHPA were added. This could be an aggregate of the formed AgNPs which was not well protected by polymer shell layer.

Formation of the polymer layer coating on the surface of the AgNPs could be explained as shown in Figure 6, in which some DHPA moieties on DHPAC reduced ionic silver to AgNPs and quinone moieties. Some DHPA moieties simultaneously adhered to the formed AgNPs via dopamine-like adhesion mechanism which was well proven in some previous report [15, 16, 21, 22]. The quinone moieties from oxidized DHPAC easily reacted with amine groups of other

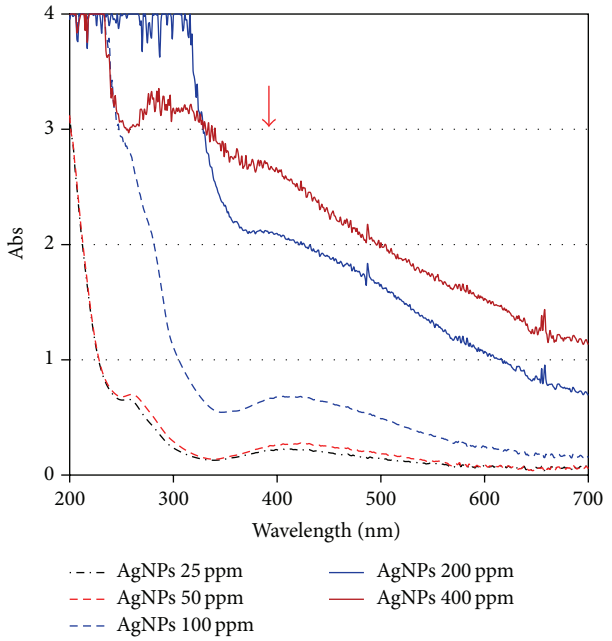



Figure 3: UV-Vis absorbance of Ag core-DHPAC shell NCs solution.

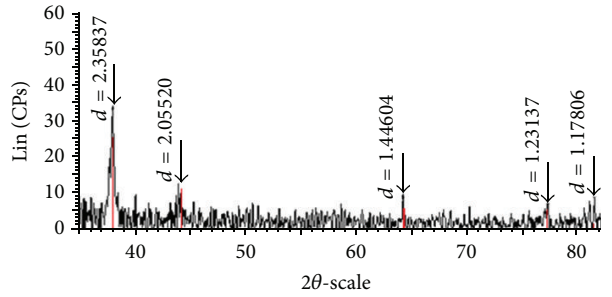



Figure 4: XRD pattern of the Ag core-DHPAC shell NCs.

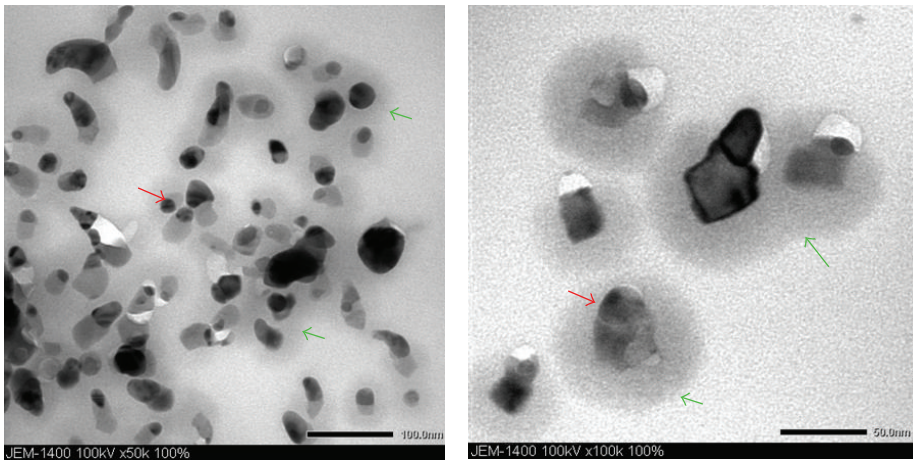



Figure 5: TEM images of the Ag core-DHPAC shell NCs: red and green arrows indicate Ag core and polymer shell layer, respectively.

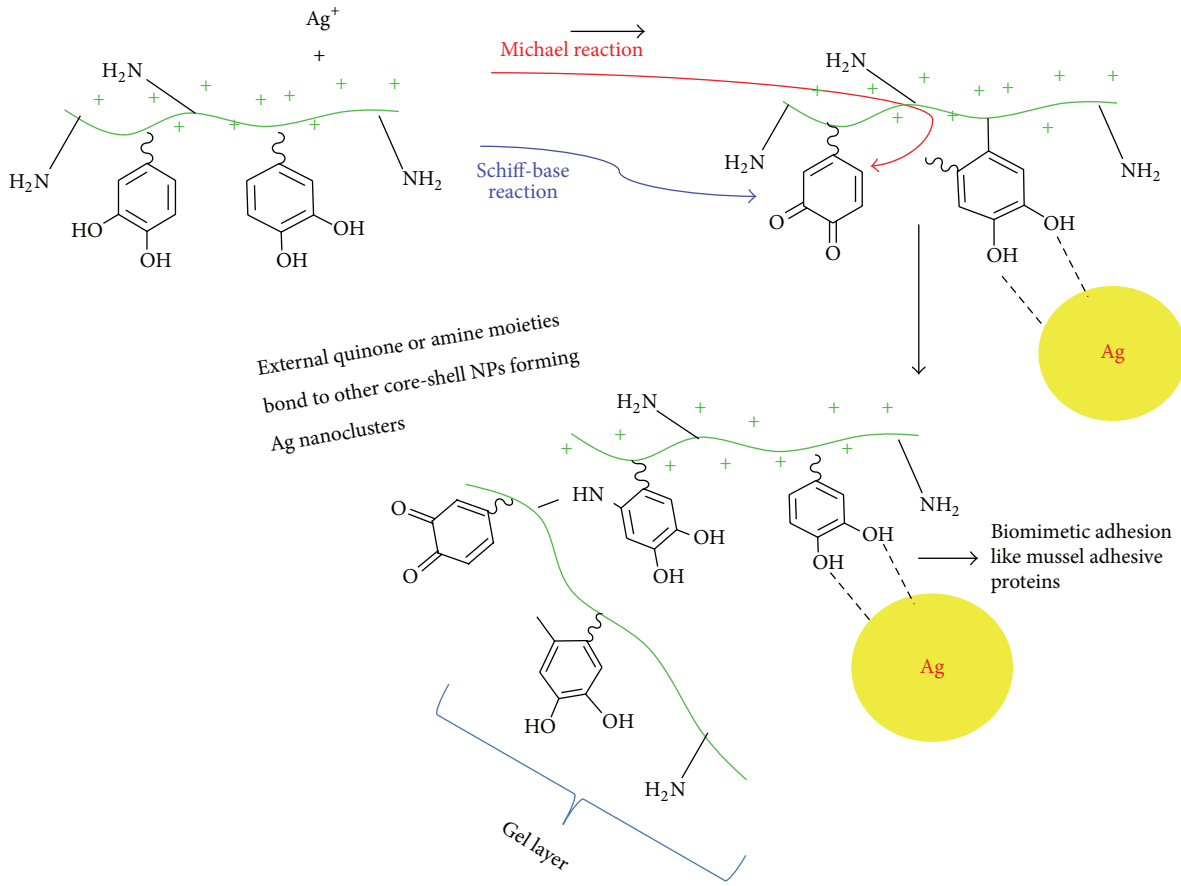



Figure 6: Core-shell AgNPs formation via biomimetic adhesion and chemical reactions.

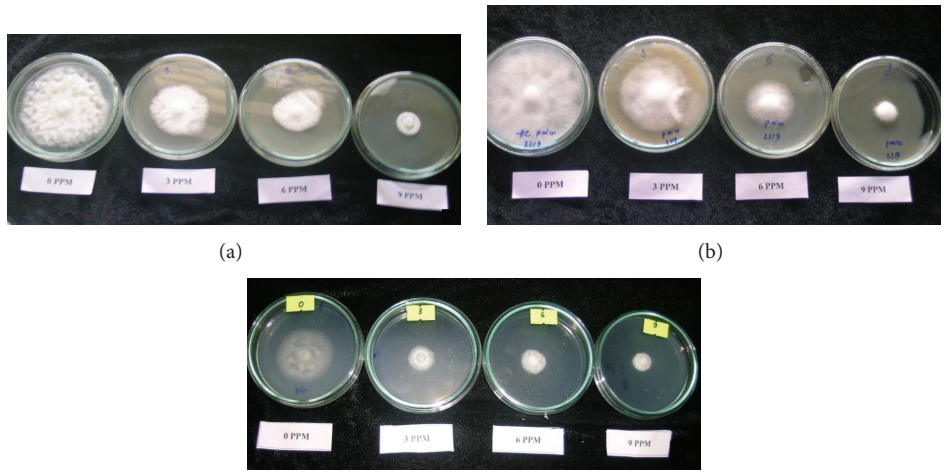
DHPAC chains via Michael reaction between amine group of chitosan and double bond next to carbonyl of oxidized catechol or Schiff-base reaction between amine group of chitosan and carbonyl of oxidized catechol which resulted in formation of a polymeric hydrogel network. In the process, some functional groups on the core-shell AgNPs could bond together to form a nanocluster containing these AgNPs.

*3.3. Antibacterial Activity of Ag Core-DHPAC Shell NCs.* The Ag core-DHPAC shell NCs exhibited strong growth inhibition of plant-pathogenic fungi such as *P. capsici*, *P. nicotianae*, and *P. colocasiae*. Figure 7 showed results obtained from antifungal experiments of the AgNCs colloidal solutions. There was strong growth of these fungi in control sample without AgNCs. A high colony diameter of the fungi was recorded. In the presence of small amount of AgNCs (3 ppm), growth of the fungi was significantly decreased.

Figure 7 showed growth inhibition of these fungi, in which *P. capsici* fungus was inhibited at 80% in the presence of 9 ppm AgNCs. Growth inhibition of *P. nicotianae* and *P. colocasiae* was approximated. Fifty percent growth inhibition of these fungi was recorded at six ppm of AgNCs approximately, which was a low concentration compared to effective dose (ED50) of inhibition for plant-pathogenic *Corticium salmonicolor* at 27.2 ppm of AgNPs [23]. In additional experiment, antifungal activity of oligochitosan has also been conducted. In the same concentration with DHPAC used in preparation of AgNCs, the chitosan was diluted in the same manner to the highest concentration at 45 ppm. In the concentration, chitosan did not show antifungal activity. It was in agreement with a previous report in which oligochitosan polymer solution showed antibacterial activity at high concentration (100 ppm) [22].

Different behaviors might be due to differences in organizations, structures, and functions of these plant-pathogenic fungi or due to different interaction of the core-shell AgNPs and oligochitosan with the fungi (Figure 8).

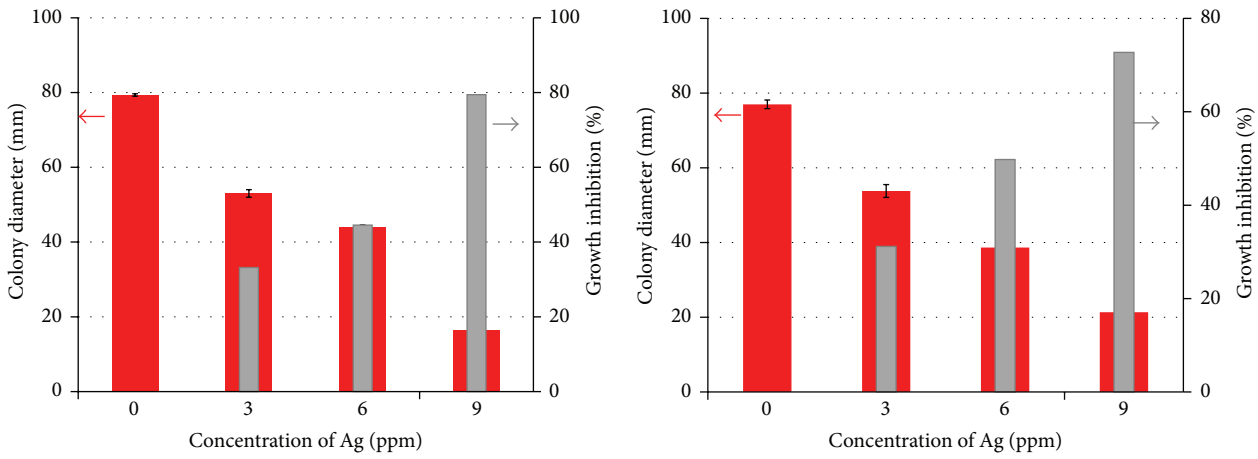
For the Ag core-DHPAC shell NCs, chitosan-based shell layer might improve antifungal activity of the AgNCs because of cationic polymer possessing antimicrobial or antipathogenic activity. Xu et al. reported that oligochitosan exhibited the highest inhibitory growth with *P. capsici* compared to other phytopathogens. Fifty percent inhibitory growth of *P. capsici* was reported at 100 ppm of oligochitosan [24]. Although the activity was much lower than that of AgNPs, the polymer layer could act as an active targeting site and result in increasing interaction of the cationic chitosan shell layer on Ag core in the NCs and phospholipid layer on bacterial membrane via electrostatic interaction. This brought the Ag core-DHPAC shell NCs to the surface of the microbes and sustainedly released $Ag^+$ ions could kill the




(a) (b)

(c)

Figure 7: Growth of the fungi in diffrent PDA media with and without AgNCs: *P. capsici* (a), *P. nicotianae* (b), and *P. colocasiae* (c).




(a) (b)

Figure 8: Growth inhibition of Ag core-DHPAC shell NCs on the plant-pathogenic fungi: *P. capsici* (a) and *P. nicotianae* (b).

fungi. Slade and Pegg reported that several kinds of *Phytophthora* fungi were killed by $Ag^+$ in the range 46–460 nM (5–50 ppb) [25]. Sustained release of $Ag^+$ ions from the Ag core-DHPAC shell NCs could be significant to inhibit growth of the plant-pathogenic fungi and protect plants from *Phytophthora* fungi.

## 4. Conclusion

In this work, silver core-chitosan shell nanoclusters were prepared via chemical reduction using chitosan derivative as a reducing and protecting agent. Low concentration of the nanoparticles exhibited a powerful activity against plant pathogens, such as *P. capsici*, *P. nicotianae*, and *P. colocasiae*. Positive results show the potential application as an inexpensive ecofungicide for agriculture.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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