Mycobiology 39(1) : 26-32 (2011)
DOI:10.4489/MYCO.2011.39.1.026

Inhibition Effects of Silver Nanoparticles against Powdery Mildews on Cucumber and Pumpkin

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(Received October 21, 2010. Accepted February 16, 2011)

Powdery mildew is one of the most devastating diseases in cucurbits. Crop yield can decline as the disease severity increases. In this study, we evaluated the effect of silver nanoparticles against powdery mildew under different cultivation conditions *in vitro* and *in vivo*. Silver nanoparticles (WA-CV-WA13B) at various concentrations were applied before and after disease outbreak in plants to determine antifungal activities. In the field tests, the application of 100 ppm silver nanoparticles showed the highest inhibition rate for both before and after the outbreak of disease on cucumbers and pumpkins. Also, the application of 100 ppm silver nanoparticles showed maximum inhibition for the growth of fungal hyphae and conidial germination in *in vivo* tests. Scanning electron microscope results indicated that the silver nanoparticles caused detrimental effects on both mycelial growth and conidial germination.

KEYWORDS : Agricultural chemical, Inhibition effect, Powdery mildew, Silver nanoparticles

Powdery mildew is one of the most destructive foliar diseases of cucurbits. On cucurbits, the disease can be caused by two fungal species, *Golovinomyces cichoracearum* or *Sphaerotheca fusca*, which are obligate biotrophic ectoparasites that induce identical symptoms but can be distinguished easily under light microscopy [1].

A wide variety of vegetable crops are affected by powdery mildews, including artichoke, beans, beets, carrot, cucumber, eggplant, lettuce, melons, peas, peppers, pumpkins, radicchio, radishes, squash, tomatoes, and turnips. The powdery mildews are a group of pathogens that can cause disease over a wide range of environmental conditions. However, several environmental factors may directly affect the development of this disease in cucurbits including temperature, relative humidity and light. Temperature and humidity must be examined together because it is the water vapor pressure deficit that has the greatest effect on host-parasite interactions [2]. For example, temperatures between 75~85°F and elevated levels of relative humidity (80~95%) in the absence of rainfall promote the development of this disease.

Severe infection by powdery mildew before the flowering stage can reduce the yield of cucumber fruit by 20~40%. Leaf infestation by this pathogen interferes with photosynthesis and respiration, leading to reduced fruit set, inadequate ripening, and poor flavor development [3]. The disease causes a whitish, talcum-like powdery growth on leaf surfaces, petioles and stems. Infected leaves usually wither and die, and the plants senesce prematurely [4]. The disease is a major production problem in many areas of the world, and the reduction of fruit quality and crop yield is the most striking aspects of disease loss.

Genetic resistance is used extensively as a control measure in cucumber and pumpkins, and it is being incorporated into other cucurbit crops. Yield potential should be considered when selecting varieties because some resistant varieties produce less fruit than susceptible varieties that have not been treated with fungicides. Resistant varieties of pumpkin and winter squash are under development. Successive cucurbit plantings should be physically separated because older plants can serve as a source of conidia.

Silver ions are very reactive. They inhibit microbial respiration and metabolism and they cause physical damage [5, 6]. Moreover, silver ions may intercalate into bacterial DNA once they enter the cell which prevents further proliferation of the pathogen [7]. Silver has been used to treat medical ailments for over 100 years due to its natural antibacterial and antifungal properties. It is also used in many applications as a pure free metal or as a compound because it possesses antimicrobial activity against pathogens but is nontoxic to humans.

Recently, nanotechnology has amplified the effectiveness of silver particles as antimicrobial agents. The nanosilver particles typically measure 25 nm. Reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. In fact, nanotechnology helps in overcoming the limitations of size and can change the outlook of the world regarding science [8]. Sil-

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ver nanoparticles have extremely large relative surface areas which increases their contact with bacteria or fungi, vastly improving its bactericidal and fungicidal effectiveness. The larger surface area-to-volume ratio of silver nanoparticles increases their contact with microbes and their ability to permeate cells. When in contact with bacteria and fungus, they will adversely affect cellular metabolism and inhibit cell growth. Silver suppresses respiration, basal metabolism of electron transfer systems, and transport of substrates in the microbial cell membrane. The nano-silver inhibits multiplication and growth of those bacteria and fungi which cause infection, odour, itchiness and sores. Nanoparticle development has restored interest in the antimicrobial effects of metals, which declined following the widespread application of modern synthetic antibiotics. However, studies on the antimicrobial activity of silver nano particles have been performed mostly against the animal pathogens [9].

Here, we report that silver nanoparticles can be used effectively in the control of powdery mildews and the prevention of deleterious infections. Our results support the hypothesis that silver nanoparticles can be prepared in a simple and cost-effective manner and are suitable for formulating new types of fungicidal materials.

Materials and Methods

Nano silver solution and fungicide. Silver nanoparticle used in this study (WA-CV-WA13B, CV) was obtained from Bio-Plus Co. Ltd (Pohang, Korea) at 1,000 ppm initial concentration (Table 1). Different working concentrations of silver nano particles (10 ppm, 30 ppm, 50 ppm, and 100 ppm) were prepared by diluting the original stock solution. All solutions were stored at 4°C until use. Two different fungicides i.e. NSS-F (Dongbangagro, Co., Seoul, Korea) and Fenari (Dongbu HiTek, Seoul, Korea) were used as positive controls.

Field assay. In order to determine the efficacy of silver nanoparticals against powdery mildew in the field, an experiment was carried out in Gothan, Chuncheon, Kangwon-do after cucumbers were infected with the disease naturally. Silver nanoparticle CV was used at four different concentrations (10 ppm, 30 ppm, 50 ppm and 100 ppm). The aerial spray method was used to apply silver nanoparticles around the shoot portion of the whole plants 3~4 weeks before the outbreak of the disease and after disease occurrence. Fungicides NSS-F (Dongbangagro, Co.) and Fenari (Dongbu HiTek) were used as positive controls and distilled water was used as negative control. Disease index was calculated by counting the numbers of infected leaves out of 150 leaves among the treated plants. Also, in order to determine the efficacy of silver nanoparticles, experiment was carried out in a pumpkin field in Sembat, Chuncheon, Kangwon-do with the same procedures used for cucumber. Fungicides NSS-F and Fenari were used as positive controls and water was used as a negative control as described above. Each experiment was repeated three times and disease index was calculated by counting the numbers of infected leaves out of 150 leaves among the treated plants.

***In vitro* assay and scanning electron microscope (SEM) analysis.** Infected leaves of approximately 5 cm × 5 cm were collected aseptically and brought into the laboratory for *in vitro* inhibition analysis of powdery mildew using SEM. The diseased portion was cut out from the leaf and kept in a petri dish (90 × 15 mm). Five mL of four different concentrations (i.e., 10 ppm, 30 ppm, 50 ppm and 100 ppm) of silver nanoparticles CV was applied over the surface of leaves using a sprayer. Water was used as a control. Treated leaves were incubated for four days at room temperature. The treated leaves were then observed by SEM provided by SEM (LV-SEMS-3500N Hitachi, Korea Basic Science Institute-Chuncheon).

Data analysis. The results were obtained one week after the last treatment for after-the-disease-outbreak treatment, and the results were obtained four weeks after the last treatment for before-the-disease-outbreak treatment. Disease incidence (%) was determined by calculating the number of infected leaves out of 150 leaves among the treated plants. A plant with disease symptoms was con- sidered infected.

Results

Effect of silver nanoparticles against powdery mildew in cucumbers. The results for disease incidence (%) in cucumber treated with silver nanoparticles before and after the outbreak of disease symptoms were shown in Fig. 1. The average disease incidence observed in the control plants was 82.0%. All plants treated with silver nanoparticle CV showed disease suppression compared to the control. The disease incidence was significantly lower in plants treated with high concentrations of silver nanoparticle. Inhibition increased with increasing concentration of silver nanoparticles. The chemical fungicide "Fenari" showed the lowest disease incidence (3%). Other commercial fun-

Table 1. Characteristics of silver nanoparticles used in this study

Type	Physical form	Average particles size (nm)	Silver contents (μg/mL)	Solvent
WA-CV-WA13B (CV)	Dark brown colloid	7~25	40,000~50,000	Distilled water

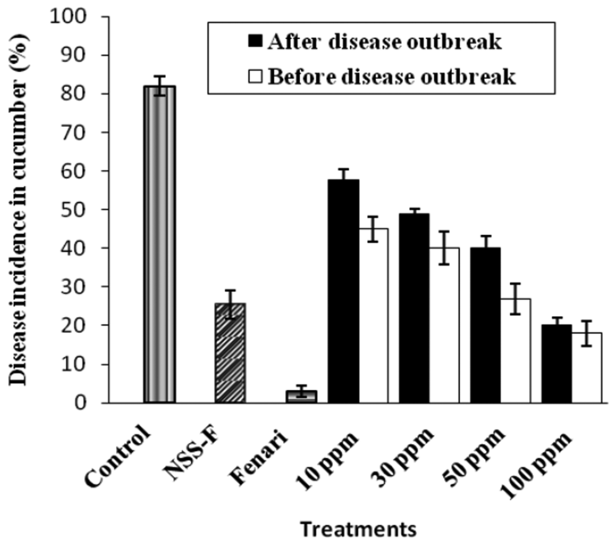

Fig. 1. Effect of silver nanoparticles WA-CV-WA13B against powdery mildew in cucumber. Results were obtained one wk after the last treatment for after-the-disease-outbreak treatment and the other results were obtained four wk after the last treatment for before-the-disease-outbreak treatment. Commercial fungicides NSS-F (Dongbangagro, Co., Seoul, Korea) and Fenari (Dongbu HiTek, Seoul, Korea) were used as positive controls. Distilled water was used as a negative control. Data were obtained from triplicate assays and presented as mean ± SD.

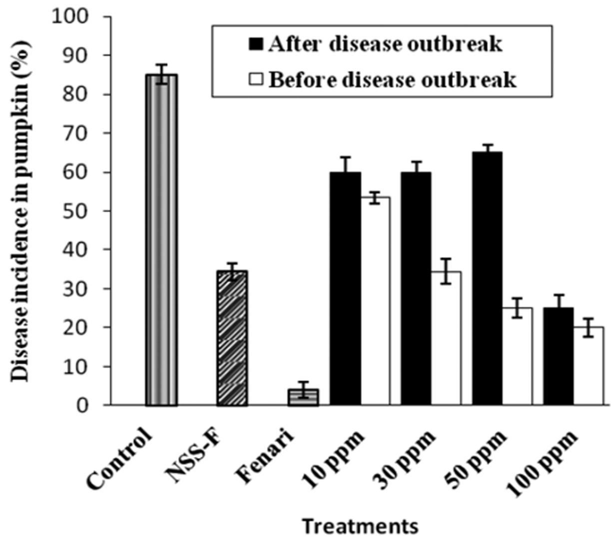

Fig. 2. Effect of silver nanoparticles WA-CV-WA13B against powdery mildew in pumpkin. Results were obtained one wk after the last treatment for after-the-disease-outbreak treatment and the other results were obtained four wk after the last treatment for before-the-disease-outbreak treatment. Commercial fungicides NSS-F (Dongbangagro, Co., Seoul, Korea) and Fenari (Dongbu HiTek, Seoul, Korea) were used as positive controls. Distilled water was used as a negative control. Data were obtained from triplicate assays and presented as mean ± SD.

gicide NSS-F treated plants showed 25.5% disease incidence. The comparative analysis of disease incidence, when plants were treated with different concentrations of silver nanoparticle before and after disease outbreak on plants, was also assessed. The disease incidence was higher in the plants treated after disease outbreak on plants. The disease incidence was observed as 57.8, 48.8, 40.2 and 20% in 10, 30, 50 and 100 ppm concentrations of silver nanoparticles treated after disease outbreak on plants. In similar way, the disease incidence was observed as 45, 40, 27 and 18% in 10, 30, 50 and 100 ppm concentrations of silver nanoparticles treated before the disease outbreaks on plants, respectively. Therefore, results showed the application of silver nanoparticle was more effective when applied before any disease symptoms occurred on plants. Also, 100 ppm concentration of silver nanoparticles was more effective than that of commercial fungicide NSS-F in both before and after the disease outbreak conditions.

Effect of silver nanoparticles against powdery mildew in pumpkin. The disease incidence of silver nanoparticles CV was analyzed against powdery mildew in pumpkins (Fig. 2). The average disease incidence was maximum, i.e. 85% in control plants. Positive control "Fenari" showed the lowest disease incidence (4%) against the powdery mildew, and another commercial fungicide NSS-F showed 34.4% disease incidence, which is higher compared to that of 100 ppm silver nanoparticles application in both before and after disease outbreak conditions. The efficacy of 50 ppm silver nanoparticles was also higher than that of NSS-F when the treatment was done before disease outbreak on plants. However, the disease incidence was similar with that of NSS-F in 30 ppm silver nanoparticles treatment. The disease incidence was observed the highest in case of silver nanoparticles treatment done after disease outbreak in plants. The disease incidence was significantly low when treatment was done before disease appears on plants. Suppression of disease was observed significantly high in all four concentrations of silver nanoparticles when it was applied before disease outbreak in plants. The disease incidence was observed as 60, 60, 65 and 25% in 10, 30, 50 and 100 ppm concentrations of silver nanoparticles treatment after the disease outbreak on plants.

The inhibition of powdery mildew was observed significantly high in case of treatment done before disease outbreaks on pumpkin. The disease incidence was observed in 10 ppm, 30 ppm, 50 ppm and 100 ppm silver nanoparticles were 53.4, 34.4, 25 and 20% respectively, treated before disease outbreak on plants. Commercial fungicide NSS-F showed lower disease suppression rate compared to 100 ppm treatment on pumpkins on both before and after the disease outbreak conditions. The result showed that the inhibition of powdery mildew on both plants depends on the concentration of silver nanoparticles and treatment timing. Successful reduction of powdery mildew on pumpkin can be achieved when silver nanoparticles are applied in dose dependent manner as well as before disease outbreak on host plants.

SEM analysis of silver nanoparticles against hypha and spore germination. Powdery mildew infected leaves were used to analyze via SEM the inhibition of the dis-

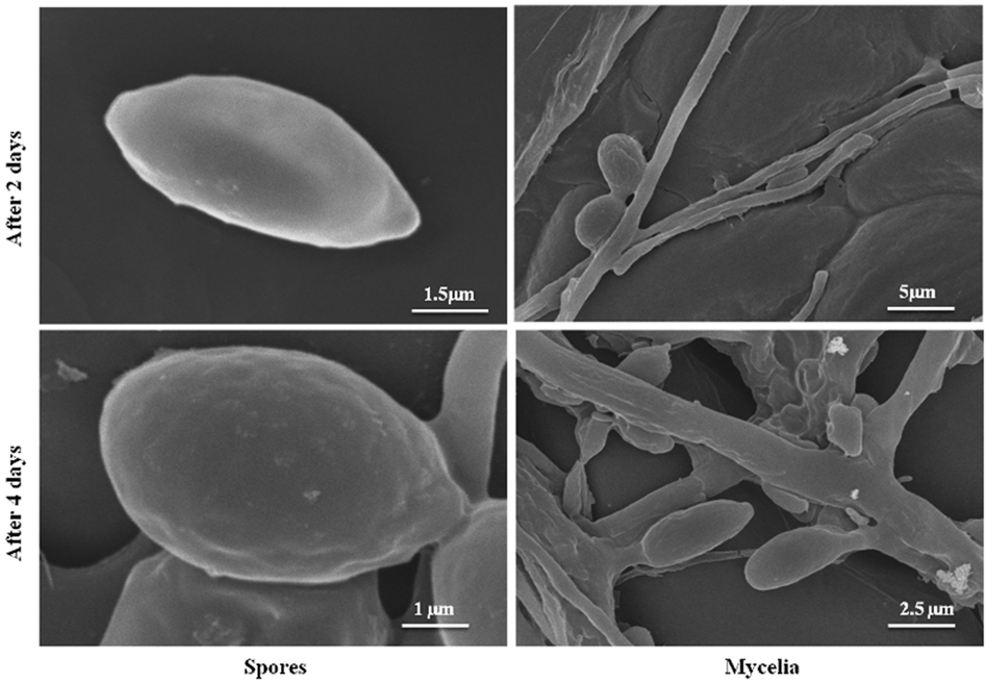

Fig. 3. Spores and mycelia of powdery mildews treated with distilled water (control) and observed with scanning electron microscope over four days with two-day interval.

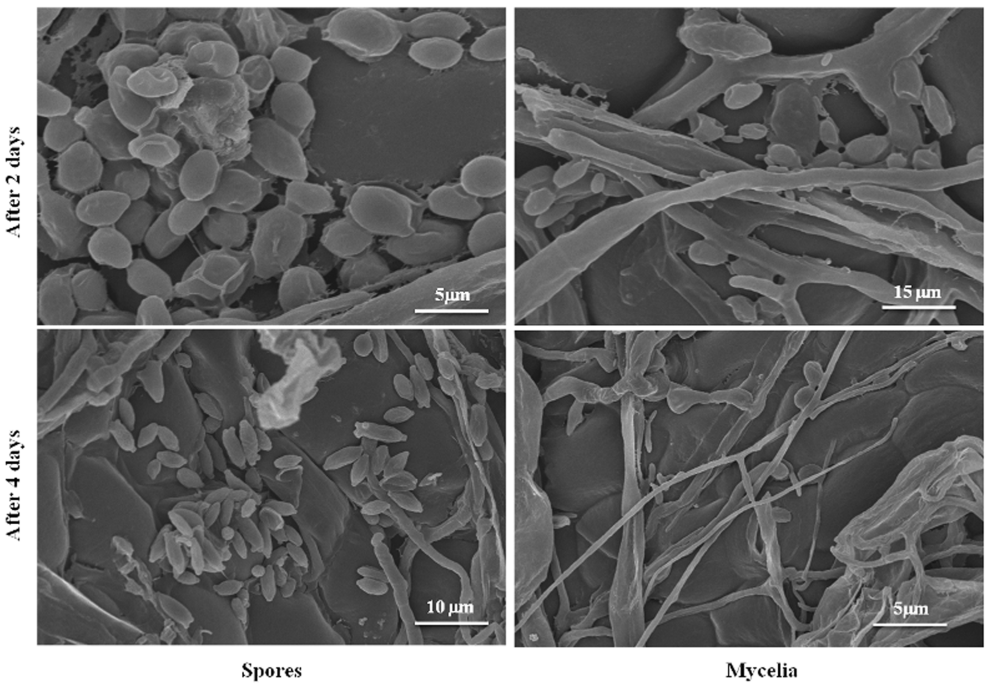

Fig. 4. Spores and mycelia of powdery mildews treated with 10 ppm silver nanoparticles WA-CV-WA13B and observed with scanning electron microscope over four days with two-day interval.

ease by silver nanoparticles. Control leaves treated with distilled water had slightly wrinkled spores and mycelia 2 days after treatment but they regained their original shape by 4 days after treatment (Fig. 3). When powdery mildew was treated with 10 ppm silver nanoparticles, there were wrinkled and sunken spores and mycelia (Fig. 4). The condition became more severe over time. Similar conditions were observed in spores and mycelia treated with 30 ppm and 50 ppm silver nanoparticles (Figs. 5 and 6). When powdery mildew was treated with 100 ppm silver

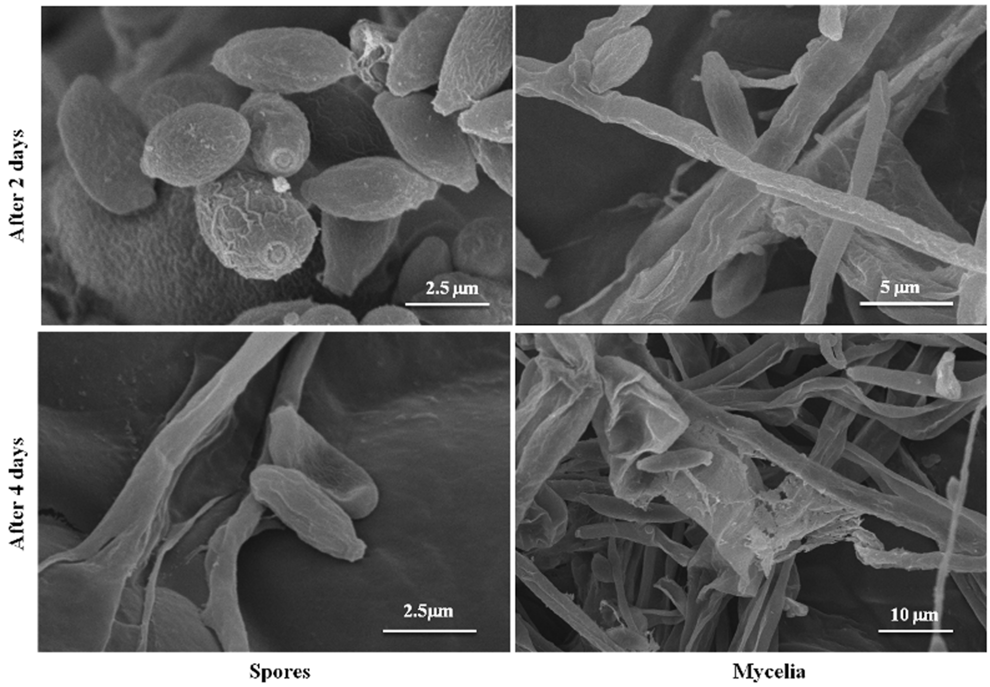

Fig. 5. Spores and mycelia of powdery mildews treated with 30 ppm silver nanoparticles WA-CV-WA13B and observed with scanning electron microscope over four days with two-day interval.

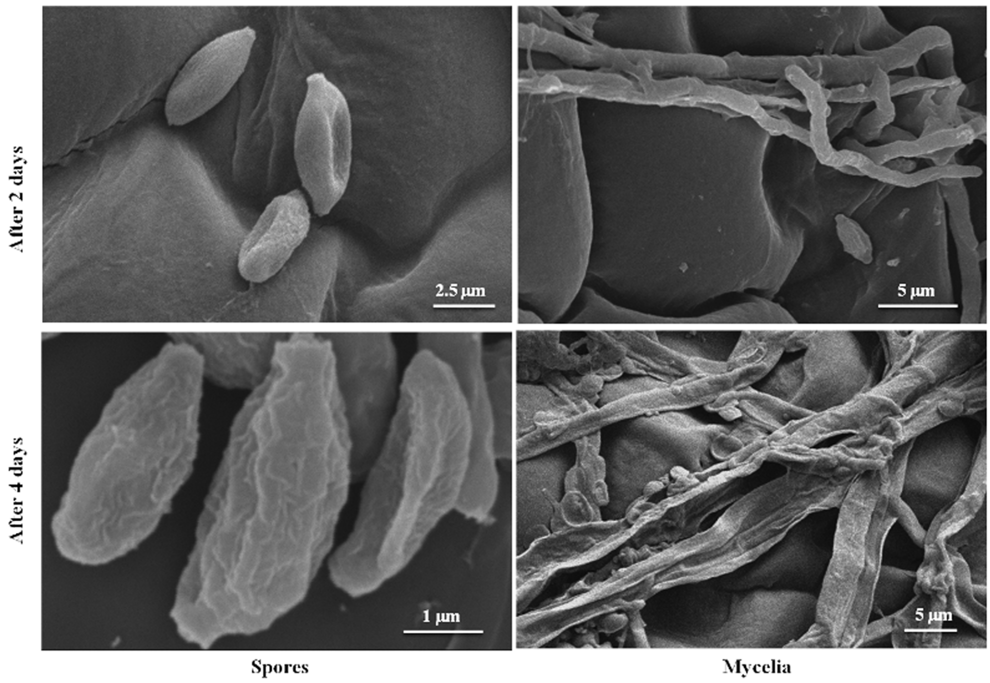

Fig. 6. Spores and mycelia of powdery mildews treated with 50 ppm silver nanoparticles WA-CV-WA13B and observed with scanning electron microscope over four days with two-day interval.

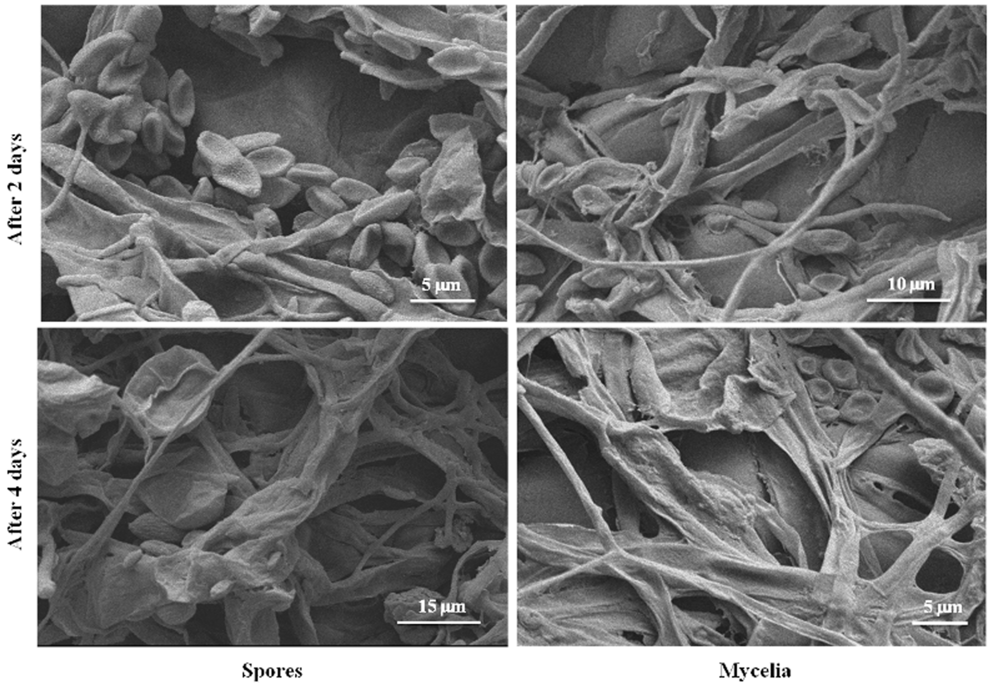

Fig. 7. Spores and mycelia of powdery mildews treated with 100 ppm silver nanoparticles WA-CV-WA13B and observed with scanning electron microscope over four days with two-day interval.

nanoparticles, cellular deformities occurred from day two after treatment and the condition became more severe over time (Fig. 7). In most cases, the death and lysis of spores and mycelia were observed.

Discussion

Little is known about the effects of silver on the phytopathogenic fungi, because most studies have focused on bacterial and viral pathogens of animals. In this study, we evaluated the inhibition effects of silver nanoparticles against powdery mildew in cucumbers and pumpkins in the field. Our results clearly demonstrated that the silver nanoparticles inhibit the fungus which causes powdery mildew. Previous studies suggested that nanometer-sized silvers possess different properties, which might come from morphological, structural and physiological changes [10]. Silver nano particles are highly reactive because they generate Ag^+ ions, while metallic silver is relatively unreactive [11]. Nanoparticles penetrate into microbial cells, which imply lower concentrations of nano-sized silvers are sufficient for microbial control. This would be efficient, especially for some organisms that are less sensitive to antibiotics due to poor penetration of some antibiotics into cells [12]. A previous study observed that silver nanoparticles disrupt transport systems, including ion efflux [11]. The dysfunction of ion efflux can cause rapid accumulation of silver ions, which interupts cellular processes such as metabolism and respiration by reacting with molecules. Also, silver ions produce reactive oxygen species via their reaction with oxygen, which are detrimental to cells, causing damage to proteins, lipids, and nucleic acids [13, 14].

In the present experiment, silver nanoparticles of 50 ppm and 100 ppm had significant inhibition effects against powdery mildew, in both field tests. As we observed, the treatment below 100 ppm solution had shown low inhibition rate when it was applied after disease outbreak. But when silver nanoparticle was applied 3~4 weeks before disease outbreaks, even 50 ppm concentration of silver nanoparticles can inhibit powdery mildew effectively. This suggests that disease suppression can be achieved with a low concentration of silver nanoparticles when it is applied before disease outbreak in the field. Therefore, this study demonstrated that silver nanoparticles can control powdery mildew in field conditions.

Acknowledgements

This research was supported by grants from the Ministry of Food, Agriculture, Forestry and Fisheries, and in part, the Agriculture and Life Sciences Research Institute (ALSRI) of Kangwon National University. We would like to thank Bio-Plus Co. Ltd. (Pohang, Korea) for providing silver nanoparticle used in this study.

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