Plant Pathol. J. 16(4) : 206-210 (2000)

The Plant Pathology Journal

Evaluation of Electrolyzed Oxidizing Water as a Control Agent of Cucumber Powdery Mildew

Yong-Hwan Lee*, Kwang-Hong Cha, Sook-Ju Ko, In-Jin Park, Boung-In Park[1] and Ki-Young Seong[2]

Chonnam Agricultural Research and Extension Services, Naju 520-715, Korea

[1]Kyoung-Oo Tech, Inchon 401-071, Korea

[2]Applied Plant Science Division and Institute of Biotechnology, Chonnam National University, Kwangju 500-757, Korea

(Received on May 25, 2000)

The effect of the electrolyzed oxidizing water on *Sphaerotheca fuliginea* was investigated with germination and sporulation of the fungal conidia. The sporulation was inhibited by the electrolyzed oxidizing water of pH 2.5, 3.5, and 4.5, but was not inhibited by the distilled water adjusted pH with 1N-HCl solution. However, the electrolyzed oxidizing water did not affect conidial germination. The oxidation-reduction potential at pH 2.5 and pH 3.5 of electrolyzed oxidizing water were 1130 mV and 1060 mV, respectively, but those of distilled water adjusted with HCl solution were 550 mV and 490 mV, respectively. When the electrolyzed oxidizing water of ORP over 1100 mV was sprayed on cucumber leaves at 3 days and 7 days intervals after transplanting, the disease severities of powdery mildew were about 8.5% and 19.2%, respectively. Disease severity of a standard control (triflumizole 30% WP, 500 mg/L) was about 3.0%, while that of plants without electrolyzed oxidizing water was to 45.8%.

Keywords : electrolyzed oxidizing water, *Sphaerotheca fuliginea*, oxidation-reduction potential, conidia germination, sporulation.

Powdery mildew caused by *Sphaerotheca fuliginea* (Schlechtend: Fr.) Pollacci is a prevalent disease on cucumber (*Cucumis sativus* L.) grown in greenhouse (Bounassissi et al., 1989; Ohtsuka and Nakazawa, 1991; Park et al., 1996; Urquhart et al., 1994). The disease outbreaks every year in cucumber cultivation, and affects the plant growth resulting in yield reduction (Park et al., 1996). Methods for the disease control available for agricultural growers are repeated application of fungicides such as sulfur and ergosterol biosynthesis inhibitors (Ohtsuka and Nakazawa, 1991, Hashimoto et al., 1986), use of sodium silicate in hydroponic nutrient solution (Menzies et al., 1991), utilization of mildew-resistant cultivars, and use of biological control agent like *Tilleotiopsis* sp. (Urquhart et al., 1994). None of these methods, however, has provided an adequate level of disease control because of its limitation such as low temperature, occurrence of fungicide resistance, and lack of appropriate application methods (Yarood, 1950; Ohtsuk and Nakazawa, 1991; Menzies et al., 1991; Urquhart et al., 1994).

The electrolyzed oxidizing water (EOW), produced by electrolysis-ionizing reaction in which two alloyed electronodes of anode and cathode are located across the ion exchange membrane in the water bath (Shiramizu et al., 1996, Lee et al., 1998), can be a prominent candidate for disease control of plants. The EOW was widely used as a sterilizing agent in medicine (Nishimoto et al., 1996) and food (Jung et al., 1996) because it had several advantages such as a good sterilizing function with safety. However, the mechanisms and modes of sterilizing functions are not fully elucidated.

Acidic water (pH 2.5, oxidation reduction potential (ORP) of 1,000-1,200 mV) was generated in the anode while alkalic water (pH 11.5, ORP of -800 mV) was generated in the cathode. Electrolysis-ionizing reactions at the anode side can be expressed by the following reactions (Shiramizu et al., 1996)

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \qquad E^0 = 1.229\ V \qquad (1)$$

or

$$3H_2O \rightarrow O_3 + 6H^+ + 6e^- \qquad E^0 = 1.51V \qquad (2)$$

$$KCl \rightarrow K^+ + Cl^- \qquad (3)$$

$$2Cl^- \rightarrow Cl_2 + 2e^- \qquad E^0 = 1.3595V \qquad (4)$$

$$Cl_2 + 2H_2O \rightarrow 2H^+ + 2HClO + 2e^- \qquad E^0 = 1.63V \qquad (5)$$

$$Cl_2 + 4H_2O \rightarrow 6H^+ + 2HClO_2 + 6e^- \qquad E^0 = 1.64V \qquad (6)$$

$$Cl_2 + 6H_2O \rightarrow 12H^+ + 2ClO_3^- + 10e^- \qquad E^0 = 1.47V \qquad (7)$$

$$3HClO \rightarrow 3H^+ + ClO_3^- + 2Cl^- \qquad (8)$$

$$2HClO \rightarrow 2H^+ + 2ClO^- \qquad (9)$$

$$2HClO_2 \rightarrow 2H^+ + 2ClO_2^- \qquad (10)$$

*Corresponding author.
Phone) +82-62-330-2683, FAX) +82-62-336-4076
E-mail) leeyh@chonnam.rda.go.kr

Ozone and oxygen gases are generated from the pure water as shown in reactions 1 and 2, and chlorine gas is generated from potassium chloride solution as shown in reaction 4. Reaction formulae 5-10 show that chlorous acid ($HClO_2$), hypochlorous acid (HClO), and ClO_3^- are generated from chlorine gas. HClO dissolves into ClO_3^-, Cl^-, ClO^-, and H^+. $HClO_2$ dissociates into H^+ and ClO_2^-. At the anode side, H^+, ozone gas, and ClO_x^- are generated by electrolysis as a consequence. (Nishimoto et al., 1996, Jung et al., 1996). Because the anode water contains strong oxidants (ClO_x^-, oxygen gas, and ozone gas) and the ORP of anode is very high (Shiramizu et al., 1996), we called this electrolyzed oxidizing water (EOW) in this study.

In this study, to determine causes of fungicidal activity of the EOW in relation to pH and ORP, conidial germination and sporulation of *S. fuliginea* in the presence of the EOW and pH adjusted distilled water were compared. Furthermore, a practical application method of the EOW in the field was examined.

Materials and Methods

Electrolyzed oxidizing water. The electrolysis-ionized water of pH 2.5 and pH 11.5 was obtained from the products of Kyoungoo Tech Co. (Model GRA-1200, Inchon, Korea). To make the electrolyzed oxidizing water of different pHs with the interval of 1 pH unit, acidic electrolyzed water (pH 2.5) and alkalic electrolyzed water (pH 11.5) were mixed. The control solutions were made adjusting the pH with 1N-HCl or 1N-NaOH. The pH range of electrolysis-ionized water produced was from 2.5 to 6.5. The ORP of water was measured by ORP meter of Yokogawa Electric Co. (Model PH 82, Japan).

Pathogen. Conidia of powdery mildew were collected from naturally infected leaves of cucumber plants and identified as *Sphaerotheca fuliginea* according to the conidial characteristics (Boesewinkel, 1980). To dislodge old conidia, the infected leaves were shaken for 48 hr before harvesting the conidia.

Germination inhibition test. The EOW was sprayed on the glass slide with a sterilized small glass sprayer after fresh conidia were placed on the glass slide by brushing a powdery mildew-infected cucumber leaves. The glass slides were dried in the clean-bench and placed in a petri-dish with a wet filter-paper. After inoculation, the petri-dishes with a glass slide were placed in a incubator at 25°C with 12hr photo-period at a light intensity of 1,000 lux. The conidial germination was examined 48hr after inoculation under the optical light microscope. Conidia having clear germ tube were considered as germinated. This test was conducted twice.

Sporulation inhibition test. A sporulation test was conducted by Cohens procedure (1992). Leaf disks (8 mm in diameter) were removed from cotyledons using a cork borer. The disks (8 per replication) were placed in petridishes containing 0.16% water agar amended with 25 μg/ml of benzimidazole (Sigma B9131, St. Louis, Mo). The inoculum was prepared by rinsing a powdery mildew-infected leaf with water containing 0.01% Tween 20. The concentration of conidial suspension was measured with a haemocytometer. Leaf disks were inoculated by placing 10 μl of *S. fuliginea* conidia suspension (3.8×10^4 conidia/ml) on adaxial side of each leaf disk. The petridishes with the disks were incubated at 25°C with 12-hr photo-period at a light intensity of 1,000 lux. To elucidate the effects of the EOW on *S. fuliginea* sporulation, the EOW was sprayed on the disks in the petri-dishes at 1 day and 4 days after inoculation because *S. fuliginea* germinates 1 day after inoculation and starts the formation of conidia 5 days after inoculation (Endo, 1978). And then the disks were dried in the clean bench. After drying, the petridishes with the disks were incubated in a growth chamber as described previously.

The development of powdery mildew hyphae and conidia on the leaf disks was examined at 6 days and 12 days after inoculation. The frequency of different stages of powdery mildew development on the disks was determined with a stereoscope and expressed by the degree of sporulation as follow: 0 = non-infected disk; 1 = only hyphae present; 2 = hypae with conidiophores without conidia; 3 = hyphae with up to 50 conidiophores per disk; 4 = more than 50 conidiophores per disk (heavy sporulation).

Field test. To examine effect of the EOW on cucumber disease control, field trial was conducted with LeepchooNaghap variety in a PVC house. Young plants with 3-4 leaves were transplanted Oct 15, 1998 and grown in a PVC house. And the EOW was foliarly sprayed until dripping off with 3 days and 7 days intervals, and tap water was sprayed with 3 days interval from 2 weeks after transplantation (Oct 29, 1998). A commercial product, Triflumizole (Trifumine™, 30% WP) for a standard control, was diluted in tap water to the concentration of 500 mg/liter and sprayed for 3 times with 10 days interval from 7 weeks after transplantation (Nov 24, 1998). This test was designed in randomized complete blocks with three replications. Cucumbers were grown in 1.8×6 meter plots. In each plot, plants spaced 40 cm apart in two rows.

Disease severity was estimated following the Test Guideline for Pesticide Registration in Korea established by the National Institute of Agricultural Science and Technology in Suwon, Korea. One or two hundred leaves from 20 cucumber plants were sampled from each experimental unit and visually assessed 10 days interval from 3 weeks after transplanting (Nov 3, 1998), and disease severity was determined by the formula as following:

$$\text{Disease severity (\%)} = \{(4a+3b+2c+1d)/4(a+b+c+d)\} \times 100$$

where a = number of leaf with more than 50% of leaf area infected; b = number of leaf with 20.1-50.0% of leaf area infected; c = number of leaf with 5.1-20.0% of leaf area infected; d = number of leaf with 0.1-5.1% of leaf area infected; e = number of healthy leaf.

Results

Effect of the EOW on *S. fuliginea*. The EOW and the prepared solutions adjusted with 1N-HCl had no effect on conidia germination of *S. fuliginea* (Table 1). However, sporulation of *S. fuliginea* was influenced by the EOW of

Tablel 1. Effect of electrolyzed oxidizing water on the germination of *Sphaerotheca fuliginea*

Treatment[a]	pH	Conidial germination[b] (% ± Standard deviation)
Electrolyzed oxidizing water	2.5	15.3 ± 2.1
	3.5	15.3 ± 3.1
	4.5	17.3 ± 0.8
Distilled water adjusted with 1N-HCl	2.5	20.0 ± 1.7
	3.5	16.7 ± 2.9
	4.5	18.7 ± 2.5
Sterilized water	6.5	16.7 ± 2.1
Not treated[c]	–	20.7 ± 1.5

[a] Spraying after conidia inoculation. Each treatment was replicated 5 times.
[b] Conidia having clear germ tube were considered as germinated 48 hr after inoculation.
[c] No spraying after inoculation.

Table 2. Effect of electrolyzed oxidizing water on the sporulation of *Sphaerotheca fuliginea* by spraying times after inoculation of conidia

Spraying time[a] (Days)	Treatment	pH	Degree of sporulation[b] 6 days[c]	12 days
1	Electrolyzed oxidizing water	2.5	2.7	3.9
		3.5	2.8	4.0
		4.5	2.7	4.0
	Distilled water adjusted with 1N-HCl	2.5	3.1	4.0
		3.5	3.1	4.0
		4.5	3.3	4.0
	Sterilized water	6.5	3.7	4.0
4	Electrolyzed oxidizing water	2.5	1.4	1.7
		3.5	2.3	2.5
		4.5	1.9	2.8
	Distilled water adjusted with 1N-HCl	2.5	3.0	3.9
		3.5	3.0	4.0
		4.5	3.0	4.0
	Sterilized water	6.5	3.3	4.0
	Not treated[d]	–	3.5	4.0
	LSD (p=0.05)		0.4	0.16

[a] Spraying times of EOW after conidia inoculation.
[b] Average of rates for 8 leaf disks per replication on a scale from 0 to 4. 0 = non-infected disk, 1 = only hyphae present, 2 = hypae with conidiophores without conidia per disk, 3 = hyphae with up to 50 conidiophores with conidia per disk, 4 = more than 50 conidiophores per disk (heavy sporulation).
[c] Investigating date after inoculation.
[d] No spraying after inoculation.

pH 2.5, 3.5, and 4.5 (Table 2). When the EOW sprayed at 4 days after inoculation of conidia, as shown in Table 2, the sporulation was inhibited compared to not treated and the sterilized water controls. Especially, when the EOW of pH 2.5 sprayed, there were only hyphae on the disks and the conidiophores hardly formed. When the EOW of pH 3.5 and 4.5 sprayed, the conidiophores were malformed or formed without conidia. However, when the EOW was sprayed 1 day after inoculation, the conidiophore development was a little slower than not treated and the sterilized water controls 6 days after inoculation. But, the sporulation was heavy without differences compared to not treated and the sterilized water controls 12 days after inoculation. But the prepared solutions adjusted with 1N-HCl had no effect on the sporulation compared to not treated and the sterilized water controls regardless of the times of spraying.

Change of oxidation-reduction potential (ORP) according to the pH of the EOW. As the pH of the EOW and the prepared solution decreased, the ORP at different pH increased. The ORPs of pH 2.5, 3.5, and 4.5 of the prepared solution adjusted with 1N-HCl were 571, 507, and 461 mV, respectively. On the contrary, the ORPs of pH 2.5, 3.5, and 4.5 of EOW were 1130, 1060, and 985 mV, respectively (Table 3).

Control of cucumber powdery mildew by the EOW. When the EOW was foliarly sprayed with 3 days interval from 10 days after transplanting of cucumber, the disease severity of powdery mildew maintained about 10% until the end of the cultivation and the disease severity was about 20% when sprayed with 7 days interval. When the plants were not treated, disease severity increased persistently up to 45% after sudden increase between Nov 13 and Nov 23. When treated with Triflumizloe (Trifumine™, 30% WP), disease severity decreased after 1st spraying in Nov 24 and afterwards it maintained about 3% (Fig. 1).

Discussion

Several previous studies (Jung et al., 1996, Iwasawa and Nakamura, 1996, Lee et al., 1998) reported that sterilizing

Table 3. Changes of oxidation-reduction potential (ORP) in accordance with the pH of electrolyzed oxidizing water and distilled water

Treatment	pH 2.5	3.5	4.5	5.5	6.5
Electrolyzed oxidizing water	1130[a]	1065	985	915	847
Distilled water adjusted with 1N-HCl	571	507	461	448	351

[a] Oxidation-reduction potential (mV).

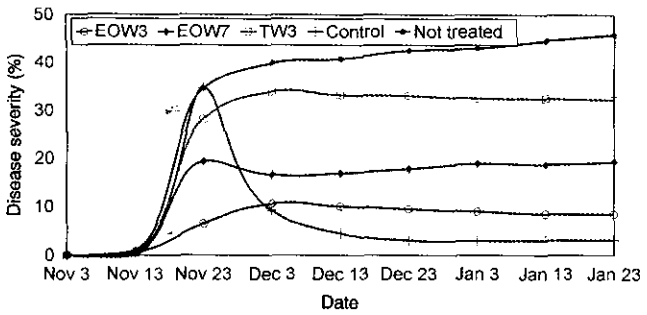

Fig. 1. Change of the disease severity of *Sphaerotheca fuliginea* by the treatments of Electrolyzed oxidizing water. EOW3, EOW sprayed with 3 days interval from Oct 29; EOW7, EOW sprayed with 7 days interval from Oct 29; TW3, Tap water sprayed with 3 days interval from Oct 29; Control, Triflumizole (30% WP, 500 mg/liter) sprayed 3 times with 10 days interval from Nov 24, 1998.

effect of the electrolyzed oxidizing water existed when the bacteria or the zoospores of *Phythophthora capsici* were soaked in the electrolyzed oxidizing water. In this experiment, it was shown that the spraying of the EOW on *S. fuliginea* did not influence conidia germination of *S. fuliginea*, but suppressed sporulation. The spraying of the EOW, therefore, may inhibit mycelial growth or conidiophore formation of *S. fuliginea*.

Electrogenic proton transport by electrochemical gradients of H^+ is a major determinant of the membrane potential of the cells including plants, bacteria, and fungi (Taiz and Zeiger, 1991). In case of fungi, generally, the cell wall of spore is about seven times as thick as that of mycelium (Elizabeth, 1996). As shown in Table 1, The EOW hardly influenced on conidia germination of *S. fuliginea*, but affected mycelial growth so that the sporulation could be inhibited (Table 2). However, the prepared solutions adjusted with 1N-HCl had no effect on both conidia germination and the sporulation. And the ORPs of the EOWs were about two times as high as those of the prepared solutions adjusted with 1N-HCl (Table 3). From this result, it could be suggested that the EOW affect the mycelial cell wall but not conidia cell wall, and the functions of cell membrane were destroyed by the high ORP of the EOW disturbing the electrochemical gradient of proton of membrane. This result is similar to the reports of Oblinger and Kraft (1979) and Smooth and Pierson (1979) that the high ORP was one of the reasons of sterilizing effect of the EOW. Because the high oxidation potential is one of the main causes of sterilizing effect of the EOW, we suggested that acidic water made by electrolysis-ionizing of tap water is appropriate to be called electrolyzed oxidizing water.

In field test, when the EOW was foliarly sprayed with 3 days and 7 days intervals, the disease severity of powdery mildew maintained about 10% and 20%. And there was no change in disease severity of powdery mildew persistently when the EOW was foliarly sprayed with 3 days interval from disease severity 50%. (data not shown). The persistence of disease severity means neither reducing nor increasing of mycelial turf of *S. fuliginea* occurred on the leaves of cucumber. The life cycle of asexual stage of cucumber powdery mildew was 5-7 days (Endo, 1978). It was indirectly shown that the spraying of the EOW with 3 days interval could inhibit the sporulation of *S. fuliginea*, but the spraying of the EOW with 7 days interval could be less effective on the inhibition of life cycle of *S. fuliginea*.

Due to heavy use of pesticides in greenhouse, a demand of safe agricultural products for consumers is increasing. In this regard, control of cucumber powdery mildew by spraying the EOW can be a good approach to diminish pesticide usage, and to maintain persistent agriculture. However, because of the destroying function of cell membrane by strong oxidation potential of the EOW, there is a possibility of a negative influence on crop cells. To make better use of the EOW on agriculture, therefore, further investigations on influences of the EOW not only to diseases but also to crops are needed.

References

Boesewinkel, H. J. 1980. The morphology of the imperfect states of powdery mildew (*Erysiphaceae*). *Bot. Rev.* 46:167-224.

Buonassissi, A. J., McDonald, L. D., Menzies, J. G. and Stace-Smith, R. 1989. Greenhouse cucumber disease survey in British Columbia. *Can. Plant Dis. Surv.* 69:72.

Cohen, R. 1993. A leaf disk assay for detection of resistance of melons to *Sphaerotheca fuliginea* race 1. *Plant Dis.* 77:513-517.

Elizabeth, M. L. 1996. Fundamentals of the fungi. Prentice-Hall, Inc., New Jersey, 574pp.

Endo, T. 1978. Epidemiological studies on the powdery midew of cucurbits, caused by *Sphaerotheca fuliginea* (Schlecht.) Poll., 3:Formation, maturation, and dissemination of conidia. *Bulletin of the Fukusima Hort. Exp. Stat.* 8:87-101.

Hashimoto, S., Sano, S., Murakami, A., Mizuno, M., Nishikawa, H. and Yasuda, Y. 1986. Fungitoxic properties of triflumizole. *Ann. Phytopath. Soc. Japan* 52:599-609.

Iwasawa, A. and Nakamura, Y. 1996. Bactericidial effect of acidic electrolyzed water- comparison of chemical acidic sodium hydrochloride (NaOCl) solution. *Kansenshogaku Zasshi* 70:915-922.

Jung, S. -W., Park, K. -J., Park, K. -J. Park, B. -I. and Kim, Y. -H. 1996. Surface sterilization effect of electrolyzed acid-water on vegetable. *Korean J. Food Sci. Technol.* 28:1045-1051.

Lee, J. H., Kwon, T. R., Moon, J. D. and Lee, J. T. 1998. Effect of acidic electrolyte water on growth and infection of *Phytophtora capsici*. *Korean J. Plant Pathol.* 14:440-444.

Menzies, J. G., Ehret, D. L., Glass, A. D. M., Helmer, T., Koch, C.

and Seywerd, F. 1991. Effects of soluble silicon on the parasitic fitness of *Sphaerotheca fuliginea* on *Cucumis sativus*. *Phytopathology* 81:84-88.

Nishimoto, Y., Morisheta, K. and Kaetsuka, M. 1996. Evaluation of electrolyzed strong acidic aqueous solution called the function water. Bunseki Kagaku, 45:701-706.

Oblinger, O. J. and Kraft, A. A. 1979. Oxidation-reduction potential and growth of *Salmonella* and *Pseudomonas fluorescens*. *J. Food Sci.* 38:1008.

Ohtsuka, N. and Nakazawa, Y. 1991. The influence of machine oil on conidia and hyphae of cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. *Ann. Phytopath. Soc. Japan* 57:598-602.

Ohtsuka, N., Sou, K., Amano, T., Ojima, M., Nakazawa, Y. and Yamada, Y. 1988. Decreased sensitivity of cucumber powdery mildew (*Sphaerotheca fuliginea*) to ergosterol biosynthesis inhibitors. *Ann. Phytopath. Soc. Japan* 54:629-632.

Park, S. -D., Kwon, T. Y., Lim, Y. -S., Jung, K. C. and Choi, B. -S. 1996. Disease survey in melon, watermelon, and cucumber with different successive cropping periods under vinylhouse conditions. *Korean J. Plant Pathol.* 12:428-431.

Shiramizu, Y., Watanabe, K., Tanaka, M., Aoki, H. and Kitajima, H. 1996. Removal of metal and organic contaminants from silicon substrates using electrolysis-ionized water containing ammonium chloride. *J. Electrochem. Soc.*143:1632-1635.

Smooth, L. A. and Pierson, M. D. 1979. Effect of oxidation-reduction potential on growth and chemical inhibition of *Clostridium botulinum* 10755A spores. *J. Food Sci.* 44:700.

Taiz, L. and Zeiger, E. 1991. Plant physiology. The Benjamin/Cummings Publishing Company, Inc., Redwood, California, 565pp.

Urquhart, E. J., Menzies, J. G. and Punja, Z. K. 1994. Growth and biological control activity of *Tilletiopsis* species against powdery mildew (*Sphaerotheca fuliginea*) on greenhouse cucumber. *Phytopathology* 84:341-351.

Yarwood, C. E. 1950. Effect of temperature on the fungicidal action of sulfur. *Phytopathology* 40:173-180.