Mycobiology 38(1) : 39-45 (2010)
DOI:10.4489/MYCO.2010.38.1.039

The Effect of Nano-Silver Liquid against the White Rot of the Green Onion Caused by *Sclerotium cepivorum*

Jin-Hee Jung[1], Sang-Woo Kim[1], Ji-Seon Min[1], Young-Jae Kim[1], Kabir Lamsal[1], Kyoung Su Kim[2]* and Youn Su Lee[1]*

[1]*Department of Plant Resources Science, College of Agriculture and Life Sciences, Kangwon National University, Chuncheon 200-701, Korea*
[2]*Department of Agricultural Biotechnology, Center for Fungal Genetic Resources and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-742, Korea*

(Received January 28, 2010. Accepted February 19, 2010)

White rot, which is caused by *Sclerotium cepivorum*, is a lethal disease affecting green onions. Three different types of nano-silver liquid (WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R) were tested in several different concentrations on three types of media to assess their antifungal activities. Results from *in vitro* experiments showed that all three of the nano-silver liquids had more than 90% inhibition rates at a concentration of 7 ppm. Greenhouse experiments revealed that all of the nano-silver liquids increased biomass and dry weights, and there were minimal changes in the population of various bacteria and fungi from the soil of greenhouse-cultivated green onions. In addition, a soil chemical analysis showed that there were minimal changes in soil composition.

KEYWORDS : Green onion, Nano-silver, *Sclerotium cepivorum*, Soil microbes

Allium fistulosum L., an herbaceous perennial plant of the family Liliaceae, originates from the western side of China. The leaves are often used for seasoning food because of their special aroma. The root and scaly bulb, which have antibacterial properties, are also widely used as expectorants, antipyretic, and diuretics among people. There are several pathogens which affect the green onion, but the most common are leaf blight, white rot, viral infection, and downy mildew. *Allium* white rot is a serious disease of onion (*Allium cepa* L.) and other *Allium* spp. that has a significant economic impact on crop yields [1]. This disease can reduce yields to uneconomic levels in four years of successive onion crops. The fungus penetrates the root epidermis then invades the cortical parenchyma [2]. Infected plants suffer from water stress and often die prior to harvest or rot in storage [3]. The pathogen persists in the soil in the absence of host plants as *Sclerotia* and can survive in this form for more than 20 years [4]. Following their production, Sclerotia are constitutively dormant for 1~3 months and will then only germinate in the presence of host plants [5].

Sclerotium is an issue in the repeated cultivation of fields, and green onion quality and productivity is declining because of a sudden increase in it [6]. Sclerotium usually replicates on the inside of the green onion that results in huge amounts of damage caused by the disease in most countries, including the USA, Canada, Europe, Asia, Africa, the middle of south of America, and Australia [7-9]. The major symptom of *Sclerotium cepivorum* is mycelium breeds around the bottom of the bulb, which increases in severity the worse the disease gets. At its worst, the bulb becomes dark and the upper part of the plant dies. Agricultural chemicals used to prevent breeding are poisonous to animals and other microorganisms and also are known to be harmful to humans.

There is an increasing need to develop an environmentally friendly material to solve these problems. Antibacterial material has various properties, but nowadays antibacterial substance has a well known merit that it can be applied widely and safely as disassembled or volatile substance. Its main spotlight is harmless for human body. For example, the use of silver is becoming more prevalent and studies on silver are also increasing [10-12]. Silver stops the metabolism of pathogenic bacteria and disinfects it. The electrical load of an emitted silver anion sterilizes the pathogenic microbes by controlling a procreation function [13, 14]. It was found that there are no bacteria which can survive after touching sliver for 6 minutes [15]. Based on this theory, it was reported that silver can disinfect almost 650 different microbes and does not harm humans because it is non-toxic but controls the smoothing metabolism function inside of the microbes [16]. Accordingly, this study analyzes the sterilization effect and control of growth using three different types of nano-silver liquid as well as their ability to prevent breeding of the *Sclerotium cepivorum.*

*Corresponding author <E-mail : younslee@kangwon.ac.kr, kyoungsu@gmail.com>

Table 1. Types of nano-silver used in this study

Type	Physical form	Average particle size (nm)	Silver contents (μg/mL)	Solvent
WA-CV-WA13B	Dark brown colloid	7~25	40,000~50,000	Pure water
WA-AT-WB13R	Colorless colloid	7~25	5,000~15,000	Pure water
WA-PR-WB13R	Colorless colloid	7~25	5,000~15,000	Pure water

Materials and Methods

Nano-silver liquid. Three types of nano-silver liquid WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R (Table 1) were provided by the Bio-plus Co. (Pohang, Korea) at a 1,000 ppm initial concentration that was then diluted into different working concentrations.

Fungi and growth media. The *Sclerotium* used was picked from the root of an infected onion collected from the vinyl house of Yangsu-Ri, Kyonggi-Do. *Sclerotium* was cultured in potato dextrose agar (PDA), malt extract agar (MEA), and corn meal agar (CMA) media for future use.

Antifungal effects of nano-silver liquid: *in vitro* and field tests. To determine the antifungal activities of nano-silver against *S. cepivorum*, three different types of nano-silver liquid with its four mixtures in eight different concentrations (1 ppm, 3 ppm, 5 ppm, 7 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm) were tested. A mycelial growth inhibition rate was determined by using the following formula.

$$\text{Inhibition rate (\%)} = \frac{(R - r)}{R} \times 100$$

where, R = Mycelial growth in control plate (diameter in cm)
r = Mycelial growth in nano silver treated plate (diameter in cm)

For the field test, nano-silver prepared at each concentration was applied to plant roots each week for a single time. After the experiment, the fresh and dry weights of the plants were measured.

Analysis of the microbe population changes in the soil from which the nano-silver-treated green onion was harvested. Soil component analysis was used to determine the effect of the nano-silver liquids on the composition of soil microbes between treated and non-treated soil samples in the field. Both treated and non-treated soil samples were air dried in a shaded field. In tryptic soy agar media, bacteria were prepared at 1.0×10^4 CFU/mL and fungi colonies were grown in PDA media with different antibiotic liquids (streptomycin sulfate salt: 0.3 g, tergitol: 500 μL, tetracycline: 0.3 g). Fungal and bacterial colonies were counted after the growth in media and the coefficient was calculated.

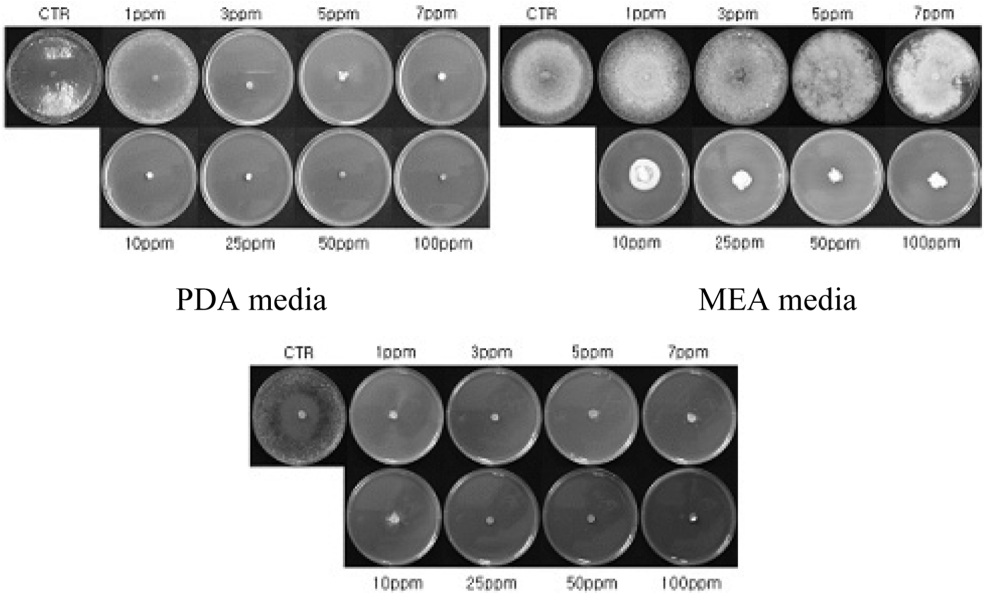

Fig. 1. *In vitro* inhibitory effects of WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R against mycelial growth of *Sclerotium cepivorum* on different media. CTR, control; PDA, potato dextrose agar; MEA, malt extract agar; CMA, corn meal agar.

Analysis of soil treated with various concentrations of nano-silver. The green onions were washed and treated with different concentrations of nano-silver. The nano-silver-treated green onions were naturally dried and 5 g of stem and root were burned and the ashes were fixed with a total volume of 25 mL aqua regia ($HCl : HNO_3 = 3 : 1$). The prepared aqua regia liquid was used to measure the inductively coupled plasma and detect the consistency of the nano-silver. Nano-silver prepared from the aqua regia addition was made similarly to the standard scale of 0.05~1 ppm, which was measured and used for the experiments.

Results

Examining the ability of nano-silver to control the growth of *Sclerotium cepivorum*. We examined green onion infected with *S. cepivorum* and the ability of nano-silver to control the growth of it in the lab. Diseased green onions were removed from a farmhouse in Yangsu-ri, Gyungki-do and the growth of a mycelium was observed. The three different nano-silver liquids were each used at a concentration of 1 ppm, 3 ppm, 5 ppm, 7 ppm, 10 ppm, 25 ppm, 50 ppm, and 100 ppm on PDA, MEA and CMA culture plates (Fig. 1).

The inhibition rate of each concentration of nano-silver WA-CV-WA13B was observed in PDA media, and it was found that 82% inhibition was observed with a 7 ppm concentration. Likewise an inhibition rate of 89% was observed for a 3 ppm concentration of nano-silver. In MEA media, the growth inhibition was only observed at concentrations over 10 ppm. Inhibition of growth by the nano-silver liquid was highest on the CMA media. MEA had less of an inhibitory effect than PDA. We confirmed that WA-CV-WA13B inhibited mycelium growth at the concentration of 10 ppm. The inhibitory effect of WA-AT-WB13R on each media was more effective than WA-CV-WA13B. The inhibition rate was 86% on PDA with a 5 ppm concentration and 93% on CMA with a 3 ppm concentration. An inhibition rate of 81% of the control percentage on an MEA plate at a 25 ppm concentration on nano-silver WA-PR-WB13R, 81% inhibition was recorded on PDA at a 7 ppm concentration, 84% on CMA with a 10 ppm concentration, and 81% on MEA at a 25 ppm concentration. Over all, nano-silver had the best inhibitory effect on PDA. The differences of growth observed for each media demonstrated that all of the ingredients found in every media have an effect on growth of a mycelium (Table 2).

Each media used with the WA-CV-WA13B and WA-AT-WB13R mixtures showed good inhibition rates. The inhibition rate observed at a 3 ppm concentration of nano-silver liquid on CMA media was 83% and on PDA media with a 7 ppm concentration of nano-silver was 82%. Likewise, a 25 ppm concentration of nano-silver on MEA media showed a growth inhibition rate of 85%. The antagonistic effect of nano-silver against fungi was more notable in the CMA media compared to the other types. Other media added with WA-CV-WA13B and WA-PR-WB13R mixture also showed good inhibition rate. At a 10 ppm concentration of nano-silver liquid on PDA media the inhibition rate was 78% and on CMA media with a 3 ppm concentration of nano-silver the inhibition rate was 80%. The mixture of WA-AT-WB13R and WA-PR-WB13R demonstrated good inhibition rates. The inhibition rate observed with a 5 ppm concentration of nano-silver liquid on PDA media was 80% and on CMA media with a 3 ppm concentration of nano-silver was 80%. WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R mixtures also had good inhibition rates. The inhibition rate observed with a 25 ppm concentration of nano-silver liquid on PDA media was 80% and on CMA media with a 1ppm concentration of nano-silver it was 68%. MEA media with a 3 ppm concentration of liquid had a growth inhibition rate of 81%. These results suggest that nano-silver is more antagonistic against fungi on the CMA media (Table 3).

Table 2. Inhibitory effects of nano-silver WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R against *Sclerotium cepivorum* on three different types of media

	Inhibition rate (%)								
	WA-CV-WA13B			WA-AT-WB13R			WA-PR-WB13R		
	PDA	MEA	CMA	PDA	MEA	CMA	PDA	MEA	CMA
CTR	0	0	0	0	0	0	0	0	0
1	1	0	46	0	0	26	0	0	20
3	69	0	89	66	0	93	39	0	58
5	68	1	92	86	4	94	75	0	56
7	82	0	89	86	19	95	81	0	72
10	87	68	100	98	54	100	79	71	84
25	93	78	100	98	81	99	96	81	72
50	100	84	100	100	85	99	100	82	78
100	100	87	100	100	85	100	100	88	87

PDA, potato dextrose agar; MEA, malt extract agar; CMA, corn meal agar; CTR, control.

Table 3. The inhibitory effects of three different nano-silver combinations against the mycelial growth of *Sclerotium cepivorum* on three different media

		Inhibition rate (%)								
		CTR	1 ppm	3 ppm	5 ppm	7 ppm	10 ppm	25 ppm	50 ppm	100 ppm
CV[a] + AT[b]	PDA	0	0	75	79	82	86	92	66	82
CV[1] + AT[2]	MEA	0	0	0	2	13	76	85	84	85
CV[1] + AT[2]	CMA	0	52	83	84	87	84	93	95	96
CV + PR[c]	PDA	0	15	62	71	74	78	76	100	100
CV + PR[3]	MEA	0	0	0	6	8	67	85	84	85
CV + PR[3]	CMA	0	6	80	82	85	85	95	98	96
AT + PR	PDA	0	0	78	80	80	71	87	98	100
AT + PR	MEA	0	0	0	0	18	52	81	82	85
AT + PR	CMA	0	41	80	84	84	89	88	99	100
CV + AT + PR	PDA	0	0	71	73	78	62	80	94	96
CV + AT + PR	MEA	0	0	11	12	29	79	81	82	80
CV + AT + PR	CMA	0	68	81	81	82	82	91	94	95

PDA, potato dextrose agar; MEA, malt extract agar; CMA, corn meal agar; CTR, control.
[a]WA-CV-WA13B.
[b]WA-AT-WB13R.
[c]WA-PR-WB13R.

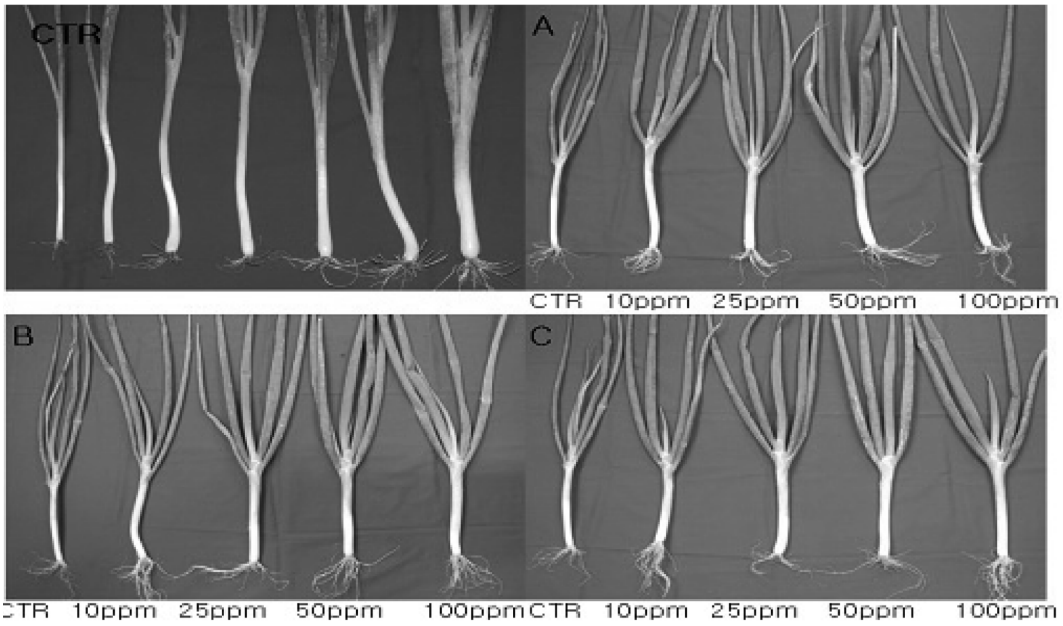

Fig. 2. The effect of nano-silver treatment on green onions. A, WA-CV-WA13B; B, WA-AT-WB13R; C, WA-PR-WB13R treated green onions. CTR, untreated control.

Field tests. We applied different concentrations of nano-silver (10 ppm, 23 ppm, 50 ppm, and 100 ppm) at the base of each plant 4 times each week for up to 4 weeks. After 5 months, the roots of the plants were observed and the dry weight was measured after drying it in drying machine at 80°C. The fresh weight of the harvested green onion was also measured. After the application of nano-silver, treated and non-treated green onions were harvested and the dry weights of the plants were taken. Both the fresh weight and dry weight of the treated plants increased. The green onions treated with WA-CV-WA13B showed the highest dry and fresh weights compared to the other treatments. The onion treated with nano silver liquid has more weight than non- treated or control ones (Fig. 2).

Analysis of the microbe population changes from the soil in which green onions treated with nano-silver were harvested.

Changes in soil microbe populations: We investigated the soil fungi and bacteria by comparing the microorganism population in soil samples taken at the places where

Table 4. The effect of nano-silver treatment on the fresh and dry weights of green onions

	WA-CV-WA13B					WA-AT-WB13R				WA-PR-WB13R			
	CTR	10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm
Fresh weight (g)	11.5	28.3	16.8	22.3	21.2	16.1	17.0	18.3	22.4	19.2	19.0	19.0	18.2
Dry weight (g)	0.965	1.998	1.346	1.849	1.666	1.295	1.323	1.564	1.162	1.391	1.102	1.152	1.378

CTR, control.

Table 5. Changes in the population of various bacteria from green onion cultivated greenhouse soil

	Colony number/plate at 1.0×10^4 CFU/mL												
	CTR	WA-CV-WA13B				WA-AT-WB13R				WA-PR-WB13R			
		10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm
Before	28.3 ± 3.1	22.7 ± 4.2	31.3 ± 5.5	51.3 ± 7.4	32.0 ± 2.6	20.7 ± 8.4	35.7 ± 4.5	23.7 ± 8.5	28.7 ± 4.2	22.3 ± 5.5	22.7 ± 9.5	24.7 ± 7.2	49.0 ± 1.0
After	23.0 ± 4.6	16.0 ± 8.7	16.3 ± 1.5	17.0 ± 1.0	13.7 ± 2.1	22.0 ± 2.0	14.3 ± 2.5	15.0 ± 4.0	18.7 ± 6.7	19.3 ± 5.0	12.7 ± 3.1	15.7 ± 7.2	12.7 ± 2.5

CTR, control.

Table 6. Change in the population of various fungi from green onion cultivated greenhouse soil

	Colony number/plate at 1.0×10^2 CFU/mL												
	CTR	WA-CV-WA13B				WA-AT-WB13R				WA-PR-WB13R			
		10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm
Before	7.7 ± 0.6	3.0 ± 1.7	4.0 ± 1.0	0.7 ± 0.6	2.3 ±	2.3 ± 1.5	0.7 ± 0.6	1.0 ± 1.0	1.3 ± 0.6	0.7 ± 0.6	2.3 ± 0.6	0.7 ± 0.6	1.3 ± 1.5
After	7.7 ± 0.6	4.3 ± 1.2	6.0 ± 1.0	1.3 ± 1.5	3.3 ± 0.6	0.7 ± 0.6	1.0 ± 1.0	2.0 ± 1.7	4.3 ± 1.5	1.3 ± 0.6	1.7 ± 0.6	0.3 ± 0.6	1.7 ± 0.6

CTR, control.

Table 7. Analysis of soil treated with various concentrations of nano-silver

Sample	pH (1 : 5)	EC (1 : 5) (μS/cm)	P_2O_5 (mg/kg)	Organic compounds (%)	T-N (%)	Exc. Cation (cmol(+)/kg)				CEC (cmol(+)/kg)
						K	Ca	Mg	Na	
A[b]-10 ppm	6.53	364.10	987.10	4.42	0.23	1.11	6.80	3.16	0.35	14.06
A-25 ppm	6.46	423.00	1072.17	4.31	0.22	1.42	6.69	3.71	0.39	15.29
A-50 ppm	6.56	318.00	938.06	4.68	0.23	1.19	7.02	3.12	0.45	14.87
A-100 ppm	6.75	289.50	930.07	4.79	0.25	0.87	6.78	2.66	0.27	13.66
B[c]-10 ppm	6.76	314.00	1047.84	4.96	0.24	1.32	6.57	2.64	0.32	14.15
B-25 ppm	6.83	410.00	1034.65	6.23	0.25	1.48	8.81	3.22	0.46	17.05
B-50 ppm	6.60	357.00	975.77	4.68	0.35	0.98	9.79	3.19	0.56	17.17
B-100 ppm	6.34	334.60	905.55	4.53	0.25	0.87	7.23	2.85	0.32	14.12
C[d]-10 ppm	6.40	317.00	1103.57	5.59	0.27	1.52	10.67	2.51	0.32	18.11
C-25 ppm	6.58	267.10	987.84	4.92	0.22	1.35	8.63	2.69	0.44	15.97
C-50 ppm	6.55	443.00	920.23	4.82	0.24	1.07	6.77	2.06	0.23	14.09
C-100 ppm	6.53	357.90	839.61	4.93	0.23	1.15	6.67	2.47	0.22	14.69
CTR[a]	6.65	334.60	905.55	5.94	0.25	1.27	7.68	2.65	0.31	15.86
After 2 months	6.88	228.50	646.11	4.07	0.21	1.34	5.38	2.21	0.87	12.88

EC, electric conductivity; CEC, cation exchange capacity; CTR, control.
[a]Untreated.
[b]WA-CV-WA13B.
[c]WA-AT-WB13R.
[d]WA-PR-WB13R.

nano-silver liquid was used to treat green onions. This experiment was conducted to determine if nano-silver treatment affected useful microbes (Tables 5 and 6). The number of bacteria in the soil from treated individuals

generally decreased in comparison to the soil from non-treated individuals. Although non-treated soil microbes did not decrease but some increase was observed. Not only did fungi colony increase but the number of live materials also increased. The fresh weight decreased at the beginning but it increased at the end of the experiment. The nano-silver components did not reduce the number of soil microbes and it did not play a role in the extinction of any soil microbes.

Soil ingredients analysis: The soil used to cultivate the green onion was analyzed, and after two months, the soil treated with nano-silver liquid was not significantly different than non-treated soil. As shown in Table 7, an electric conductivity (EC) of 334.60 was observed in controls and a maximum of 443.00 was observed among the treated soil, and the minimum observed after two months was 289.50. In the P_2O_5 analysis, the treated soil had increased levels of P_2O_5 in comparison to the control which was 905.55 and it was decreased to 646.11 after 2 months of treatment. Contents of the organic compounds dropped from 5.94 to 4.07 after 2 months. The T-N content was not different between treat and non-treated soil, the content of Ca and Mg decreased, and K and Na slightly increased. A positive ion EC (mg/100 g) decreased by 2.98 less than the control (Table 7).

Soil treated with nano-silver did not show many differences in comparison to the non-treated control. Specifically, the pH value of treated and non-treated soil was not significantly different. In soil component analysis, it was found that nano-silver did not decrease the soil ingredients and that it was safe for controlling different fungi from an environmental perspective too.

Analysis of soil treated with various concentrations of nano-silver. Nano-silver was used to treat a green onion field to test for its effectiveness according to the concentration available. The best results were found in soil treated with a concentration of 25 ppm. Likewise 10 ppm, 50 ppm, and 100 ppm treated soil showed a 0.11 mg, 0.33 mg, and 0.24 mg silver concentration, respectively.

In the field analysis, an increase in the treatment of nano-silver concentration in green onion showed decreased level of silver when it was observed after experiment. This suggests that nano-silver liquid and silver contained in the green onion plant should be examined in future.

This research has shown that nano-silver is harmless in the human body if safety measures are applied and that nano-silver liquid can increase plant productivity and reduce environmental pollution.

Table 8. Silver concentrations found in nano-silver-treated green onion plants after collection from the field

Sample	Value of measure (mg/L)	Concentration of silver (mg/kg)
Control average	0.00	0.00
10 ppm average of treatment	0.04	0.11
25 ppm average of treatment	0.10	0.49
50 ppm average of treatment	0.07	0.33
100 ppm average of treatment	0.05	0.24

Discussion

In Korea, the green onion is affected by many *Sclerotium* forming fungi. Among them *Sclerotium cepivorum* is the most prevalent. Therefore, in this study we attempted to control the disease caused by this fungi using nano-silver liquid. These results suggest that nano-silver is effective for fungi growth inhibition. In our studies, nano-silver liquid provided by Bio-plus Co., Korea was used and diluted to make different concentrations of nano-silver. The nano-silver liquid was then used to analyze the inhibition of fungi on different medium including PDA, MEA, and CMA. *Sclerotium cepivorum* was inoculated at the center of each different media plate containing different concentrations of nano-silver liquid. The inhibition rate was calculated by applying the given formula for each replicate in comparison to the control. As a result, it was found that nano-silver liquid WA-CV-WA13B (CV) had the highest inhibition rate on CMA media at the 3 ppm concentration, and that WA-AT-WB13R (AT) had the same result. However, WA-PR-WA13R (PR) had a higher inhibition rate on PDA media at the 7 ppm concentration. When all three of the nano-silver liquid mixtures were applied, the highest inhibitory effect occurred on the CMA media. Similar results were observed in the field when green onion plants were treated with different concentrations of nano-silver (10 ppm, 25 ppm, 50 ppm, and 100 ppm). In this case, WA-CV-WA13B showed the effective inhibition according to biomass observed from the dry weight of the onion (Table 4).

To analyze the changes in the microbe population in the soil where green onions were collected and treated with or without nano-silver liquid. The bacterial population was decreased more in the treated soil in comparison the non-treated soil, and fungi populations other than *S. cepivorum*, which was not found, increased. In the early stage bacteria decreased but later on it increased. The only soil microbes were affected by the nano-silver liquid was *S. cepivorum*. In comparison to other microbes, nano-silver affected ecological aspects of certain microbes. According to soil component analysis, there was no difference in the pH value of nano-silver liquid treated soil after 2 months. When the amount of nano-silver was calculated in the plants grown in nano-silver treated soil, it was found that the concentration of nano-silver used for treatment and the concentration of nano-silver found in the plants was inversely proportional. Therefore, more experiments are needed to determine the actual concentration of nano-silver needed to control microorganisms.

We conclude that the nano-silver liquid is effective for the control of *Sclerotium cepivorum*. Nano-silver liquid for the prevention of various plant pathogenic fungi is highly recommended to farmers. Additionally, the use of nano-silver does not cause any harm to human beings, and it is safe for the environment and agricultural products. In conclusion, we can say that by using nano-silver liquid, environmental pollution and the excessive use of chemical compounds in the field can be reduced. It is expected that the application of nano-silver at low concentrations will be economic, eco friendly, and decrease farm management costs.

Ackwoledgements

This research was supported by grants from the Ministry for Food, Agriculture, Forestry and, Fisheries, and in part, the Agriculture and Life Sciences Research Institute (ALSRI) of Kangwon National University.

References

1. Coley-Smith JR. White rot disease of Allium: problems of soil-borne diseases in microcosm. Plant Pathol 1990; 39:214-22.
2. Abd El-Razik AA, Shatla MN, Rushdi M. Studies on the infection of onion plants by *Sclerotium cepivorum* Berk. Phytopathologische Zeitschrift 1973;76:108-16.
3. Entwistle AR. Allium white rot and its control. Soil Use Manag 1990;6:201-9.
4. Coley-Smith JR, Mitchell CM, Sansford CE. Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli*. Plant Pathol 1990;39:58-69.
5. Coley-Smith JR. Alternative methods of controlling white rot disease of Allium. In: Chet I, editor. Innovative approaches to plant disease control. New York: Wiley; 1987. p. 161-77.
6. Cho WD, Kim WG, Hong SK, Kim WS. *Sclerotium* sp. associated with occurrence of a new disease, globular *Sclerotium* rot of garlic. Plant Pathol J 2003;18:372.
7. Cho WD, Kim WG. *Sclerotium* sp. occurrence in the vegetable crops of species of *Allium*. Plant Pathol J 1996;12:251-4.
8. Crowe FJ, Hall DH. Soil temperature and moisture effects on *Sclerotium* germination and infection of onion seedlings by *Sclerotium cepivorum*. Phytopathology 1980;70:74-8.
9. Mordue JE. *Sclerotium cepivorum*. CMI descriptions of pathogenic fungi and bacteria, No. 512. Kew: Commonwealth Mycological Institute; 1976.
10. Kang HY, Jung MJ, Jeong YK. Antibacterial activity and the stability of an Ag^{+} solution made using metallic silver. Korean J Biotechnol Bioeng 2000;15:521-4.
11. Lim YT, Choi HK, Ahn YH. Antimicrobial activity assessment of functional plastics which contained Ag-Hydroxyl Apatite agent. Korean J Biotechnol Bioeng 2001;16:123-7.
12. Cho KH, Park SG. Study on the antibacterial effects of silver nanoparticles prepared by sonication method. J Korean Ind Eng Chem 2004;15:952-5.
13. Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Escherichia coli*. Can J Microbiol 1974; 20:883-9.
14. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res 2000;52:662-8.
15. Kim JH. Nano silver chemotherapeutic agents and its applications. NICE 2004;22:656-60.
16. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. Nanobiotechnol 2005;16:2346-53.