Effect on Colony Growth Inhibition of Soil-Borne Fungal Pathogens by Available Chlorine Content in Sodium Hypochlorite

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(Received on July 4, 2018; Revised on November 12, 2018; Accepted on December 9, 2018)

Our study investigated the available chlorine content, contact time and difference among strains of each pathogen for sodium hypochlorite (NaOCl) to control chemically against soil-borne fungal pathogens, such as Phytophthora rot by Phytophthora cactorum, violet root rot by Helicobasidium mompa, and white root rot by Rosellinia necatrix, causing die-back symptom on apple trees. As a result, the colony growth of Phytophthora cactorum was inhibited completely by soaking over 5 s in 31.25 ml/l available chlorine content of NaOCl. Those of H. mompa and R. necatrix were inhibited entirely by soaking over 160 s in 62.5 and 125 ml/l available chlorine content in NaOCl, respectively. Also, inhibition effect on available chlorine in NaOCl among strains of each soil-borne pathogen showed no significant difference and was similar to or better than that of fungicides.

Keywords: apple tree die-back, disease control, sodium hypochlorite, soil-borne diseases

Handling Editor: Sang, Mee Kyung

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crop protection agent, however, NaOCl is not yet registered as a control agent in Korea, it is restricted to apply in fields. Nevertheless, because the effect of the registered control agents is insignificant, it is necessary to develop a definite control agent and method for soil-borne diseases which cause dieback in apple trees.

In this study, the inhibition available chlorine content, minimum contact time, and inhibition effect among isolates for NaOCl on colony growth of the 3 soil-born fungal pathogens, compared with registered control agents was investigated in vitro.

Materials and Methods

**Phytopathogens.** Six isolates of *Phytophthora cactorum* (KACC40166, 40174, 40175, 40176, 40183, and 40448), 2 isolates of *Helicobasidium mompa* (KACC40169 and 40836), 4 isolates of *Rosellinia necatrix* (KACC40168, 40445, 40446, and 40447) were distributed from the National Agrobiodiversity Center (https://genebank.rda.go.kr) in Rural Development Administration. Also, 1 isolate of *Helicobasidium* sp. (CBARES2015V1) and 2 isolates of *Rosellinia* sp. (CBARES2015W1 and W2) were isolated from apple trees (Lee, 1995; Shikata and Mitsueda, 1978; Singleton et al., 1992; Tadao, 1984). The used medium for culture and experiment was potato dextrose agar (PDA; Difco, Becton, Dickinson and Company, MD, USA).

**Tested chemical and fungicides.** The chemical used was sodium hypochlorite (NaOCl, 8.0% available chlorine content, Junsei, Japan) for growth inhibition of each pathogen. The fungicides used are as follows; azoxystrobin (WP, a.i. 10%) for *Phytophthora cactorum*, thiophanate-methyl (WP, a.i. 70%) and tolclofos-methyl (WP, a.i. 50%) for *Helicobasidium mompa* and *Helicobasidium* sp. and fluazinam (WP, a.i. 50%), benomyl (WP, a.i. 50%), isoprothiolane (GR, a.i. 12%) for *Rosellinia necatrix* and *Rosellinia* sp.

**Experimental design.** In order to determine the available chlorine content of NaOCl, cultured agar plug of each phytopathogen colony with Cork borer 2 (Ø 6.2 mm, Usbeck, Radevormwald, Germany) was placed on sterilized paper discs (Ø 8 × 1.5 mm, Advantec, Japan) on PDA medium and then a cultured agar plug on a paper disc were placed. Six available chlorine content of the chemical were chosen for the *in vitro* experiments: 1.25, 3.125, 12.5, 31.25, 62.5, 125 ml/l of 8.0% NaOCl in sterilized water. Each available chlorine content was injected into a paper disc by 70 μl while being kept cool on ice, and incubated at 23 ± 1°C for 5 to 15 days in MIR-154 (Panasonic, Tokyo, Japan).

To investigate the inhibitory contact time in NaOCl for each pathogen, 5 agar plugs of each pathogen were soaked in the determined available chlorine content in NaOCl for 5, 10, 20, 40, 80, 160, 320, 640, and 1,280 s, respectively. They were taken out, put on sterilized absorbent paper, cultured on PDA, and then incubated at 23 ± 1°C for 5 to 15 days.

Inhibition rate for each available chlorine content of NaOCl was as follows; (control value – value of each available chlorine content of NaOCl) / control value × 100.

Finally, isolates of the pathogens were tested for showing the inhibition difference within the available chlorine content of NaOCl determined above as well as the currently registered fungicides.

As the medium preparation, 100 ml of the PDA medium was sterilized and cooled by 50-60°C, and then the standard or 2-fold amount of each crop protection agent were added. Especially, isoprothiolane was dispensed by 30 ml of PDA medium after adding standard amount 25.5 g or 2-fold amount 51 g in 100 × 40 mm plant culture dish (SPL Lifesciences Co., Ltd., Gyeonggi, Korea). All treatments were performed 3 times in 3 replications.

**Data analysis.** The colony growth diameter of each treatment was measured and the inhibition rate compared to non-treatment was calculated. The diameter of the colony growth was measured by the average of long axis and short axis and experiments were tested by 3 times with 5 repetitions per treatment.

Means could be compared to the respective control using the Duncan's test ($P < 0.05$) by using CoStat 6400 program (CoHort software, USA).

**Results and Discussion**

Available chlorine content and contact time in NaOCl for colony growth inhibition of soil-borne fungal pathogens. *P. cactorum* KACC40166 isolate was completely inhibited over 31.25 ml/l of NaOCl. *H. mompa* KACC40836 and *R. necatrix* KACC40168 isolate were completely inhibited over 31.25 and 62.5 ml/l of NaOCl despite difference among repetitions, respectively (Fig. 1). In addition, as shown in Table 1, *P. cactorum* KACC40166 isolate showed a significant inhibition rate of 66% in the colony diameter on available chlorine content of 12.5 ml/l of NaOCl, compared with the control and no colony formation over 31.25 ml/l of NaOCl was showed. *H. mompa* KACC40836 isolate showed significant inhibition rate of 11%, 16%, and 21% in the colony diameter on 1.25, 3.125, and 12.5 ml/
of NaOCl, respectively, compared to the control and no colony formation over 31.25 ml/l of NaOCl was showed. There was no significant difference for the colony growth diameter between 1.25 and 3.125 ml/l as well as 3.125 and 12.5 ml/l of NaOCl. *R. necatrix* KACC40168 isolate showed significant inhibition rate of 36% and 61% in the colony diameter on 31.25 and 62.5 ml/l of NaOCl, respectively, compared to the control. There was no statistical difference for the colony growth diameter between 31.25 and 62.5 ml/l of NaOCl and no colony formation over 125 ml/l of NaOCl was showed. From these results the colony inhibition effect of soil-borne pathogens was different according to available chlorine content of NaOCl, as if the sensitivity of NaOCl varies according to plant pathogens (Cayanan et al., 2009; Hong et al., 2003; Santos-Rufo and Rodriguez-jurado, 2016; Taylor et al., 2000; Thompson, 1965). Chun et al. (1997) reported that 2.6% of NaOCl killed most of bacteria and fungi in rice seeds. Shin et al. (2014) showed that conidia of *Gibberella fujikuroi* were significantly inhibited by 0.008-0.01% of NaOCl and the control value on 0.3% and 0.5% of NaOCl in the infected rice seeds were 93.1% and 93.8%, respectively. Sauer and Burroughs (1986) reported inhibition of *Aspergillus* spp. at 1-5% concentration NaOCl. Therefore, this available chlorine content of NaOCl were similar range to the colony inhibition for *P. cactorum, H. mompa*, and *R. necatrix*.

The results on contact time that inhibits colony formation of each pathogen isolate by the available chlorine content determined above are shown in Table 2. Compared with the control treatment, the *P. cactorum* KACC40166 isolate showed the significant colony inhibition rate of 6-25% and 100% after soaking in 12.5 ml/l of NaOCl for 5-20 s and over 40 s, respectively. In addition, the isolate incubated after soaking in 31.25 ml/l of NaOCl over 5 s showed the significant inhibition rate of 100% without colony formation. The *H. mompa* KACC40836 isolate showed no significance after soaking in 31.25 ml/l of NaOCl for 5-10 s. The isolate treated for 20-160 s, whereas, showed the significant inhibition rate of 22-80% and tended toward the longer the soaking time, the higher the inhibition rate. That

### Table 1. Colony inhibition rate for a typical isolate of soil-borne fungal pathogens by available chlorine content of NaOCl on PDA

| Available chlorine content of NaOCl (ml/l) | *P. cactorum* KACC40166 |  | *H. mompa* KACC40836 |  | *R. necatrix* KACC40168 |  |
|-------------------------------------------|------------------------|---|------------------|---|------------------------|---|
| Colony growth diameter (mm)                | Inhibition rate (%)     | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) |
| Control                                   | 28.1 a                  | -                          | 31.7 a                  | -                          | 31.4 a                  | -                          |
| 1.25                                      | 26.8 a                  | 5                          | 28.3 b                  | 11                         | 28.7 ab                 | 9                          |
| 3.125                                     | 25.5 a                  | 9                          | 26.8 bc                 | 16                         | 34.1 a                  | -9                         |
| 12.5                                      | 9.7 b                   | 66                         | 25.0 c                  | 21                         | 28.8 ab                 | 8                          |
| 31.25                                     | 0 c                     | 100                        | 0 d                     | 100                        | 20.0 bc                 | 36                         |
| 62.5                                      | 0 c                     | 100                        | 0 d                     | 100                        | 12.4 c                  | 61                         |
| 125                                       | 0 c                     | 100                        | 0 d                     | 100                        | 0 d                      | 100                        |

Mean separation by DMRT at *P* < 0.05.
treated over 320 s. showed the significant inhibition rate of 100% without colony formation. No significant difference showed among the range of 5-40 s as well as between 40 and 80 s. Treatments in 62.5 ml/l of NaOCl for the 5 s showed no significant difference. On the other hand, treatments for 10-80 s showed the significant inhibition rate of 23-82% without difference among the range of either 5-20 s or 10-40 s. That treated over 320 s showed the significant inhibition rate of 100% without colony formation.

Cayanan et al. (2009) reported that the zoospores of *P. cactorum* showed complete inhibition after treatment in free chlorine of 0.3 ml/l for 6 min. Meanwhile, Jeffers (1992) reported that the apple root stock MM.106 treated in 1.05% of NaOCl for 10 min. were fewer plants diseased by *Phytophthora* root rot as well as increased significantly both shoot length and fresh weight of root than the control that soaked in water. Thus, the root stock treated for 60 min. showed no significance with phytotoxic, compared to the control soaked in water.

### Table 2. Colony inhibition rate for a typical isolate of soil-borne fungal pathogens by soaking time from the determined available chlorine content of NaOCl

| Soaking time (sec.) | *P. cactorum* KACC40166 | 12.5 ml/l of NaOCl | 31.25 ml/l of NaOCl | 31.25 ml/l of NaOCl | 62.5 ml/l of NaOCl | 62.5 ml/l of NaOCl | 125 ml/l of NaOCl |
|---------------------|--------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|-----------------|
| Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) |
| Control | 25.1 a | 28.7 a | 24.2 a | 26.8 a | 27.2 a | 33.8 a | 17.8 b | 22.4 b | 34 |
| 5 | 23.6 b | 6 | 22.1 ab | 9 | 23.2 ab | 13 | 20.3 b | 25 | 23.0 b | 32 |
| 10 | 20.5 c | 18 | 21.7 ab | 10 | 20.6 bc | 23 | 18.4 b | 32 | 17.5 c | 48 |
| 20 | 18.8 d | 25 | 19.0 b | 22 | 20.5 bc | 24 | 17.9 b | 34 | 18.0 c | 47 |
| 40 | 0 e | 100 | 18.6 bc | 23 | 17.2 c | 36 | 16.9 b | 38 | 17.3 c | 49 |
| 80 | 0 e | 100 | 15.3 c | 37 | 4.9 d | 82 | 18.4 b | 32 | 0 d | 100 |
| 160 | - | - | 4.8 d | 80 | 0 e | 100 | 4.2 c | 85 | 0 d | 100 |
| 320 | - | - | 0 e | 100 | 0 e | 100 | - | - | - | - |
| 640 | - | - | - | - | - | - | - | - | - | - |
| 1,280 | - | - | - | - | - | - | - | - | - | - |
| Mean separation by DMRT at *P* < 0.05.
isolate showed no significant difference between 12.5 ml/l of NaOCl and the fungicide without distinction of treatment dose. *P. cactorum* KACC40176 isolate was inhibited significantly by rather 12.5 ml/l of NaOCl than the fungicide and *P. cactorum* KACC40174 isolate showed no significant difference between 31.25 ml/l of NaOCl and 2-fold dose treatment of the fungicide and both *P. cactorum* KACC40176 and 40183 isolate showed no significance between 12.5 and 31.25 ml/l of NaOCl treatment (Table 3). As only azoxystrobin WP was registered as a control agent and possible the occurrence of the fungicide-resistant isolate with annual use (2015 APS Annual Meeting, http://www.apsnet.org/meetings/Documents/2015_meeting_abstracts/aps2015abP667.htm), therefore, we suggest that 12.5-31.25 ml/l of NaOCl treatment would be effective to control the Phytophthora root rot without the concern for resistant isolate.

From the results of colony inhibition rate for *Helicobasidium* sp. CBARES20151, *H. mompa* KACC40169, and KACC40836 isolate, the 3 isolates showed significant inhibition rate of 75-87% and all 100% in 31.25 and 62.5 ml/l of NaOCl treatment, respectively, compared to control. Thus, those treated by each available chlorine content of NaOCl showed significant difference compared to standard and double dose of 2 control fungicides, thiophanate-methyl and tolclofos-methyl. Compared to control, the 3 isolates showed significant inhibition rate of 30-46%, regardless of thiophanate-methyl treatment dose. Also, *H. mompa* KACC40169 and KACC40836 isolate showed significant inhibition rate of 29-39%, but *Helicobasidium* sp. CBARES20151 isolate showed no significant difference, regardless of tolclofos-methyl treatment dose (Table 4). As reports of FRAC Code list 1 (2017), these results indicate that the resistant isolates for thiophanate-methyl and tolclofos-methyl were possible to occur, according to use the fungicides over 20 years. In fact, Fernández-Ortuño and Schnabel (2012) reported *Botrytis cinerea* that showed

| Treatments            | KACC40166 | KACC40174 | KACC40175 | KACC40176 | KACC40183 | KACC40448 |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                       | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) |
| Control               | 30.9 a    | -         | 28.2 a    | -         | 28.9 a    | -         | 30.0 a    | -         | 26.9 a    | -         | 25.1 a    | -         |
| 12.5 ml/l of NaOCl    | 20.2 a    | 35        | 19.3 ab   | 32        | 15.9 b    | 45        | 5.9 b     | 80        | 6.5 bc    | 76        | 20.7 b    | 18        |
| 31.25 ml/l of NaOCl   | 0 b       | 100       | 0 c       | 100       | 0 c       | 100       | 0 b       | 100       | 0 c       | 100       | 0 d       | 100       |
| 1× azoxystrobin       | 20.7 a    | 33        | 14.7 ab   | 48        | 16.2 b    | 44        | 21.4 a    | 29        | 14.7 b    | 45        | 14.5 c    | 42        |
| 2× azoxystrobin       | 20.2 a    | 35        | 12.4 bc   | 56        | 15.4 b    | 47        | 21.4 a    | 29        | 12.6 b    | 53        | 13.8 c    | 45        |

Mean separation by DMRT at *P* < 0.05.

| Treatments              | KACC40169 | KACC40836 | CBARES20151 |
|-------------------------|-----------|-----------|-------------|
|                         | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) |
| Control                 | 15.3 a    | -         | 12.3 a      | -         | 9.5 a      | -         |
| 31.25 ml/l of NaOCl     | 3.9 c     | 75        | 2.9 c       | 76        | 1.2 c      | 87        |
| 62.5 ml/l of NaOCl      | 0 d       | 100       | 0 d         | 100       | 0 c        | 100       |
| 1× thiophanate-methyl   | 8.8 b     | 43        | 6.9 b       | 44        | 6.4 b      | 33        |
| 2× thiophanate-methyl   | 8.2 b     | 46        | 6.6 b       | 46        | 6.7 b      | 30        |
| 1× tolclofos-methyl     | 9.9 b     | 35        | 8.8 b       | 29        | 9.1 a      | 4         |
| 2× tolclofos-methyl     | 9.3 b     | 39        | 7.6 b       | 38        | 8.4 a      | 12        |

Mean separation by DMRT at *P* < 0.05.
the resistance for thiophanate-methyl as well as Van Bruggen and Arneson (1984) reported *Rhizoctonia solani* that showed the resistance for tolclofos-methyl. Therefore, we suggest that 31.25-62.5 ml/l of NaOCl treatment would be effective to control the violet root rot diseased by *H. mompa*.

From the results of colony inhibition rate for *Rosellinia* sp. CBARES20152 and CBARES20153, *R. necatrix* KACC40168, KACC40445, KACC40446, and KACC40447 isolate, the other isolates except for *R. necatrix* KACC40445 showed significant inhibition rate of 20-33% in 62.5 ml/l of NaOCl treatment. All of the 6 isolates showed significant inhibition rate of 100% in 125 ml/l of NaOCl treatment. For standard dose and 2-fold dose of the fungicides, such as benomyl, fluazinam and isoprothiolane, all of the 6 isolates showed significant inhibition rate of 100% in benomyl treatments. However, the other isolates except for *R. necatrix* KACC40446 showed significant inhibition rate of 55-79% and 74-94% in fluazinam and isoprothiolane treatment, respectively. Difference between standard and 2-fold treatments in each fungicide showed no significance (Table 5). From this result, the fungicide, benomyl, was the most effective fungicide to inhibit the colony of white root rot. Yukita (2003) reported that soaking treatment for 20 min in 500-fold diluted solution of a fungicide, Fluazinam (a.i. 39.5%), showed high control value at both laboratory and field experiments against *H. mompa* and *R. necatrix*. Nevertheless, Eguchi et al. (2008) reported that white root rot of pear trees re-occurred 2 years after fluazinam treatment in Japan. Meanwhile, Takaya et al. (1976) reported that soaking treatment for 24 h in 1,000-fold diluted solution of a fungicide, benomyl (a.i. 50%), showed significant control effect against white root rot as well as Komori and Nakamura (1985) reported that 1,000 ppm of benomyl or isoprothiolane inhibited completely the isolate of *R. necatrix*.

Recently, FRAC Code list 1 (2017) reported that all of the tested fungicides were concerned by occurrence of resistant isolates. In fact, there were reported that a resistance isolate for benomyl in *Rosellinia necatrix* occurred (López-Herrera and Zea-Bonilla, 2007), a cross-resistance isolate for isoprothiolane in *Magnaporthe oryzae* occurred (Ishii and Hollomon, 2015), a resistance mutant isolate for fluazinam in *Botrytis cinerea* induced (Shao et al., 2015).

In conclusion, our study showed that pathogens caused soil-borne diseases, Phytophthora root rot, violet root rot, and white root rot, were effectively inhibited by 12.5-31.25 ml/l, 31.25-62.5 ml/l, and 62.5-125 ml/l of NaOCl, respectively.

**Acknowledgments**

This work was carried out with the support of Chungeonbuk-do Agricultural Research and Extension Ser-

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**Table 5.** Colony inhibition rate of 6 isolates for *Rosellinia necatrix* and *Rosellinia* sp. by available chlorine content of NaOCl and 3 fungicides

| Treatments | KACC40168 | KACC40445 | KACC40446 | KACC40447 | CBARES20152 | CBARES20153 |
|------------|-----------|-----------|-----------|-----------|-------------|-------------|
|            | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) | Colony growth diameter (mm) | Inhibition rate (%) |
| Control    | 23.8 a | - | 17.3 a | - | 29.5 a | - | 26.0 a | - | 24.4 a | - |
| 62.5 ml/l of NaOCl | 8.1 b | 66 | 11.0 ab | 36 | 20.3 b | 31 | 20.7 b | 20 | 7.0 b | 71 |
| 125 ml/l of NaOCl | 0 c | 100 | 0 c | 100 | 0 e | 100 | 0 d | 100 | 0 b | 100 |
| 1× benomyl | 0 c | 100 | 0 c | 100 | 0 e | 100 | 0 d | 100 | 0 b | 100 |
| 2× benomyl | 0 c | 100 | 0 c | 100 | 0 e | 100 | 0 d | 100 | 0 c | 100 |
| 1× fluazinam | 5.2 bc | 78 | 6.0 bc | 65 | 6.3 ed | 79 | 5.5 c | 79 | 5.7 b | 77 |
| 2× fluazinam | 5.4 bc | 77 | 5.8 bc | 67 | 6.8 c | 77 | 5.4 c | 79 | 5.6 b | 77 |
| 1× isoprothiolane | 3.8 bc | 84 | 3.9 bc | 78 | 3.7 d | 88 | 3.5 cd | 87 | 3.8 b | 84 |
| 2× isoprothiolane | 3.8 bc | 84 | 4.0 bc | 77 | 3.6 d | 88 | 1.7 cd | 94 | 2.1 b | 91 |

Mean separation by DMRT at *P* < 0.05.
References

Bisessar, S. and Mellveen, W. D. 1992. Effects of swimming pool sanitizing chemicals on turf grass. Bull. Environ. Contam. Toxicol. 49:295-299.

Cayanan, D. F., Zhang, P., Liu, W., Dixon, M. and Zheng, Y. 2009. Efficacy of chlorine in controlling five common plant pathogens. HortScience 44:157-163.

Chun, S. C., Schneider, R. W. and Cohn, M. A. 1997. Sodium hypochlorite: Effect of solution pH on rice seed disinfection and its direct effect on seedling growth. Plant Dis. 81:821-824.

Copes, W. E., Chastagner, G. A. and Hummel, R. L. 2001. Influence of select inorganic elements and pH on the fungicidal activity of chlorine dioxide in water. SNA Res. Conf. Proc. 46:284-286.

Datnoff, L. E., Kroll, T. K. and Lacy, G. H. 1987. Efficacy of chlorine for decontaminating water infested with resting spores of Plasmopora brassicae. Plant Dis. 71:734-736.

Eguchi, N., Kawai, Y. and Yamagishi, N. 2008. Long-term evaluation of fluazinam soil drench against white root rot of Japanese pear. Annu. Rep. Kanto Tosan Plant Prot. Soc. 55:159-163.

Fernández-Ortuño, D. and Schnabel, G. 2012. First report of thiomane-methyl resistance in Botrytis cinerea on strawberry from South Carolina. Plant Dis. 96:1700-2.

FRAC. 2017. FRAC Code List 2017: Fungicides sorted by mode of action (including FRAC Code numbering). URL https://cpb-us-w2.wpmucdn.com/u.osu.edu/dist/b/28945/files/2017/04/frac-code-list-2017-final-1juq211.pdf [26 February 2019].

Hong, C. X., Richardson, P. A., Kong, P. and Bush, E. A. 2003. Efficacy of chlorothalonil for multiple species of Phytophthora in recycled nursery irrigation water. Plant Dis. 87:1183-1189.

Ishii, H. and Hollomon, D. W. 2015. Fungicide resistance in plant pathogens. Springer Japan, Ibaraki, Japan. 490 pp.

Jeffers, S. N. 1992. Preplant root treatments to reduce the incidence of Phytophthora species on dormant apple rootstocks. Plant Dis. 76:12-19.

Johnson, C. H., Rice, E. W. and Reasoner, D. J. 1997. Inactivation of Helicobacter pylori by chlorination. Appl. Environ. Microbiol. 63:4969-4970.

Kim, S.-I., Lee, S.-B. and Choi, Y.-M. 1995. Isolation and identification of antagonistic microorganisms for biological control of apple root rot disease. RDA J. Agric. Sci. 37:29-42 (in Korean).

Komori, S. and Nakamura, M. 1985. Effects of several kind of fungicides on apple white root rot. Proc. Kanto-Tosan Plant Prot. Soc. 32:135-136.

KSPP. 2009. List of plant diseases in Korea. 5th ed. The Korean Society of Plant Pathology, Suwon, Korea (in Korean).

Lee, D. H. 2002. Etiology and ecology of apple white root rot, caused by Rosellinia necatrix and its biological control. Ph.D. thesis. Kyungpook National University, Daegu, Korea.

Lee, D. H., Lee, S. W., Choi, K. H., Kim, D. A and Uhm, J. Y. 2006. Survey on the occurrence of apple diseases in Korea from 1992 to 2000. Plant Pathol. J. 22:375-380.

Lee, S. B. 1995. Etiology and epidemiology of white- and violet-root rot caused by Rosellinia necatrix and Helicobasidium mompa on apple tree and their control in Korea. Ph.D. thesis. Chungbuk National University, Cheongju, Korea (in Korean).

Lee, S. B., Chung, B. K., Jang, H. I., Kim, K. H. and Choi, Y. M. 1995. Incidence of soil-borne diseases in apple orchards in Korea. Korean J. Plant Pathol. 11:132-138 (in Korean).

Lee, S.-H., Kwon, Y., Shin, H., Kim, I.-J., Nam, S.-Y., Hong, E. Y., Kwon, S.-I., Kim, D. and Cha, J.-S. 2016. Dieback of apple tree by major soil borne diseases in Chungbuk province from 2013 to 2015. Res. Plant Dis. 22:198-201 (in Korean).

López-Herrera, C. J. and Zea-Bonilla, T. 2007. Effects of benomyl, carbendazim, fluzinam and thiophanate methyl on white root rot of avocado. Crop Prot. 26:1186-1192.

RDA. 1993. Compendium of fruit tree diseases with color plates. Rural Development Administration, Suwon, Korea. 286 pp.

Santos-Rufo, A. and Rodriguez-Jurado, D. 2016. Evaluation of chemical disinfectants in reducing Verticillium dahlia conidia in irrigation water. Crop Prot. 79:105-116.

Sauer, D. B. and Burroughs, R. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76:745-749.

Segall, R. H. 1968. Fungical effectiveness of chlorine as influence by concentration, temperature, pH, and spore exposure time. Phytopathology 58:1412-1414.

Shao, W., Zhang, Y., Ren, W. and Chen, C. 2015. Physiological and biochemical characteristics of laboratory induced mutants of Botrytis cinerea with resistance to fluzinam. Pestic. Biochem. Physiol. 117:19-23.

Shikata, H. and Mitsueda, T. 1978. A method for isolating the white root rot pathogen, Rosellinia necatrix Berl. in soil. J. Seric. Sci. Japan 47:519-526.

Shin, D. B., Goh, J., Lee, B.-C., Kang, I. J. and Kang, H.-W. 2014. Use of sodium hypochlorite for the control of bakanae disease in rice. Res. Plant Dis. 20:259-263 (in Korean).

Singleton, L. L., Mihail, J. D. and Rush, C. M. 1992. Methods for research on soilborne phytopathogenic fungi. 2nd ed. APS Press, St. Paul, MN, USA. 265 pp.

Tadao, U. I. 1984. Handbook of soil-borne diseases. Japan Plant Protection Association. Tokyo, Japan. 349 pp.

Taylor, R. H., Falkinham, J. O. 3rd, Norton, C. D. and LeCheval-
lier, M. W. 2000. Chlorine, chloramine, chlorine dioxide, and ozone susceptibility of *Mycobacterium avium*. *Appl. Environ. Microbiol.* 66:1702-1705.

Thompson, D. L. 1965. Control of bacterial stalk rot of corn by chlorination of water in sprinkler irrigation. *Crop Sci.* 5:369-370.

Van Bruggen, A. H. C. and Arneson, P. A. 1984. Resistance in *Rhizoctonia solani* to tolclofos methyl. *Neth. J. Plant Pathol.* 90:95-106.

WHO. 2011. Guidelines for drinking-water quality. 4th ed. World Health Organization, WHO Press, Geneva, Switzerland. 110 pp.

Yukita, K. 2003. Preventive effects of fluazinam on white and violet root rots of apple trees by dipping method. *Annu. Rep. Plant Prot. North Japan* 2003:81-84.