The Effect of Fungicides on Mycelial Growth and Conidial Germination of the Ginseng Root Rot Fungus, *Cylindrocarpon destructans*

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**Abstract** Ginseng root rot caused by *Cylindrocarpon destructans* is the most destructive disease of ginseng. Six different fungicides (thiophanate-methyl, benomyl, prochloraz, mancozeb, azoxystrobin, and iprodione) were selected to evaluate the inhibitory effect on the mycelial growth and conidial germination of *C. destructans* isolates. Benomyl and prochloraz were found to be the most effective fungicides in inhibiting mycelial growth of all tested isolates, showing 64.7% to 100% inhibition at a concentration of 10 μg/mL, whereas thiophanate-methyl was the least effective fungicide, showing less than 50% inhibition even at a higher concentration of 100 μg/mL. The tested fungicides exhibited less than 20% inhibition of conidium germination at concentrations of 0.01, 0.1, and 1 μg/mL. However, the inhibition effect of mancozeb on conidium germination of *C. destructans* was significantly increased to 92% to 99% at a higher concentration of 100 μg/mL, while the others still showed no higher than 30% inhibition.

**Keywords** Chemical control, *Cylindrocarpon destructans*, Fungicides, Ginseng

Ginseng is a therapeutically important perennial herb native to North America and Eastern Asia. Fourteen plants are classified under the genus *Panax*: 12 species and 2 infraspecific taxa [1]. Among the plants, Korean ginseng (*Panax ginseng*), Chinese ginseng (*P. notoginseng*), and American ginseng (*P. quinquefolius*) are three major species commonly used as herbal remedies to restore and enhance human health [2]. Ginseng has various beneficial effects on human health; for example, it reduces total cholesterol levels, improves blood circulation, and exerts anti-hypoxic effects during stress. Thus, it is considered one of the most valuable herbal medicines [3-6]. Ginseng is distributed in 35 countries, and the majority of ginseng (approximately 99%) is produced in South Korea, China, Canada, and the United States [7]. South Korea is the largest market of ginseng, with an estimated $1,140 million domestic distribution [7]. The ginseng market is expected to increase with global expansion of the health food market.

Ginseng is a perennial plant with fleshy roots. The roots require approximately 3 to 5 years of growth before harvesting, and during these long periods of cultivation in the soil, ginseng can be affected by various soil-borne pathogens including bacteria, fungi, and nematodes. Fungi are the most common pathogens, and many fungal species cause disease; for example, *Botrytis cinerea* causes gray mold and botrytis blight, *Colletotrichum panacicola* causes anthracnose, *Phytophthora cactorum* causes phytophthora blight (mildew), *Alternaria panax* causes alternaria blight, and *Cylindrocarpon destructans* causes root rot [8, 9]. *C. destructans* (teleomorph: *Neonectria radicicola*) is one of the most destructive fungal pathogens causing root rot and rusty root disease, which significantly decrease ginseng production [10-13]. The pathogen produces thick-walled resting spores called chlamydomspores. The spores can survive in soil for more than 10 years, leading to replanting disease [14].

Various approaches have been explored to manage *C. destructans* infection, including crop rotation with ginseng and rice, soil fumigation, chemical control using fungicides,
and biological control using antagonistic bacteria [15-18]. Soil fumigation before planting is the most effective treatment against *C. destructans* [14-16, 19]. However, this approach is affected by the soil environment and may not be effective on ginseng seeds already infected with the pathogen [15, 16, 18]. Integrated disease management strategies using a combination of techniques such as chemical, biological, and cultural control would be more effective than using only one control technique.

In South Korea, few studies have evaluated fungicide efficacy against *C. destructans*. The purpose of this study was to evaluate the inhibitory effect of six fungicides (thiophanate-methyl, benomyl, prochloraz, mancozeb, azoxystrobin, and iprodione) on mycelial growth and conidial germination on *C. destructans* isolates in vitro. In our study, mancozeb inhibited both mycelial growth and conidial germination, while prochloraz and benomyl significantly inhibited mycelial growth. Thiophanate-methyl was the least effective fungicide in terms of both mycelial growth and conidial germination.

A total of 11 *C. destructans* isolates were used in this study, which are listed in Table 1. Among the 11 isolates, 7 were from Eumseong, Chungbuk, 2 were from Yeoncheon, Gyeonggi, 1 was from Pocheon, Gyeonggi, and 1 was from Hongcheon, Gangwon. *C. destructans* isolates were routinely grown at 25°C on potato dextrose agar (PDA; MBcell, Seoul, Korea) for mycelial growth or V8 juice agar (80 mL V8 juice, 310 μL 10 N NaOH, and 15 g agar per liter) for conidiation. Spore suspensions of each isolate were stored in 25% glycerol at −75°C and used for each experiment. We evaluated the inhibitory effects of six different fungicides (thiophanate-methyl, benomyl, prochloraz, mancozeb, azoxystrobin, and iprodione) (Table 2) on mycelial growth and conidial germination of *C. destructans* isolates.

To analyze the inhibitory effects of the six fungicides on mycelial growth of *C. destructans* isolates, fungicide suspensions were prepared in sterile distilled water and added to autoclaved PDA (cooled to approximately 50°C) to a final concentration of 0.1, 1, 10, 100, and 1,000 μg a.i./mL for each fungicide. The mixtures were poured into petri dishes (90 mm in diameter) before solidifying. *C. destructans* isolates were grown on PDA for 7 days at 25°C. Mycelial agar plugs (5 mm in diameter) were cut from the edge of the actively growing mycelia of *C. destructans* isolates and inoculated on fungicide-amended PDA plates. Colony diameters were measured after 6 days at 25°C in darkness, and the inhibition rate was compared with that of a control without fungicide.

The inhibitory effects of six fungicides on the mycelial growth of *C. destructans* isolates are shown in Figs. 1 and 2. All fungicides showed less than 50% mycelial growth inhibition for all isolates at a concentration of 0.1 μg/mL. At a concentration of 1 μg/mL, prochloraz was the most effective fungicide, showing more than 50% growth inhibition for all the six isolates (cd19-1-2, cd51, 41077, 12yeo01-01, 12poc2-7, and 12hon1-6) (Fig. 2). Azoxystrobin showed less than 50% growth inhibition for all isolates at a concentration of 1 μg/mL. At a concentration of 10 μg/mL, the inhibitory effect of benomyl significantly increased, ranging from 77.6% to 100% for the 11 isolates. Prochloraz at 10 μg/mL also showed a relatively high inhibitory effect, ranging from 64.7% to 100% for the 11 isolates. The inhibitory effect of iprodione increased as the concentration increased, showing more than 50% growth inhibition for all isolates at a concentration of 100 μg/mL. Thiophanate-methyl was the least effective fungicide, showing less than 50% growth inhibition for all isolates, even at a concentration of 100 μg/mL. At a concentration of 1,000 μg/mL, prochloraz, benomyl, and mancozeb completely inhibited the growth of all isolates, but azoxystrobin and iprodione showed relatively weak inhibitory effects, ranging from 41.5% to 78.2% and 56.8% to 86.2% for the 11 isolates, respectively.

For the evaluation of the inhibitory effects of fungicides on conidial germination, we selected four isolates (cd19-1-2, 41077, 12yeo01-01, and 12yeo01-3) based on their origin. *C. destructans* isolates were grown in a 50-mL tube containing

### Table 1. Strains used in this study

| Isolates | Isolated year | Hosts | Location |
|----------|---------------|-------|----------|
| cd19-1-2 | 2016          | Ginseng | Eumseong, Chungbuk |
| cd30-2   | 2016          | Ginseng | Eumseong, Chungbuk |
| cd34-1   | 2016          | Ginseng | Eumseong, Chungbuk |
| cd38     | 2016          | Ginseng | Eumseong, Chungbuk |
| cd41-2   | 2016          | Ginseng | Eumseong, Chungbuk |
| cd51     | 2016          | Ginseng | Eumseong, Chungbuk |
| 41077    | 2002          | Ginseng | Eumseong, Chungbuk |
| 12yeo01-01 | 2012     | Ginseng | Yeoncheon, Gyeonggi |
| 12yeo01-3 | 2012     | Ginseng | Yeoncheon, Gyeonggi |
| 12poc2-7 | 2012          | Ginseng | Pocheon, Gyeonggi |
| 12hon1-6 | 2012          | Ginseng | Hongcheon, Gangwon |

### Table 2. Fungicides used in this study

| Chemical group | Active ingredient | Concentration (%) | Formulation |
|----------------|-------------------|-------------------|-------------|
| Thiophanate    | Thiophanate-methyl | 70.0              | WP          |
| Benimidazole   | Benomyl           | 50.0              | WP          |
| Imidazole      | Prochloraz        | 25.0              | EC          |
| Dithiocarbamate| Mancozeb          | 75.0              | WP          |
| Strobilurin    | Azoxystrobin      | 21.7              | SC          |
| Dicarboximide  | Iprodione         | 50.0              | WP          |

WP, wettable powder; EC, emulsifiable concentrate; SC, suspension concentrate.
Fig. 1. Inhibitory effect of fungicides on mycelial growth of *Cylindrocarpon destructans* isolates. Fungicide suspensions were added to autoclaved potato dextrose agar to a final concentration of 0.1 µg/mL (A), 1 µg/mL (B), 10 µg/mL (C), 100 µg/mL (D), and 1,000 µg/mL (E). Colony diameters were measured after 6 days at 25°C in darkness. All experiments were performed in triplicate and repeated three times. Data were analyzed by Duncan's multiple range test using the SigmaStat statistical software package (SPSS Inc.) and the same letters denote no significant differences at \( p = 0.05 \). Standard deviations of the means were omitted for clarity.
Effect of Fungicides on Cylindrocarpon destructans

25 mL V8 broth (80 mL V8 juice and 310 μL 10 N NaOH per liter) for 12 days at 20°C with shaking at 150 rpm. Mycelial fragments were removed by filtering through two layers of Miracloth. Conidia were washed once by centrifugation (6,000 rpm for 5 min) and suspended in sterile distilled water. Conidia were counted using a hemocytometer and diluted to 2 × 10^5 conidia/mL in sterile distilled water. The conidial suspensions were mixed with each fungicide (1 : 1, vol/vol) to final concentrations of 0.01, 0.1, 1, 10, and 100 μg a.i./mL and placed on cover glass. Germinated conidia were counted after 24 hr at 25°C in the dark using a minimum of 100 conidia per replicate. All experiments were performed in triplicate and repeated three times and analyzed by Duncan’s multiple range test.

The inhibitory effects of the six fungicides on conidial germination of C. destructans isolates are shown in Figs. 3 and 4. All fungicides (thiophanate methyl, benomyl, prochloraz, mancozeb, azoxystrobin, and iprodione) showed less than 20% germination inhibition at concentrations of 0.01, 0.1, and 1 μg/mL (data not shown). At 10 μg/mL, the inhibitory effect of mancozeb against isolate cd19-1-1 increased significantly to 79%, whereas it was less effective against the other isolates (30% inhibition). At 100 μg/mL, mancozeb strongly inhibited all tested isolates, ranging from 92% to 99% germination inhibition (Fig. 4), while the other fungicides showed low activity (no more than 30% inhibition).
Collectively, among the fungicides evaluated in our study, the dithiocarbamate fungicide mancozeb was the most effective on conidial germination and moderately effective on mycelial growth and of \( C. \) destructans isolates (Figs. 1 and 3). Mancozeb is a broad-spectrum fungicide for various fungal diseases in agriculture, acting on multiple sites in fungal cells [20]. Hassan et al. [21] evaluated the effect of several different fungicides (including mancozeb) on the mycelial growth of \( C. \) destructans and showed that mancozeb effectively decreased mycelial growth. Khorasani [22] also explored the effect of four fungicides (including thiophanate-methyl, mancozeb, iprodione, and azoxystrobin) on mycelial growth of \( C. \) destructans and showed that mancozeb was the most effective among the four fungicides. These results were consistent with our data. However, we cannot compare the inhibitory effect of mancozeb on conidial germination of \( C. \) destructans isolates with the findings of previous studies, since we are the first to evaluate this effect.

Prochloraz and benomyl decreased the mycelial growth of \( C. \) destructans isolates more than did thiophanate-methyl, azoxystrobin, and iprodione (Fig. 1). Prochloraz is an imidazole fungicide classified as a sterol demethylation inhibitor (DMI) fungicide. DMI fungicides inhibit C-14α-demethylation of 24-methylenedihydrolanosterol, which inhibits sterol biosynthesis in fungi [23]. Benomyl is a benzimidazole fungicide classified as a methyl benzimidazole carbamate (MBC) fungicide. MBC fungicides interfere with mitosis and cell division in target fungi by disrupting β-tubulin assembly [24, 25]. Several studies have evaluated the effect of these fungicides. Ziezold et al. [26] evaluated the effect of 15 fungicides (including benomyl) on the mycelial growth of \( C. \) destructans in vitro and showed that benomyl is a highly toxic fungicide. Rego et al. [27] found that of 14 fungicides evaluated, prochloraz and benomyl significantly decreased the mycelial growth of \( C. \) destructans but had no effect on conidial germination.

Thiophanate-methyl had the weakest effect on growth inhibition of \( C. \) destructans isolates (Fig. 1). Previous reports showed that the inhibitory effect of thiophanate-methyl on \( C. \) destructans growth is less effective compared with other fungicides. For example, Hassan et al. [21] found that thiophanate-methyl was significantly less effective than other tested fungicides (Chinosol, Quinosol, and mancozeb) on the growth inhibition of \( C. \) destructans, which was consistent with our results. We also found that benomyl inhibited mycelial growth in all tested \( C. \) destructans isolates (mentioned above), whereas thiophanate-methyl, which is considered an MBC fungicide with a similar mode of action as that of benomyl, showed significantly less inhibitory activity. One possible explanation for the resistance of \( C. \) destructans isolates to thiophanate-methyl is that the allele conferring resistance to thiophanate-methyl is a different allele than that conferring resistance to benomyl [28].

In this study, we explored the inhibitory effects at different concentrations of six fungicides on mycelial growth and conidial germination in \( C. \) destructans isolates in vitro. Soil fumigation has been widely used as the most effective treatment before planting against \( C. \) destructans However, other application methods of fungicides such as soil drenching and seed treatment have been rarely used due to a limited study [29, 30]. Our study provides basic information on the chemical management of \( C. \) destructans isolates and additional research on field applications are necessary for the integrated management.

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