



Synthesis and Characterization of Potential Fungicidal Silver Nano-sized Particles and Chitosan Membrane Containing Silver Particles

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Received 19 November 2008; accepted 13 April 2009

ABSTRACT

Silver nano-sized particles were synthesized with strong antifungal activity against the wood staining fungi *Ophiostoma flexuosum*, *O. tetropii*, *O. polonicum* and *O. ips*. The average size and purity of the nano-sized particles were determined by scanning electron microscopy and XRD spectra, respectively. The structural characterizations of nano-sized particles were performed using IR spectrum and EDS. Radial diffusion method was carried out to reveal the antifungal activity of silver nano-sized particles on MEA agar plates. Strong fungicidal silver nano-sized particles were mixed with chitosan solution and then the chitosan membrane was prepared by solvent evaporation method. Glutaraldehyde was used as cross-linking agent for membrane preparation. Deacetylation degree, concentration and pH of chitosan were considered as a main factor for preparation of bioactive membrane. The quality and structure of membranes were analyzed by Fourier transform infrared spectroscopy. Thermostability of the membranes was confirmed by thermogravimetric analysis. Synthesized chitosan-glutaraldehyde-Ag membrane was tested against the wood staining fungi on MEA agar plates using inhibition zone method. The results showed that the antifungal activity of chitosan membrane increased with increasing in concentration of silver nano-sized particles. According to our results, synthesized chitosan membrane has been used as a good carrier for silver nano-sized particles in wood industries and these materials could be very efficient substitutions for chemical preservatives currently used to control fungal contamination in wood industries.

Key Words:

silver nano-sized particles;
chitosan;
chitosan membrane;
wood staining fungi;
antifungal activity.

INTRODUCTION

Chitosan is an environmental friendly, nontoxic polymer derived by partial deacetylation of chitin. Many researchers synthesized chitosan membrane using different solvents with various properties, to use in various fields, such as therapeutic formulations, drug delivery, food preservation and package, waste materials treatment, biodegradable products production

and separation processes [1-6]. Chitosan, a polymer composed of β -(1 \rightarrow 4)-*N*-acetyl-*D*-glucosamine, a major component of crab, shrimp and crawfish [7]. The antimicrobial and antifungal responsibility of chitosan depends on the following reasons: chitosan deacetylation degree, molecular weight, pH of the medium and temperature [8]. Many authors have reported the

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potential antimicrobial activity of chitosan [4,8], yet the exact mechanism of antimicrobial activity of chitosan is not studied well. Among many reasons suggested by researchers it is mainly explained by the chelating character of chitosan, capability to modify the concentrations of Ca^{2+} ions and other essential minerals, trace elements important for microbial growth especially for filamentous fungal growth. Some other mechanisms have also been proposed towards the antibacterial and antifungal activity of chitosan; the antifungal inhibition could be due to the direct interaction between cell membrane and chitosan, as chitosan can directly disrupt the membrane function and the pH of chitosan plays crucial role in the interaction between cell protein and chitosan. At acidic pH the interaction between chitosan and proteins is low, and accordingly most proteins can be expected to be below their isoelectric point and positively charged at acidic pH [9]. Chitosan can also be inhibiting the cell transcription and translation indirectly by interaction of hydrolysis products with bacterial and fungal DNA, which prevents the synthesis of mRNA and proteins [8].

Silver nanoparticles are widely known for its bactericidal and fungicidal activity. Silver nanoparticles are very effective antimicrobial and antifungal agents at lower concentration [10]. The antimicrobial activity of silver is much higher than other metals, such as mercury, copper, lead, chromium and tin. Silver (I) oxide can produce free silver ions in the presence of oxygen, thus, the antimicrobial activity of silver depends on the active surface of silver. Release of free silver ions from silver (I) oxide by chitosan is more quick and rapid [8]. At lower concentration, silver nanoparticles directly damage the cell envelope by penetrating the cell and then silver binds to the DNA, this complex prevents the DNA replication by displacement of hydrogen bonds between adjacent nitrogens of purines and pyrimidines [11]. Chitosan has a high metal binding potential, especially for silver [4].

The amine and hydroxyl groups of chitosan play important role in uptake of silver cations by a chelation mechanism and sorption, respectively. Binding of silver nanoparticles into chitosan membrane mainly depends on the pH. Large quantity of silver nanoparticles binds with chitosan membrane at high-

er pH than lower pH, since the amino group is protonated at lower pH [4].

Commercial usages of chitosan membrane have been reported widely in the literature, especially used in food industries. Chitosan used to improve shelf-life period of bread, kimchi (Korean traditional vegetable fermented food), milk, noodle, rice cake, soybean curd, soybean sprouts, and starch jelly. Chitosan used as protective barriers for egg, fruits, and vegetables storage and processing aid in fruit juice production. Spoilage bacterial growth inhibited by chitosan in seafood's and seafood products by retarding the lipid oxidation [7]. Chitosan coating and films are used in storage of perishable foods [7].

According to recent findings, feasibility of silver nano-sized particles as antibacterial agent is well established while a very few investigations have been carried out on possible usage of silver nano-sized particles and chitosan as a favourite wood preservative material [6,12]. The effective role of silver nano-sized particles and chitosan against wood staining fungi is not yet been established well. The most important goal of this work is to synthesize the silver nano-sized particles with very low minimum inhibitory concentration (MIC) against wood staining fungus which validate its application in wood industries and based on our knowledge, this is the first attempt to evaluate the antifungal activity of chitosan (as a membrane form) against wood contaminating fungi. The aim of our research work is to prepare chitosan membrane containing silver nano-sized particles with strong antifungal activity against wood staining fungi. Chitosan membrane was prepared by mixing appropriate concentrations of chitosan, silver nano-sized particles and glutaraldehyde by magnetic stirring, the synthesized membrane was characterized and then tested against wood staining fungi in *in vitro* condition. This research work could be helpful to use the silver nano-sized particles and chitosan as a wood preservative material in wood industries.

EXPERIMENTAL

Fungal Strains and Materials

The following sapstaining fungal samples were used to determine the antifungal activity of silver nano-

particles and membrane: *Ophiostoma flexuosum* (363175), *Ophiostoma tetropii* (363182), *Ophiostoma polonicum* (343181) and *Ophiostoma ips* (363176). All the fungal cultures were obtained from CABI, Bioscience, UK Centre, formerly called as International Mycological Institute (IMI). The fungal cultures were grown in 2% MEA (Becton, Dickinson and Company, USA) medium as pre-inocula at 25°C for 4-7 days.

Chitosan (MW 100,000, 80% deacetylation degree) was obtained from Textile Chemistry Laboratory, Chonbuk National University, S. Korea. Analytical grade chemicals glutaraldehyde, isopropanol, hydrochloric acid, sodium borohydride, and silver nitrate were obtained from Sigma-Aldrich, USA.

Silver Nano-sized Particles Synthesis

Silver nitrate, sodium borohydride, distilled water were used as source materials for preparation of silver nanoparticles. Analytically pure grade chemicals were used for this experiment. 0.003 M of hydrated silver nitrate solution and 0.002 M of sodium borohydride solutions were prepared and chilled to 0°C, separately. The chilled silver nitrate solution was added drop by drop into the sodium borohydride solution which was taken in a reservoir under nitrogen atmosphere and stirred for 2 h. After stirring, silver ions were reduced to form the monodispersed nanoparticles in the solution. Finally, nanoparticles solution was stored at room temperature.

Membrane Preparation

At first, 2% chitosan solution was sterilized at 121°C for 20 min and the chitosan membranes were developed by solvent evaporation and film casting techniques. The appropriate amounts of the sterilized chitosan (chitosan solution was maintained with pH 5), silver nano-sized particles (10 ppm, 50 ppm and 100 ppm) and 5 vol% of glutaraldehyde solution which consists of IPA/water (90/10 vol%) mixture, 1 vol% of hydrochloric acid as a catalyst were mixed well and magnetically stirred for 2-3 min. The resultant viscous solution was spread on a clean glass plate and dried at 37°C for 48 h. Silver nano-sized particles were sonicated for 10 min before addition with chitosan mixture.

Characterization of Silver Nano-sized Particles and Membrane

Morphological characteristics of the synthesized nano-sized particles and mapping were analyzed by scanning electron microscope coupled with EDX and elements mapping. Air-dried samples were used for SEM analysis. The samples were coated with gold by ion sputtering (Jeol JFC-1200 fine coater) and observed under SEM (JSM-5200). X-Ray diffraction (XRD) patterns for the silver nano-sized particles were obtained using a (D-Max 3A, Rigaku XRD) diffractometer in the 2 θ range from 30 to 80 with Cu, K α radiation. The structural characterization of the silver nanoparticles and membranes were investigated by FTIR. FTIR spectra of the samples were recorded at room temperature using Fourier transform infrared spectroscopy (Spectrum GX, USA) in the region from 400 cm⁻¹ to 3000 cm⁻¹. Thermal behaviour of the membranes was examined by Perkin Elmer instrument. The TGA measurements were carried out under a nitrogen atmosphere with a heating rate of 20°C/min from 30°C to 800°C.

Antifungal Activity

Evaluation of Antifungal Activity of Silver Nanoparticles and Chitosan Membrane

Percentage inhibition of sapstaining fungi was measured by radial mycelial growth method in presence of various concentrations of silver nano-sized particles. Four different concentrations (1 ppm, 10 ppm, 50 ppm, and 100 ppm) of silver nano-sized particles were used in antifungal activity test. Two percentage MEA media were prepared with above mentioned percentages of silver nano-sized particles solution, separately. Six millimeters agar plugs from the pre-inoculum of *O. flexuosum*, *O. tetropii*, *O. polonicum* and *O. ips* were transferred to 2% MEA amended with different concentrations of the tested mixture. Control plates for all 4 fungal samples were maintained without antifungal agents. All plates were incubated at 20°C for 7 days. The observation of mycelial growth was followed until 7 days and data's were registered.

Inhibition zone method was carried out to evaluate the antifungal and antibacterial activity of chitosan membrane. To make small pieces of membrane, all membranes were kept in distilled water for 1 h and membranes were punched to make small pieces

(approximately 6 mm in diameter) and then they were air-dried inside a laminar air flow chamber. Fungal samples *O. flexuosum*, *O. tetropii*, *O. polonicum* and *O. ips* were inoculated in 2% MEA plates before placing the membranes. All membrane samples were placed in 3 corners of each fungus, separately. Plates were incubated at 20°C for 7 days.

Statistical Analysis

Additionally, statistical analysis was conducted to evaluate the fungicidal activity of silver nano-sized particles. Four replicates were maintained for 4 fungal samples at all concentrations including control. The difference between the mycelial growths of replicates were not significant, thus the average was used for antifungal index analysis. Antifungal index was calculated based on the method of Zhong [13]:

$$\text{Antifungal index (\%)} = (1 - Dt / Dc) \times 100$$

where Dt = diameter of mycelial growth zone in test plate; Dc = diameter of mycelial growth zone in control plate. Results with significant difference $P < 0.05$ were considered statistically [14].

RESULTS AND DISCUSSION

Characterization of Silver Nano-sized Particles and Membrane

Figure 1 shows typical SEM image of smooth surface, spherical shape silver nano-sized particles and

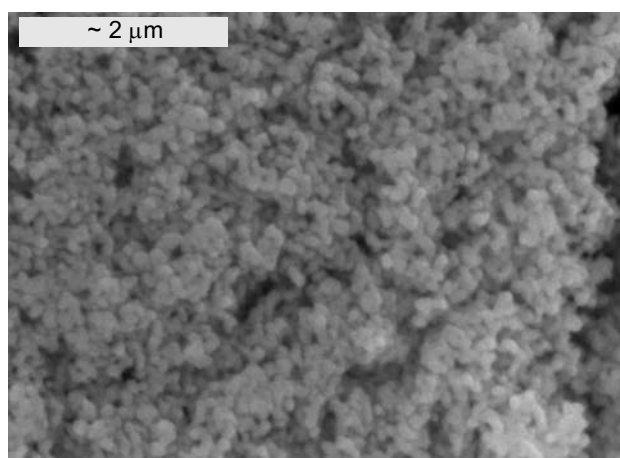


Figure 1. SEM image of silver nanoparticles.

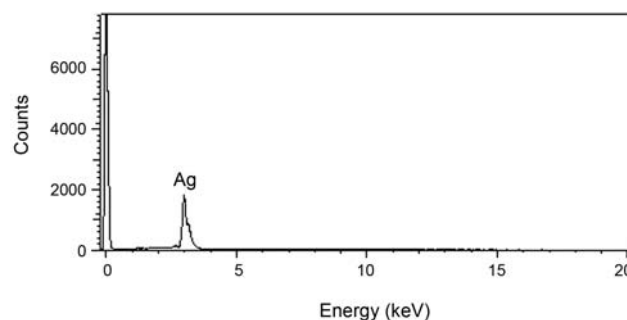


Figure 2. EDS profile of silver nanoparticles.

the average size of the nanoparticles found to be 100 to 200 nm. Figure 2 shows the EDS profile of silver nano-sized particles which indicate the formation of silver nano-sized particles without any external oxide contamination. The crystal nature of synthesized silver nano-sized particles was confirmed by XRD profile. Four characteristic peaks were appeared for silver nano-sized particles in XRD profile (Figure 3). These peaks are exactly matched with the standard values of metallic silver. XRD and EDS results show that nanoparticles were produced without any impurities. Silver nano-sized particles existence was confirmed by IR spectrum (Figure 4). A strong band was observed at 2378 cm^{-1} along with two other shoulder peaks at 1621 cm^{-1} and 1318 cm^{-1} . 2378 cm^{-1} indicates the strong aliphatic C-H stretching vibration and the additional shoulder peaks correspond to silver ions.

The thickness differences between the different

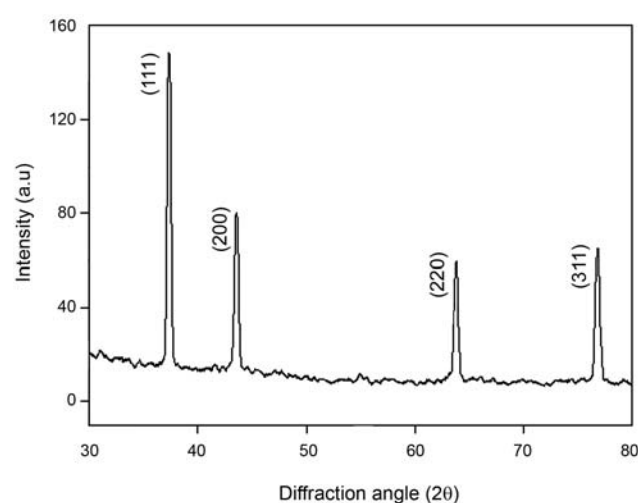


Figure 3. XRD pattern of silver nanoparticles.

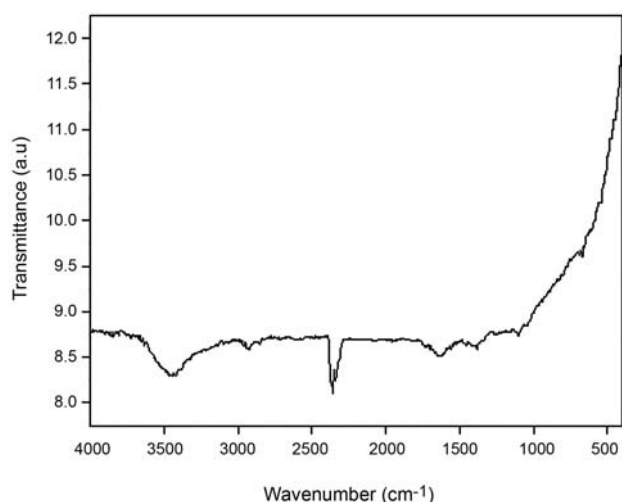


Figure 4. IR spectrum of silver nanoparticles.

membranes were identified by naked visualization. Pure chitosan membrane was comparatively thinner than chitosan-glutaraldehyde membrane and chitosan-glutaraldehyde membrane was darker than chitosan membrane. It is already reported that thickness of the membrane depends on long drying time [5]. Thin membranes were very flexible and transparent than thick membranes. Thicker membranes were rigid. Figure 5 displays the FTIR spectra of pure chitosan membrane and blended chitosan membranes. The characteristic bands of pure chitosan membrane are described as follows (Figure 5): the bands observed at 1155, 1067, 1030, and 894 cm^{-1} are attributed for the stretching vibration of C–O–C linkages in the saccharide structure. The bands appeared at 1324 cm^{-1} and 1380 cm^{-1} reflect the stretching vibration of the C–N

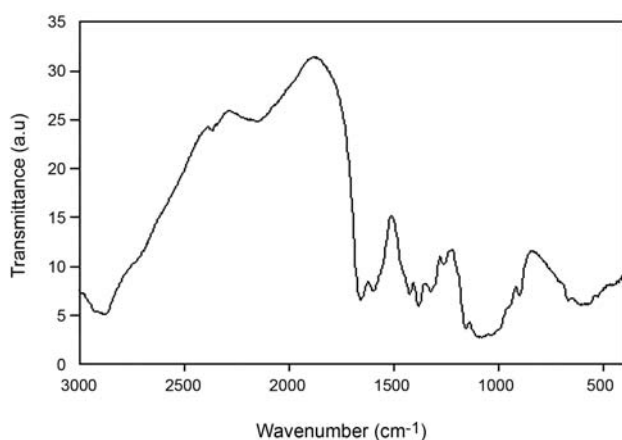


Figure 5. FTIR spectrum of chitosan-glutaraldehyde membrane.

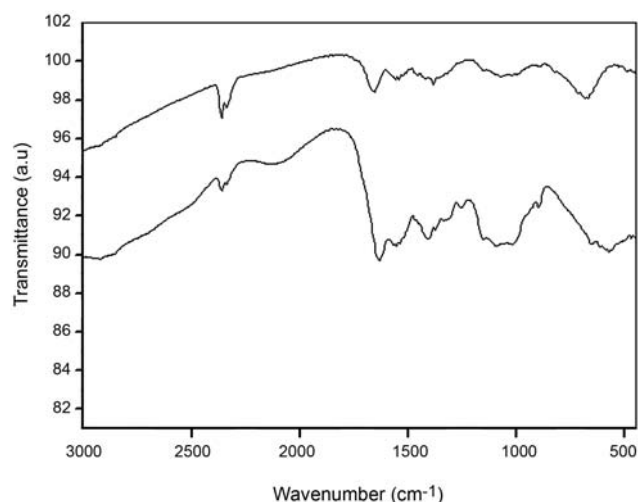


Figure 6. FTIR spectra of chitosan-glutaraldehyde-Ag membrane.

bond (amide III) and the C–H binding modes of methylene. A strong peak observed at 1600 cm^{-1} is assigned for the NH_2 absorption band [13]. All the bands described above were observed for the blended membranes. Addition of silver nitrate shifted the characteristic peak of amide from 1632 cm^{-1} to 1661 cm^{-1} and ensures the incorporation of silver ions into the membrane (Figure 6). A shift was effectively caused by the coordination interaction between NH_2 groups of the chitosan and silver ions, called as an inductive effect [4]. Besides, the homogeneous distribution and presence of silver nanoparticles in synthesized membrane were confirmed by elements mapping and EDX analysis (Figures 7 and 8). Carbon peak in EDX may be attributed to the chitosan in the membrane and Ag peaks indicate its presence.

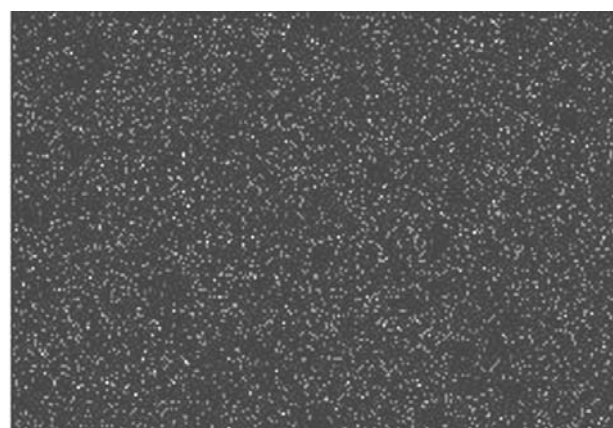


Figure 7. Elemental mapping of chitosan-silver membrane.

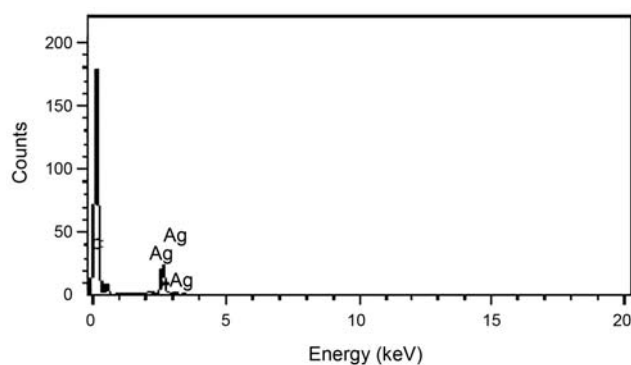
Table 1. Thermostability of chitosan and chitosan-glutaraldehyde membrane.

| Temperature (°C) | Chitosan membrane weight loss (%) | Chitosan-glutaraldehyde membrane weight loss (%) |
|------------------|-----------------------------------|--|
| 100 | 10.14 | 7.12 |
| 200 | 22.54 | 13.77 |
| 250 | 27.99 | 25.26 |
| 400 | 62.19 | 49.50 |
| 650 | 70.08 | 60.37 |

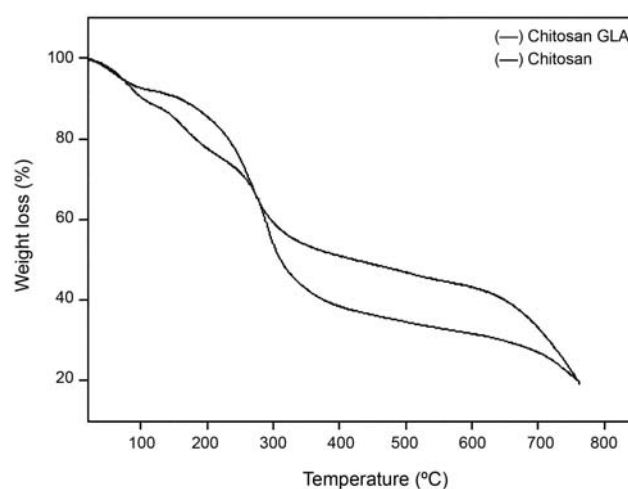
Thermostability of the prepared membranes was confirmed by thermogravimetric analysis (Figure 9). Weight loss of chitosan and chitosan-glutaraldehyde membranes were shown in two stages. First stage of weight loss was observed between 50°C to 350°C, over 50% of weight loss was observed above 350°C. This loss may be due to the loss of adsorbed and bounded water [15]. A second stage of weight loss observed between 400°C to 650°C, it was attributed to the degradation of chitosan. A much lower weight loss was observed for the chitosan-glutaraldehyde membrane than pure chitosan membrane. 60.37% of weight loss was observed at 650°C in chitosan-glutaraldehyde membrane (Table 1), which indicates the higher thermostable character of chitosan-glutaraldehyde membrane.

Antifungal Activity of Silver Nano-sized Particles and Membrane

Wood can easily be infected by a wide range of fungi, including wood-decay fungi and staining fungi. Wood staining is caused by sapstaining fungi due to the production of melanin in ray parenchyma tissues and cell lumens of fungal hyphae. The strength of wood is not affected by sapstaining fungi, but it can cause

**Figure 8.** EDX profile of chitosan-silver membrane.

serious damage in natural colour of wood. *Ophiostoma sp.* is one of the major genera of sapstaining fungi. Many authors have studied wood decay fungi and sapstaining fungi control on wood by chemical, biological agents and natural products. Mycelial growth of *Ophiostoma flexuosum*, *O. tetropii*, *O. polonicum* and *O. ips* were reduced on media amended with different concentrations of silver nano-sized particles (Figures 10-13). The results showed that mycelial inhibition of all tested fungi was started from minimum concentration of silver nano-sized particles used in the media. *O. flexuosum* was highly sensitive fungus towards silver nanoparticles; no growth was observed when high concentration (100 ppm) used (Figure 11). *O. ips* exhibits slight tolerance against silver nano-sized particles. Thirty millimeters of *O. ips* mycelial growth was observed in MEA medium containing 100 ppm silver nano-sized particles (Figure 10). While 24 mm and 14 mm in diameter mycelial growth of *O. polonicum* and *O. tetropii* were observed in MEA media containing 100 ppm silver nano-sized

**Figure 9.** Thermogravimetric analysis of chitosan and chitosan-glutaraldehyde.

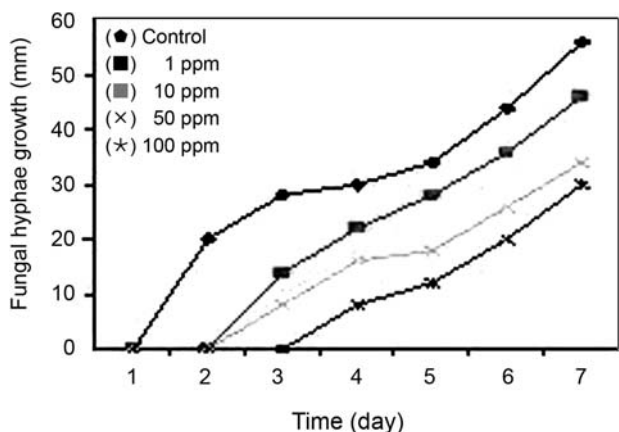


Figure 10. Effect of silver nanoparticles on mycelial growth of *O. ips*.

particles, respectively (Figures 12 and 13).

According to statistical analysis, among four different concentrations of silver nano-sized particles solution, the mycelial growth was highly inhibited at the concentration of 50 ppm and 100 ppm, *O. tetropii* was highly sensitive towards all concentrations of silver nano-sized particles. 56% to 81% of growth inhibition was observed in *O. tetropii* plates (Table 2). While 100 ppm of silver nano-sized particles completely inhibited the growth of *O. flexuosum* in MEA agar plates (Table 2). In *O. ips* plates, 39% and 46% of growth reduction were observed at 50 ppm and 100 ppm concentration, respectively (Table 2). Growth inhibition percentage of *O. polonicum* increased gradually which was dependent on the increasing concentration of silver nano-sized particles 39%, 53%, and 68% growth reduction were observed

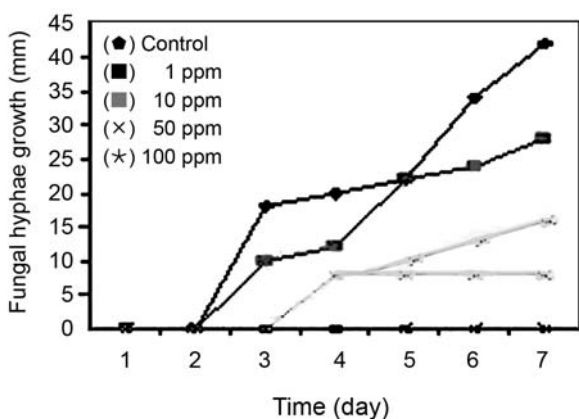


Figure 11. Effect of silver nanoparticles on mycelial growth of *O. flexuosum*.

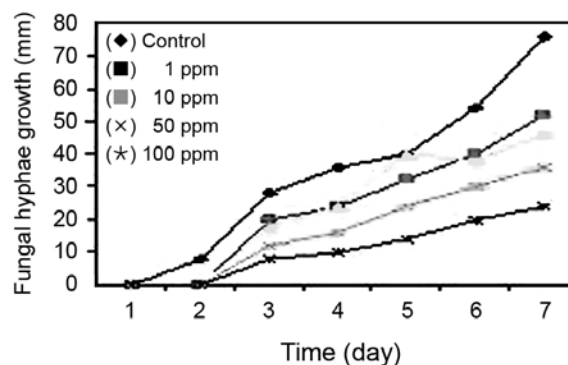


Figure 12. Effect of silver nanoparticles on mycelial growth of *O. polonicum*.

at plates amended with 10, 50, and 100 ppm silver nano-sized particles plates, respectively (Table 2). Based on our results, the inhibition of radial growth of all tested fungi was due to the antifungal properties of silver nano-sized particles.

Chitosan is widely used as a natural product to control fungal contamination in food industries and so on. The antifungal activity of chitosan-glutaraldehyde-silver nano-sized particles has shown in Figure 8. It is clearly evident that chitosan-glutaraldehyde membrane containing silver nano-sized particles showed strong antifungal activity against wood staining fungi group, *Ophiostoma flexuosum*, *Ophiostoma tetropii*, *Ophiostoma polonicum*, and *Ophiostoma ips* (Figures 14-17). *Ophiostoma polonicum* and *O. flexuosum* were highly sensitive towards chitosan-glutaraldehyde-Ag nanoparticle membrane than *O. ips* and *O. tetropii* (Figures 14-17). Many mechanisms were proposed for the antifungal property of chitosan, among those mechanisms, the main reasons

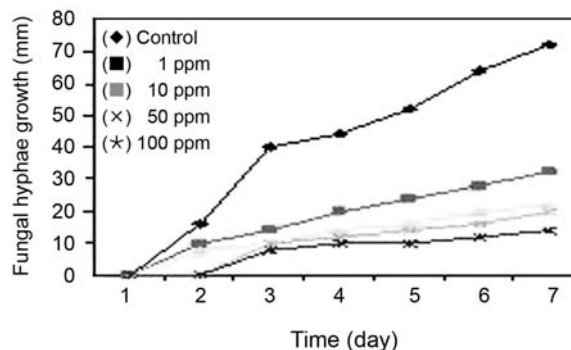


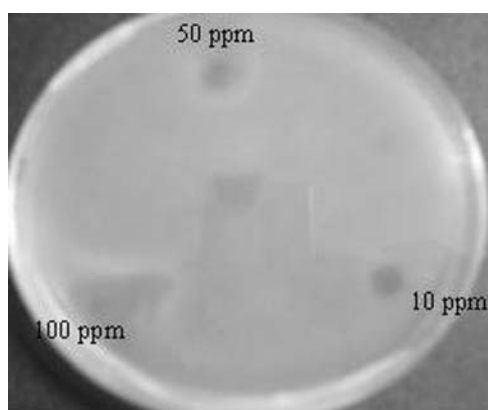
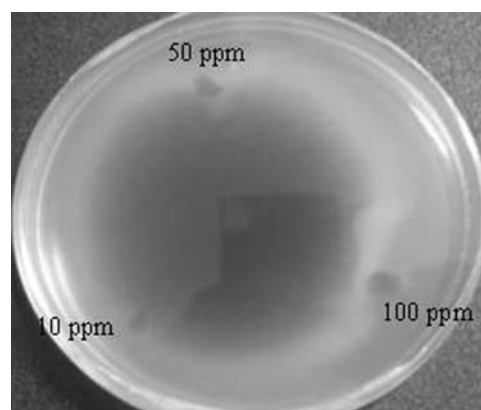
Figure 13. Effect of silver nanoparticles on mycelial growth of *O. tetropii*.

Table 2. Percentage inhibition of fungal growth in 2% MEA agar plates in the presence of silver nano-sized particles.

| Fungal samples | Control (without silver nanoparticles) | Silver nanoparticles (percentage inhibition %) | | | |
|---------------------|---|--|--------|--------|---------|
| | | 1 ppm | 10 ppm | 50 ppm | 100 ppm |
| <i>O. ips</i> | 0 | 18 | 29 | 39 | 46 |
| <i>O. flexuosum</i> | 0 | 33 | 62 | 81 | 100 |
| <i>O. polonicum</i> | 0 | 32 | 39 | 53 | 68 |
| <i>O. tetropii</i> | 0 | 56 | 69 | 72 | 81 |

may be due to the interaction of chitosan with cell membrane, proteins and DNA [8]. The inhibition rate of pure chitosan was not significant (date not shown) compared to chitosan-silver complex. Some fungal species are highly resistant towards pure chitosan, since those species contain chitosan biopolymer in its cell wall. *Aspergillus sp.* is the best example for chitosan resistant fungus. Based on our results, wood staining fungal species are also not sensitive towards pure chitosan. However, chitosan was applied with other antifungal agents, such as silver, the complex has remarkable antifungal activity. According to literature, the chitosan with some antimicrobial agents, such as silver, zinc, thiourea markedly inhibited the bacterial growth relative to fungal growth [16]. The antifungal activity of chitosan-silver ions solution may be due to the interaction of complex with protein, which leads to the inactivation of protein and direct interaction with DNA, this interaction creates the mutation and stops the replication ability of DNA. This solution can also go through the cell wall easily,

since those materials are very small in size. This passage can induce the cell lysis. The antifungal activity of chitosan mainly depends on the concentration and pH of chitosan. The antimicrobial activity of chitosan has been studied well [4,5,8,9,12]. Literature results showed that antifungal activity of chitosan decreased with increasing in concentration of chitosan [12], this is due to the formation of cross-linked structure of chitosan amino groups through their strong intramolecular hydrogen bonding, which diminished the possibilities of interaction between fungal cell membrane and amino group of chitosan [12]. Microbial growth inhibition of fungi by chitosan also depends on the direct disturbance of cell membrane and chitosan can act as a chelating agent, which can prevent the normal nutritional pathway of fungi [12]. Hu et al. [17] reported the antimicrobial activity of silver particles with chitosan solution. The chitosan concentration and pH of chitosan solution were chosen based on previous reports and our laboratory results [4,16]. As shown in Figures 10-13, the antifungal activity of

**Figure 14.** Photograph of the antifungal test results of the chitosan-glutaraldehyde membranes with different concentrations of silver against *O. tetropii*.**Figure 15.** Photograph of the antifungal test results of the chitosan-glutaraldehyde membranes with different concentrations of silver against *O. ips*.

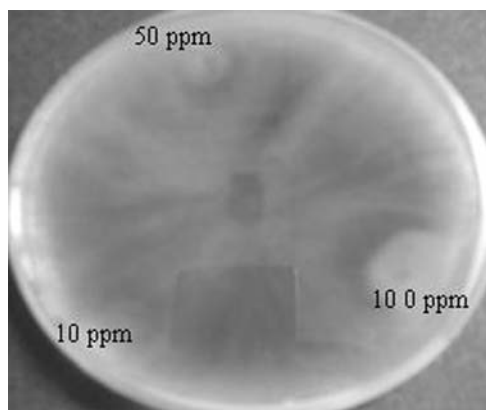


Figure 16. Photograph of the antifungal test results of the chitosan-glutaraldehyde membranes with different concentrations of silver against *O. polonicum*.

membrane was increased with increasing concentration of Ag nano-sized particles. As mentioned above, the antibacterial activity of chitosan has been studied well, but the antifungal activity of chitosan varies depending on the fungal species tested against chitosan. The antifungal activity of pure chitosan was not significant. It could be due to the repulsion or weak interaction between the chitosan charge and negative charge fungal cell surface. The antifungal response of chitosan membrane and chitosan solutions is not similar. The differences between antibacterial response of chitosan membrane and chitosan solutions were already reported [4]. The inhibition zone of chitosan membrane was increased with the increasing concentration of silver nano-sized particles. Thus, the results support the role of silver nano-sized particles in antifungal activity. The antifungal activity of Ag^+ has been well studied. The potential role of Ag in wood preservation was well studied by Dorau et al. [11]. The antifungal activity of silver ions could be described as following reasons: disruption of transmembrane energy metabolism and membrane electron transport chain by formation of insoluble compounds in the cell wall, the formation of insoluble compound may be due to the inactivation of cell wall sulphhydryl group; silver ions can create mutation in fungal DNA by displacing the hydrogen bonds; silver ions can dissociate the enzyme complexes which are essential for respiratory chain and membrane permeability, disruption of membrane bounded enzymes and lipids could cause the cell lysis. The chitosan pH

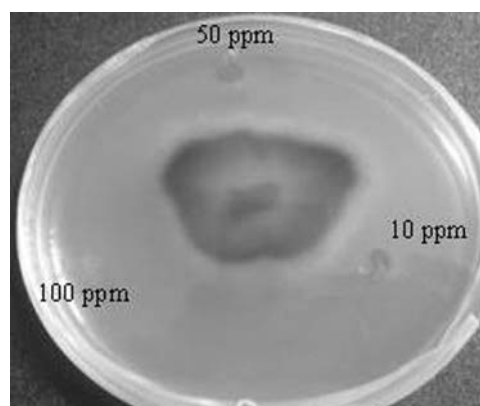


Figure 17. Photograph of the antifungal test results of the chitosan-glutaraldehyde membranes with different concentrations of silver against *O. flexuosum*.

5 is also highly influenced the higher interaction between the chitosan amine groups with electron pair of silver nanoparticles. The different concentrations of silver nano-sized particles play a major role in the antifungal activity. There was no higher significant difference identified between the antifungal activity of silver ions at 10 ppm and 50 ppm, however, 100 ppm silver nano-sized particles containing membrane had remarkable antifungal activity against all tested sapstaining fungal samples. The usage of different concentrations of silver nano-sized particles, given a comprehensible note on the feasible amount of silver, may be applied into chitosan membrane to produce significant antifungal activity. Our experiment results showed that chitosan membrane is a good carrier for silver nano-sized particles and silver nano-sized particles can be easily incorporated with chitosan membrane by treating with silver nano-sized particles solution, these silver incorporated membranes are highly active antifungal materials. The potential of chitosan-silver membrane and solution against wood staining fungi has been studied well. The growth of sapstaining fungi were reduced by chitosan-silver membrane and radial growth of sapstaining fungi were also reduced by silver nano-sized particles at minimum concentrations.

CONCLUSION

A hundred ppm of silver nanoparticles were com-

pletely inhibited the growth of *O. flexuosum* and more than 50% growth reduction observed in other tested fungal samples. Same results were observed in the chitosan membrane containing silver nano-sized particles. Thermostability of membrane was gradually increased with addition of cross-linking agent glutaraldehyde. In view of our experimental results, chitosan has been identified as a good carrier for silver ions in wood industries. The coating of chitosan membrane incorporated with silver nano-sized particles could reduce the fungal contamination in wood in industries and it could be used as a preservative material. This investigation has given the preliminary information to determine the antifungal activity of chitosan-silver membrane at laboratory level. With these laboratory level results, further investigations are currently conducted in the field to evaluate the antifungal potential of chitosan-silver membrane against wood staining and wood decay fungi.

ACKNOWLEDGEMENT

This study was supported by Technology Development Programme for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea (No. 105070-03-3-SB010).

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