A New Composition of Nanosized Silica-Silver for Control of Various Plant Diseases

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The present study addressed the efficacy of nanosized silica-silver for controlling plant pathogenic microorganisms. The nanosized silica-silver consisted of nano-silver combined with silica molecules and water soluble polymer, prepared by exposing a solution including silver salt, silicate and water soluble polymer to radioactive rays. The nanosized silica-silver showed antifungal activity against the tested phytopathogenic fungi at 3.0 ppm with varied degrees. In contrast, a number of beneficial bacteria or plant pathogenic bacteria were not significantly affected at 10 ppm level but completely inhibited by 100 ppm of nanosized silica-silver. Among the tested plant pathogenic fungi, the new product effectively controlled powdery mildews of pumpkin at 0.3 ppm in both field and greenhouse tests. The pathogens disappeared from the infected leaves 3 days after spray and the plants remained healthy thereafter. Our results suggested that the product developed in this study was effective in controlling various plant fungal diseases.

Silicon (Si), which is the second most abundant element on the earth, is known to be absorbed into plants to increase disease resistance and stress resistance (Brecht et al., 2003; Ma et al., 2001). In particular, an aqueous silicate solution, used to treat plants, is reported to exhibit excellent preventive effects on pathogenic microorganisms causing powdery mildew or downy mildew in plants, and as well, is known to promote the physiological activity of plants, accelerating the growth of plants and inducing disease resistance and stress resistance in plants (Garver et al., 1998; Kanto et al., 2004). However, since silica has no direct disinfection effects on pathogenic microorganisms in plants, it does not exhibit any effect on established diseases. Further, the effects of silica significantly vary with the physiological environment, and thus, they do not reach a

predetermined level required for registration as an agricultural chemical.

Silver (Ag) is known as a powerful disinfecting agent for killing unicellular microorganisms by inactivating enzymes having metabolic functions in the microorganisms by oligodynamic action (Kim et al., 1998). In addition to silver, although heavy metals, such as copper or zinc, may exert the same action, silver has the strongest antimicrobial effects. Also, silver is known to exhibit superb inhibitory effects on algae growth. Research into silver as a substitute for chlorine or other toxic microbicides has been continuously progressing. Moreover, various inorganic antimicrobial agents that use silver have been developed to date.

Presently available silver-based inorganic antimicrobial agents are produced in the forms of silver-supported inorganic powders, silver colloids, metal silver powders, etc., of which silver-supported inorganic powders are the most used and thus are representative of a typical inorganic antimicrobial agent (Mallick et al., 2004; O'Neill et al., 2003; Thomas and McCubin, 2003).

Silver in an ionic state exhibits high antimicrobial activity (Kim et al., 1998; O'Neill et al., 2003; Thomas and McCubin, 2003). However, ionic silver can be disadvantageous because it is unstable due to its high reactivity and thus easily oxidized or reduced into a metal depending on the surrounding atmosphere. Hence, ionic silver causes discoloration by itself or allows other materials to cause undesirable coloration, and it does not continuously exert antimicrobial activity. Meanwhile, silver in the form of a metal or oxide, which is stable in the environment, may be disadvantageous because it is undesirably used in a relatively increased amount due to its low antimicrobial activity.

Silver, having the above advantages and disadvantages, is presently receiving attention in the form of nano-particles. Various methods of preparing the nano-particles include mechanical grinding, co-precipitation, spraying, sol-gel manufacture, electrolysis, inverse microemulsion, etc (Marignier et al., 1985; Mallick et al., 2004). However, these methods are disadvantageous because the size of the particles formed is difficult to control, or high cost is

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required to prepare fine metal particles. For example, the sizes, shapes and size distribution of the particles are impossible to control in the co-precipitation method, since the particles are prepared using an aqueous solution phase. Through the electrolysis and sol-gel methods, high preparation costs are required, and also, mass production is difficult. Although the inverse microemulsion method allows sizes, shapes and size distribution of the particles to be easily controlled, but it may not be used in practice due to its complicated preparation processes.

On the other hand, methods of preparing nanometer sized particles using exposure to radioactive rays are provided, which are advantageous because sizes, shapes and size distribution of the particles are easily controlled, and the particles may be prepared at room temperature. Also, preparation processes are simple, and therefore, mass production is possible at low costs.

Several studies reported a method of preparing a nanometer sized silver colloid using exposure to radioactive rays (Fujita et al., 1962; Marignier et al., 1985). According to the above reports, a silver salt is dissolved in tertiary distilled water, added with sodium dodecylsulfate (SDS), polyvinyl-alcohol (PVA) or polyvinylpyrrolidone (PVP) as a colloid stabilizer, purged with nitrogen, and then exposed to radioactive rays, to prepare a silver colloid. However, the silver colloid thus prepared has a particle size of 100 nm or larger, and has poor stability, and must be used in a high concentration to exhibit antimicrobial actions on microorganisms, in particular, fungi.

Accordingly, we prepared nano-sized silica-silver particles by mixing a silver salt, silicate and a water-soluble polymer, and exposing the mixture into radioactive rays. The preparation comprises nano-silver combined with silica and a water-soluble polymer to generate uniform and stable formulation with excellent antimicrobial effects at a very low concentration. The objective of the present study was to provide the optimum formulations of nano-sized silica-silver particle to effectively control plant pathogenic microorganisms.

Materials and Methods

Preparation of nanosized silica-silver. One gram of sodium silicate (Na_2SiO_3), 1 g of silver nitrate ($AgNO_3$), 1 g of polyvinylpyrrolidone (PVP), and 12 ml of isopropyl-alcohol (IPA) were added to distilled water and dissolved therein so that the total volume was 200 ml. The resultant solution was bubbled for 20 min using nitrogen gas, and then exposed to gamma rays at 25 kGy to prepare nano-sized silica-silver. The structure of the prepared composite was examined by field emission-transmission electron microscopy (FE-TEM; JEM-2100F, JEOL Co. Ltd., Japan)

according to a method described by Oh et al. (2006). To confirm whether the particles thus prepared were nano-silver particles, test groups as shown in Table 1 were prepared and allowed to stand at room temperature for 24 hr, after which color change was observed.

Antifungal effect of nanosized silica-silver. Potato dextrose agar (PDA, Difco Co. Ltd.) medium was autoclaved, and then 25 ml were aliquotted into each of a plurality of petri dishes. Before the aliquotted medium was cured (at about 40 to 60°C), it was mixed with silica from the test group A, mixed with the nanosized silica-silver prepared in above description from the test group C, mixed with 20 nm sized silver particles from the test group B, and mixed with 100 nm sized silver particles from the test group D, and then cooled, to prepare each medium (Fig. 3). The concentration of the mixed material of each test group was set to 0.3, 3 and 6 ppm. Into an PDA plate containing nanosized silica-silver comprising a silica molecules and water-soluble polymer at concentrations of 0, 0.3, 3, 10 and 100 ppm, a circular piece (with diameter of 5 mm) from a solid culture of *Botrytis cinerea*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Magnaporthe grisea* or *Pythium ultimum* was inoculated. After incubation at 25°C for 2 to 6 days, the growth inhibition of the microorganisms was examined. A spore germination test was according to a method described by Yadav et al. (2005).

Antibacterial effects of nanosized silica-silver. To assay the inhibitory effects on the bacterial growth, the varying concentrations of nanosized silica-silver, which was combined with silica molecules and water soluble polymer, were tested on *Escherichia coli*, *Bacillus subtilis* 1021, *Pseudomonas syringae* pv. *syringae* 2440, *Xanthomonas campestris* pv. *vesicatoria*, *Azotobacter chrococuum* SL206, and *Rhizobium tropici*. After loading 100 ml of LB medium into a 500 ml Erlenmeyer flask, *Escherichia coli* was cultured at 37°C, and the other bacteria were cultured at 30°C, for 15 to 16 hrs under aerobic condition on rotary shaker at 190 rpm. After incubation, 20 ml of culture fluid of each strain was inoculated into an LB agar plate containing nanosized silica-silver at concentrations of 0.3, 3, 10 and 100 ppm, respectively. Subsequently, the *E. coli* strain was cultured at 37°C, and the other bacteria were cultured at 30°C for 6 to 7 days.

Antifungal effect on powdery mildew. To assay controlling effects of the nanosized silica-silver on pathogenic fungi in field (Jodong-ri, Beolgok-myeon, Nonsan, Korea), the present experiment was carried out in a plastic film greenhouse of green squash plants infected with powdery mildew. Nanosized silica-silver of 0.3 ppm was uniformly

sprayed onto green squash plants infected with powdery mildew. After the nanosized silica-silver was applied, the progress of powdery mildew was observed for 3 weeks.

Chemical injuries from nanodized silica-silver at high concentration on plants. To assay the chemical injuries on plants due to application of nanosized silica-silver, an undiluted solution and 10, 100, and 1000 times diluted solutions of nanosized silica-silver were sprayed on the surfaces of leaves including new leaves of cucumber (Joenbaecdadaki, Hungnong) and pansy (Magestic Giant Yellow, Sakada). After 3 days, chemical injuries on plants were observed.

Results

Production of nanosized silica-silver. Fig. 1b showed the prepared nanosized silica-silver under FE-TEM. The nanosized silica-silver particles had uniform particle sizes having a particle size of 1 to 5 nm. The nanosized silica-silver particles may be independently separated or be formed into loose spherical clusters due to intermolecular

attraction. The test groups A and B in Table 1 were prepared by exposing the dissolved solution to radioactive rays, and the test groups C and D are the solutions in which Ag^+ ions were present without exposure to radioactive rays. The test groups SW and DW were controls containing no silver ions or silver particles.

As apparent from Table 1, the test groups SW, D and DW were colorless without color change even after allowing to stand for 24 hr, which means that silver ions, chlorine ions, or silver ions and chlorine ions were all absent. However, the colorless test group C turned to reddish brown, which means that silver ions were formed into AgCl along with chlorine ions in tap water. In addition, the test groups A and B showed yellow without color change, which means that stable nano-silver particles combined with silica molecules and water soluble polymer were formed via exposure to radioactive rays and AgCl precipitates were not formed even in the presence of chlorine ions. The color change is shown in Fig. 2.

The absorption spectrum of the solutions of the test groups DW, B and D in Table 2 among which only the test group B absorbed light of 403 nm is the unique wavelength
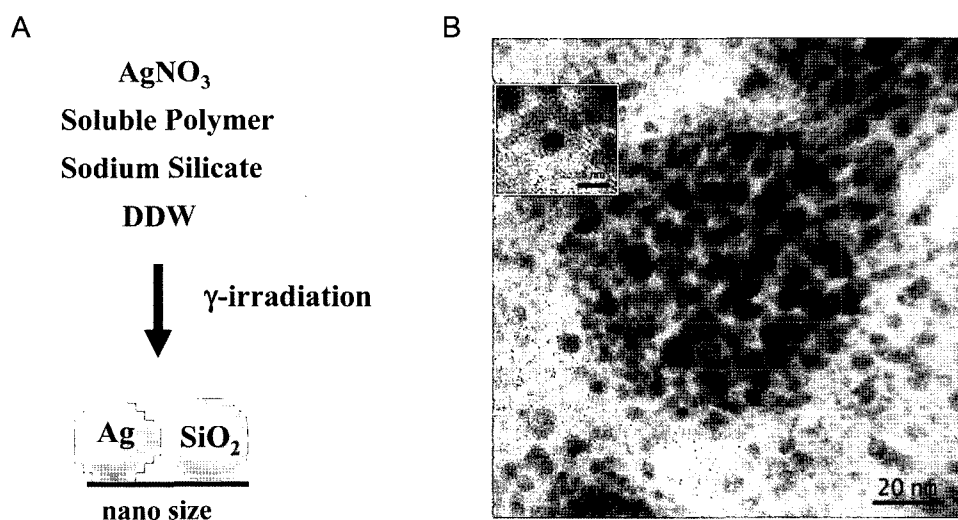
A

AgNO₃

Soluble Polymer

Sodium Silicate

DDW

⬇ **γ-irradiation**

| Ag | SiO₂ |

nano size

B

Fig. 1. A schematic flowchart for the preparation of nanosized silica-silver (A), and a transmission electron micrograph of the nanosized silica-silver (B). Bar indicates 20 nm.

Table 1. Stability of the nanosized silica-silver in tap water condition

Test Group	Add Solution	DDW	Tap Water	Color Change
SW	0	0	45 ml	Colorless → Colorless
A	NSS	0	45 ml	Yellow → Yellow
B	NSS	45 ml	0	Yellow → Yellow
C	AgNO₃	0	45 ml	Colorless → Reddish Brown
D	AgNO₃	45 ml	0	Colorless → Colorless
DW	0	45 ml	0	Colorless → Colorless

NSS: 3200 ppm nanosized silica-silver solution 90 μl, AgNO₃: 3200 ppm AgNO₃ solution 90 μl

SW   A   B   C   D   DW
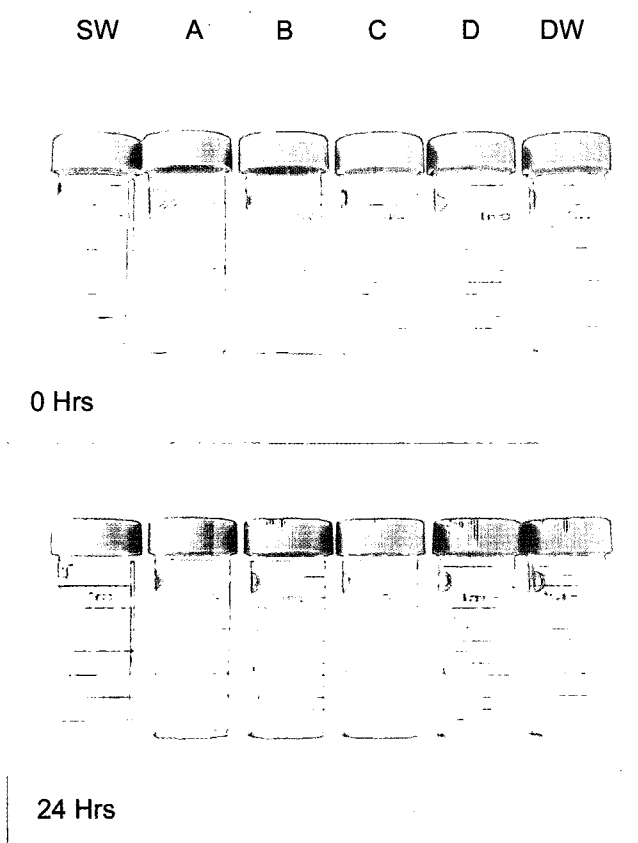
0 Hrs

24 Hrs

Fig. 2. The stability of colloidal nanosized silica-silver in an aqueous environment. The experimental condition indicates in Table 1.

of nano-silver, whereas the test groups DW and D did not absorb light at the same wavelength (data not shown). From the result of the absorption spectrum, measured after the solution was allowed to stand, it was confirmed that stable nanosized silica-silver particles comprising silica molecules and water soluble polymer were formed by exposing the
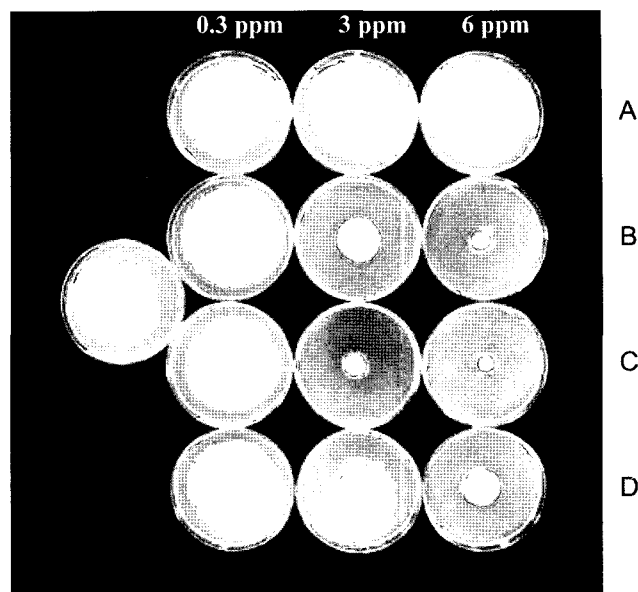

0.3 ppm   3 ppm   6 ppm

A

B

C

D

Fig. 3. The antifungal effect of nanosized silica-silver on *Botrytis cinerea*. (A) including only silica had the same result as a control group. The (B) and (D), including 20 nm sized silver and 100 nm sized silver, respectively. (C) including the nanosized silica-silver.

solution comprising sodium silicate, silver nitrate and PVP to radioactive rays.

In vitro **antimicrobial activity test of nanosized silica-silver.** As shown in Fig. 3, the test group A including only silica showed the same result as a control group, regardless of the concentrations. The test groups B and D, including 20 nm sized silver and 100 nm sized silver, respectively, had the same results as a control group at a concentration of 0.3 ppm. However, the test group C including the nanosized silica-silver prepared in this study had a higher inhibitory effect on the growth of *B. cinerea* than those of test groups including 20 nm sized silver and 100 nm sized silver, even

Table 2. Effective concentration of nanosized silica-silver on suppression of microbial growth

Microorganisms	Growth inhibition (%) according to concentration of the nanosized silica-silver			
	0.3 ppm	3.0 ppm	10 ppm	100 ppm
Pythium ultimum	15.5	66.7	100	100
Magnaporthe grisea	1.4	27.0	100	100
Colletotrichum gloeosporioides	11.8	21.6	100	100
Botrytis cinerea	2.6	82.7	100	100
Rhyzoctonia solani	54.8	94.8	100	100
Bacillus subtilis	0	0	50	100
Azotobacter chrococuum	0	0	0	100
Rhizobium tropici	0	0	0	100
Pseudomonas syringae	0	0	0	100
Xanthomonas compestris pv. *vesicatoria*	0	0	0	100

NSS (3ppm)

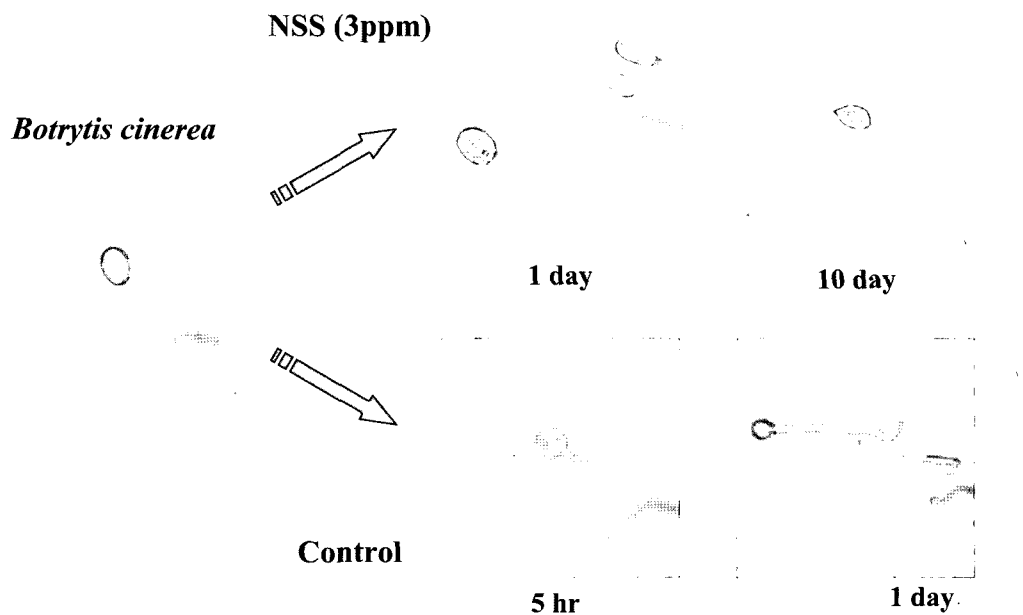*Botrytis cinerea*

1 day

10 day

Control

5 hr

1 day

Fig. 4. The inhibitory effect of nanosized silica-silver on spore germination of *B. cinerea* and the inhibitory effect varying with a time period. Nanosized silica-silver treatments was addition of 3 ppm in 2×10^4 spores/ml diluted solution and was incubated at 25°C.

at a low concentration of 3 ppm. The smaller size of nano silver, more effectively suppress fungal growth. Furthermore, as seen in Fig. 4, the nanosized silica-silver inhibited spore germination of *B. cinerea* at a low concentration of 3 ppm. In non-treatments, 10% spore germination occurred after 5 hour, whereas in nanosized silica-silver treatments any spores were not germinated until 10 days. The nano silver-silica 3.0 ppm effectively inhibited several pathogenic fungi tested in this study with varied degrees, while some fungi were effectively suppressed even at 0.3 ppm. However, most bacteria either useful or plant pathogenic were not suppressed at 10 ppm, but strongly inhibited at 100 ppm.

Plant disease control of nanosized silica-silver in field. Control of powdery mildew was examined 0, 3, and 7 days after nanosized silica-silver application. Although powdery mildew was widespread on the leaves of green squash at day 0, controlling effects close to 100% were observed after the nanosized silica-silver was applied (Fig. 5). After about 3 weeks, powdery mildew was not observed (data not shown).

Phytotoxicity of nanosized silica-silver. Fig. 6 showed the chemical injuries of on the entire surfaces of leaves including new cucumber leaves and pansy flowers by nanosized silica-silver applied at a high concentration (3200 ppm).

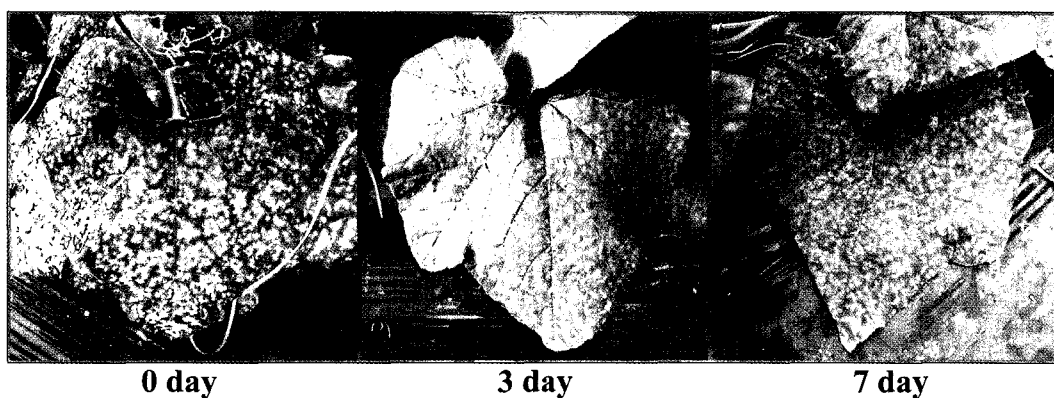0 day   3 day   7 day

Fig. 5. The antifungal effect of nanosized silica-silver on green squash suffering from powdery mildew in field. The powdery mildew infected green squash leaves were treated with 0.3 ppm nanosized silica-silver colloidal solution. The pictures were took the same leaf.
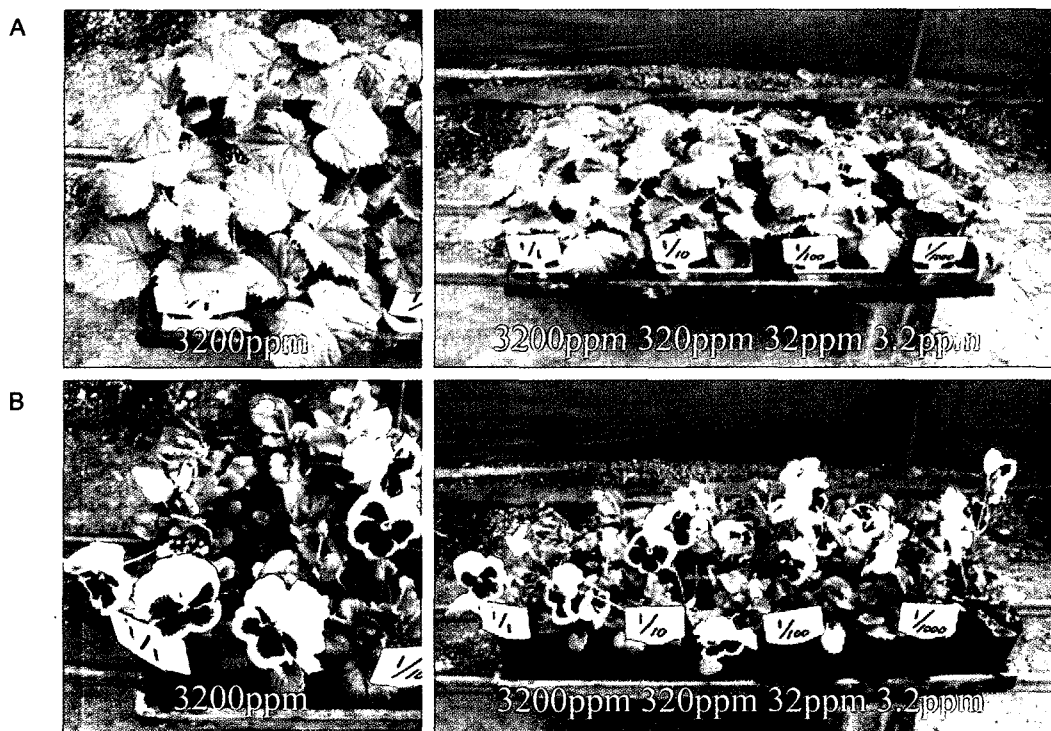
Fig. 6. The chemical injuries caused by a high concentration of nanosized silica-silver on plants. Cucumber plants (A) and pansy plant (B) were sprayed with 50 ml nanosized silica-silver, respectively. The concentration of applied solution is from 3.2 to 3,200 ppm.

Compared to the control group, typical chemical injury phenomena, such as wrinkle of new leaves, did not appear in the treatment groups using the diluted solutions (3.2-3200 ppm). Any phytotoxic phenomena was not detected in the solution applied to plants. These results were the same as when applied to other plants.

Discussion

A schematic flowchart showing the preparation of nano-sized silica-silver was provided to show the comprising silica molecule and water-soluble polymer, according to the present method (Fig. 1A). The solution, formed after being exposed to gamma rays, was yellow due to nano-silver particles (Fig. 2), which indicated that the silica molecule and the water-soluble polymer were combined with silver particle through the above reaction, yielding stable nano-sized silica-silver particles. In the present study, the term "nanosized silica-silver" means a composite comprising nanosized silver particle and silica molecules that are combined with water-soluble polymer. According to a specific aspect, nanosized silica-silver may be prepared by exposing a solution comprising silver salt, silicate and water-soluble polymer to radioactive rays. One example of the composite is a structure in which nanosized silver particles formed from silver ion and silica molecules

formed from silicate is, individually or together, surrounded by water-soluble polymer via exposure to radioactive rays. The nanosized silica-silver in a colloidal state may be present as nano-particles separated from each other or be formed into loose spherical clusters (Fig. 1B). Therefore, the clusters are simply separated when the temperature increases. The nanosized silica-silver absorbs light at 403 nm which is the unique wavelength of nano-silver (Mallick et al., 2004), and has a uniform nanoparticle size as shown in Fig. 1b. The particle size of the nanosized silica-silver preferably ranged from 1 to 5 nm. The nanosized silica-silver contained in the composition of the present study has high preservability, and is used in the state of being diluted in tap water (Table 1) or agricultural water (data not shown). Compared to silver ions that are precipitated in the form of silver chloride along with chlorine ions in tap water, the nanosized silica-silver may be more easily handled and may reduce controlling costs. Silver in the ionic state is easily oxidized and precipitated in the form of AgCl while browning in the presence of Cl⁻ ions. Thus, the state of silver may be confirmed using tap water having Cl⁻ ions. In the case where silver is present in the state of Ag⁺ ions, it precipitates. Meanwhile, when silver is present in stable nano-silver particles, it turns yellow (Fig. 2).

The smaller size of nano silver more effectively suppressed fungal growth (Fig. 3A). In general, 1 to 5 nm sized

particles may pass through a protoplasmic membrane, and silica is well absorbed into fungi (Wainwright et al., 1986). When the nanosized silica-silver is absorbed into fungal cells, silver nanoparticles function to increase disinfecting activity, and silica, which induces dynamic resistance to diseases to increase resistance, acts to form a physical barrier to pathogenic fungi (Kim et al., 2003), and thus, recurrence of diseases may be prevented for a considerably long period after disinfection of pathogenic microorganisms (Fig. 3 and 5). The results of this study showed that the product is effective to control of various diseases at lower than 3.0 ppm that is not a concentration to suppress pathogens on agar medium (Table 2). We considered that following plant pathogenic fungi could be treated and controlled using the nanosized silica-silver include: *Blumeria* spp., *Sphaerotheca* spp., *Phytophthora* spp., *Rhizoctonia* spp., *Colletotrichum* spp., *Botrytis* spp., *Magnaporthe* spp. and *Pythium* spp. In addition, the composition for controlling plant pathogens in this study may control pathogenic bacteria in plants at a concentration higher than 10 ppm (Table 2). The composition attempted in this study inhibits the growth and development of both Gram-positive and Gram-negative bacteria, in which the inhibitory effect on the growth and development of Gram-positive bacteria is higher than that on the growth and development of Gram-negative bacteria.

Although the composition presented in this study does not exhibit a controlling effect on bacteria at a low concentration, for example, 10 ppm, and particularly, 3 ppm or less, it exhibits a high controlling effect on fungi at the same concentration (Table 2). Dissimilarity of its anti-microbial activity between the two microbial groups (fungi and bacteria) is possibly due to the different mechanism of silica absorption and the cell wall composition. Thus, when the composition of the present method is applied at a concentration of 5 ppm or less, and preferably, 3 ppm, or in some cases, 0.3 ppm, it may selectively inhibit only pathogenic fungi in plants without action on bacteria beneficial to plants.

As described above, the present study provides a composition for controlling pathogenic microorganisms in plants. Nanosized silica-silver of the present study exhibits a wide range of antimicrobial activity, and can control both spores and hyphae (Fig. 3 and 4). In addition, the nanosized silica-silver manifests efficient controlling effects at low concentrations, and may maintain the controlling effects for a long period upon a single application. Furthermore, the nanosized silica-silver does not cause chemical injuries and is non-toxic to the human body (O'Neill et al., 2003) and to plants even wih a high concentration (Fig. 6). The nanosized silica-silver can selectively control the microorganisms depending on its concentration. Since Ag and SiO_2 were

known environmentally safe and even benefit for human health (Shankar et al., 2003; Yau et al., 2004) and the cost of nanosized silica-silver is much lower than commercial fungicides, it is believed that the formulation is highly useful to manage various fungal plant diseases in eco-friendly sustainable agriculture. Studies on modes of action and biological spectrum of the new composition are being further investigated.

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