Efficacy of Fluopicolide against *Phytophthora capsici* Causing Pepper Phytophthora Blight

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In this study, we evaluated the efficacy of fluopicolide to inhibit *Phytophthora capsici* *in vitro*, and to control pepper Phytophthora blight in a greenhouse and pepper fields. Fluopicolide was tested on various developmental stages of *P. capsici* 06-143 (a sensitive isolate to metalaxyl) and JHAW1-2 (a resistant isolate to metalaxyl). Mycelial growth and zoosporangium germination of both isolates were completely inhibited at 4.0 µg/ml of the fungicide *in vitro*. The EC₅₀ (effective concentrations reducing 50%) of *P. capsici* 06-143 against zoospore were 0.219 µg/ml, while those of JHAW1-2 were 3.829 µg/ml. When fluopicolide was applied at 100 and 1,000 µg/ml 7 days before inoculation with *P. capsici* 06-143 in the greenhouse test, the disease was controlled completely until 6 days after inoculation. However, the curative effect of fluopicolide was not as much as the protective effect. When fluopicolide was applied by both soil drenching and foliar spraying, the treatments strongly protected pepper against the Phytophthora blight disease. Based on these results, fluopicolide can be a promising candidate for a fungicide to control *P. capsici* in the pepper fields.

Keywords : fluopicolide, pepper Phytophthora blight, fungicidal efficacy

The blight disease caused by *Phytophthora capsici*, is one of the most devastating soil-borne diseases that limit the pepper production in Korea (Hwang and Kim, 1995). Several methods including chemical application, cultural practices, and crop rotation are used to control the disease in the fields of Korea. Especially, the fungicide application has been known to be economically effective with the other methods (Matheron and Porchas, 2000). Many fungicides have been used for the control of the diseases as preventive, curative and systemic compounds, when applied as only one or as mixtures. However, the intensive use of fungicides in Korea

has resulted in the increase of the *Phytophthora* isolates showing the reduced sensitivity to metalaxyl (Kim et al., 2007; Lee et al., 2009; Yeon et al., 2008). The development of resistance to several fungicides in *P. capsici* has led completely to develop new fungicides (Parra and Ristaino, 2001).

Fluopicolide is a novel fungicide of the new chemical class, acyl picolide, developed by Bayer CropScience (Latorse et al., 2006). It exhibits a high level of controlling activity against a range of economically significant plant pathogens of the Oomycetes that cause a number of important diseases in crops, for example, grape downy mildew caused by *Plasmopara viticola* and potato and tomato late blight caused by *P. infestans* (Bardsley et al., 2006; Gouot, 2006). Furthermore, fluopicolide has a novel biochemical mode of action and does not show cross resistance with other fungicide groups (Latorse et al.; Toquin et al., 2006). Although information on fluopicolide efficacy against pepper Phytophthora blight in laboratory, greenhouse and field might provide a good practical implement to management of the disease, there was little information about practical application of fluopicolide to pepper plants. Therefore, in this study, we evaluated the efficacy of fluopicolide in inhibiting *P. capsici* at several stages of its life cycle, and its protective, curative and persistent effect in a greenhouse and pepper fields.

Materials and Methods

Fungicides. Stock solution was prepared by dissolving fluopicolide technical grade 98.2% and metalaxyl technical grade 98.0% in dimethyl sulfoxide (DMSO). The solution was diluted with distilled water to keep DMSO concentration below 1%. For evaluating the efficacy in the greenhouse and the field, the dilution used for a greenhouse tests was amended with 0.03% Tween-20. Copper hydroxide (a.i. %, WP) and dimethomorph (a.i. %, WP) were used as the standard fungicide to compare the efficacy of fluopicolide in the field test.

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Pepper plants and *P. capsici* pathogens. The pepper (cv. Nokkwang) was used as a host plant of Phytophthora blight in this study. For the greenhouse test, pepper was grown in a pot (a plant per 0.2-L pot) in the greenhouse. When six leaves were fully developed, plants were used to evaluate the efficacy of the fungicide in greenhouse.

P. capsici 06-143 and JHAW1-2 were isolated from the infected plants in the pepper field in Chungbuk, Korea and were maintained on V-8 juice agar medium at 4°C. Through the sensitivity test against metalaxyl, *P. capsici* 06-143 and JHAW1-2 were confirmed to be sensitive and resistant to metalaxyl, respectively.

In vitro efficacy tests. The investigation for its inhibitory effects of fluopicolide on mycelial growth, zoosporangium germination and zoospore release from zoosporangium was performed. The inhibitory effect of the fungicide on the mycelial growth of *P. capsici* was assessed by the agar dilution method. A mycelial disc (in diameter of 5 mm) was taken from the growing edge of the 4 day-old cultures, and inoculated on V-8 juice agar medium amended with each fungicide. At least six concentrations with three replicates for each fungicide were used to obtain the respective fungitoxicity curves. Control plates without fungicides were amended with an equivalent amount of solvent only. The effect of the fungicide on mycelial growth was determined by measuring the diameter of mycelial colonies after incubation for 5 days after inoculation (DAI) at 20°C in the dark.

Zoosporangia of *P. capsici* 06-143 and JHAW1-2 were prepared for investigating the efficacy of fluopicolide against zoosporangium germination and zoospore release from zoosporangium. For harvesting zoosporangia of *P. capsici* 06-143 and JHAW1-2, each isolate grown on V-8 juice agar medium at 20°C and dark condition for 5 days was inoculated on oat meal agar medium. After incubation for 5 days at the same condition mentioned above, aerial mycelia were scratched by sterile rubber, and followed by irradiation under a fluorescent light (14 hrs/day) for 2 days. Ten ml of sterile distilled water was added to mycelial mats of each isolate grown on oat meal agar medium for 5 days at 20°C. The zoosporangia were collected and adjusted to the concentration of 1×10^4 zoosporangia/ml. To assess the efficacy of fluopicolide in inhibiting zoospore release from zoosporangium, the fungicide was applied to the suspension of zoosporangia by each indicated concentration, and placed at 4°C for 3 hrs. After incubation at 15°C for 3 hrs, the numbers of empty zoosporangia among 100 zoosporangia were counted with 3 replications. The inhibition of zoosporangium germination was assessed on a glass slide. As mentioned above, fluopicolide in DMSO was added into the suspension of zoosporangia of *P. capsici* (1×10^4

zoosporangia/ml) for the indicated concentration on a glass slide. The slides, on which the mixture was placed, were kept in a humidity box at 25°C and the number of germinated zoosporangia was examined 6 hrs later.

Greenhouse test. The protective and the curative effects of fluopicolide on *P. capsici* were determined in a greenhouse. The fungicide was drenched into soil at the concentrations of 1,000, 100, 10 and 1 mg/ml, respectively, at 7, 3, and 1 day before inoculation (DBI) for the protective activity. For the curative activity, it was treated by soil drenching 1 and 3 DAI. The zoosporangial suspension was prepared as mentioned, and adjusted to 1×10^4 zoosporangis/ml. After the suspension of *P. capsici* 06-143 was poured, the pots were placed in greenhouse until Phytophthora blight of pepper was developed 12 days later. The disease incidence of Phytophthora blight was investigated 6 and 12 DAI, respectively.

Field test. The control activity of fluopicolide against Phytophthora blight and the effect of application period were determined in the field located at Yeongi, Chungnam. All experiments were designed by randomized block design with 3 replicates. The fungicide was applied in the pepper fields by two spray programs. In the first program, the fungicide was applied 4 times by 10-day interval through spraying to the upper part of pepper. The fungicide was drenched into soil twice by 15-day interval in the other program. The disease incidence in the field was determined gated 20 days after the last spraying application of the fungicides. As a standard fungicide, dimethomorph was applied 4 times by spraying with 10-day interval. To determine the effect of application period on the control activity, fungicides such as fluopicolide, copper hydroxide and dimethomorph were applied 4 times by 7-day interval and twice by 14-day interval. The disease incidence was investigated 7 days after the last application of fungicides in 7-day interval program.

Results

Efficacy of fluopicolide on the mycelial growth, zoosporangium germination and zoospore release. The isolates 06-143 and JHAW1-2 of *P. capsici*, showed different responses to metalaxyl application. As shown in Fig. 1, EC₅₀ (effective concentration reducing the mycelial growth by 50%) values of *P. capsici* 06-143 were 0.219 µg/ml, while those of *P. capsici* JHAW1-2 were 3.829 µg/ml. However, fluopicolide effectively controlled the mycelial growth of both isolates, regardless of the sensitivity to metalaxyl. EC₅₀ values of *P. capsici* 06-143 and JHAW1-2 to the fungicide were 0.245 and 0.222 µg/ml, respectively.

Fig. 1. Effect of fluopicolide and metalaxyl on the mycelial growth, zoosporangium germination, and zoospore release from zoosporangium of *Phytophthora capsici* 06-143 and JHAW1-2. Three graphs of left side showed the efficacy of fluopicolide on zoosporangium of *P. capsici* isolates, 06-143 and JHAW 1-2, sensitive and resistant to metalaxyl. Graphs on the right side showed the efficacy of metalaxyl on controlling *P. capsici* 06-143 and JHAW 1-2.

Furthermore, while metalaxyl did not inhibit zoosporangium germination and zoospore release from zoosporangium in both isolates, fluopicolide did inhibit them, showing EC_{50} values as 1.374 and 1.231 $\mu\text{g/ml}$ against zoosporangium germination of *P. capsici* 06-143 and JHAW1-2, respectively, and EC_{50} values as 1.169 and 0.338 $\mu\text{g/ml}$ against zoospore release of *P. capsici* 06-143 and JHAW1-2, respectively. Mycelial growth and zoosporangium germination never occurred in both of isolates by the treatment more than 4 $\mu\text{g/ml}$ of fluopicolide.

Efficacy of fluopicolide in the greenhouse trials. When fluopicolide was applied at the concentration of 1, 10, 100, and 1,000 $\mu\text{g/ml}$ just one DBI with *P. capsici* 06-143, an excellent control efficacy of the fungicide was obtained against *Phytophthora* blight in the greenhouse (Fig. 2A). This efficacy was kept until even 12 days later. When it was applied protectively 3 and 7 DBI, 10 and 1 $\mu\text{g/ml}$ of fluopicolide did not control the disease. In the treatment of 100 and 1,000 $\mu\text{g/ml}$ fluopicolide applied 3 and 7 DBI,

respectively, the disease was controlled perfectly until 6 DAI. Although the control value of 1,000 $\mu\text{g/ml}$ fluopicolide treated even 7 DBI was 100% until 12 DAI, each of 100 $\mu\text{g/ml}$ of fluopicolide applied 3 and 7 DBI was decreased to 80 and 40%, respectively. When the fungicide was curatively applied at the concentration of 1,000 $\mu\text{g/ml}$ one DAI, it showed 80 and 60% of control value 6 and 12 DAI, respectively. When dimethomorph and metalaxyl were applied protectively 7, 3 and 1 DBI, each control value showed same trend (Fig. 2B and C). While there was no curative effect of dimethomorph, 100 and 1,000 $\mu\text{g/ml}$ of metalaxyl showed 66.7 and 100% of control value until 6 DAI, respectively.

Effect of fungicide-application period on the activity of fluopicolide. There was no difference in the activity of fluopicolide against *Phytophthora* blight between the application with 7-day interval and 14-day interval (Fig. 3). The control value of fluopicolide treated in 7-day interval program was 84.5%, and that was 81.2% even though it was

Fig. 2. Control efficacy of fungicides against *Phytophthora capsici* 06-143 causing pepper *Phytophthora* blight. The fungicide was applied by soil-drenching preventively 7, 3, and 1 day before inoculation with *P. capsici* 06-143. For assessing curative effect of fluopicolide, it was applied 1 and 3 days after inoculation with the fungal pathogen. Zoospores of *P. infestans* 06-143, which were harvested from mycelial mat incubated on oatmeal agar medium and adjusted to 1.0×10^4 zoospores/ml, were poured into soil. A; fluopicolide, B; dimethomorph, C; metalaxyl.

Fig. 3. Effect of the application period on the control activity of fluopicolide in the pepper field. Each fungicide was applied by 7-day and 14-day interval, respectively.

applied by 14-day interval. Also in dimethomorph, there was no difference between two application methods, although its control value was lower than that of fluopicolide. In the case of copper hydroxyde, however, the control value of the 7-day interval application was superior to that of 14-day interval.

Table 1. Control efficacy of fluopicolide on the pepper *Phytophthora* blight in fields

Fungicides	Application	Disease incidence (%)	Control value (%)
Fluopicolide	4 times, Leaf spray with 10-day interval	9.0 b	88.5
Fluopicolide	2 times, Soil drenching with 15-day interval	1.3 a	98.3
Dimethomorph	4 times, Leaf spray with 10-day interval	36.7 c	53.0
Untreated control	—	78.0 d	

Control efficacy of fluopicolide in field test. Fluopicolide prevented *Phytophthora* blight in pepper effectively. As shown in this study, when fluopicolide was applied 4 times by foliar-spraying with 10-day interval, its control value was 88.5% (Table 1). Soil-drenching of fluopicolide with 14-day interval provided 98.3% of control value.

Discussion

Fluopicolide is a new acyl picolide fungicide with a novel mode of action and very effective on a broad spectrum of Oomycetes such as *P. infestans* and *Plasmopara viticola* (Bardsley et al., 2006; Gouot, 2006). Regardless of some informations about the efficacy of fluopicolide against several diseases of Oomycetes, the efficacy of fluopicolide against pepper *Phytophthora* blight has not been known enough to use in the fields. The present study demonstrated the efficacy of fluopicolide. The fungicide provided an excellent control in greenhouse and field tests for controlling *Phytophthora* blight on pepper.

Fluopicolide was reported to be active at several stages of life cycles in *P. infestans* and *P. viticola*. (Latorse et al., 2006). Zoospores were immobilized after treatment with fluopicolide and then swelled and burst. Ultimately, it inhibited zoospore mobility at the concentration of $1.0 \mu\text{g/ml}$ or below. By the treatment of fluopicolide, both indirect germination such as zoospore release from zoosporangium and direct germination of zoosporangium in *P. infestans* was prevented, too. The curative effect of fluopicolide was observed in potatoes and vines against the pathogens (Latorse et al., 2006). Against *P. capsici* causing *Phytophthora* blight, fluopicolide also had excellent efficacy. EC_{50} values were $1.23\text{--}1.37 \mu\text{g/ml}$ against zoosporangium germination, and $0.34\text{--}1.17 \mu\text{g/ml}$ against zoospore release, respectively. An excellent protective activity in the green-

house test which the fluopicolide was applied by soil-drenching even at 1 µg/ml resulted from those characteristics of the fungicide. Not only above stages of life cycle in *P. capsici* but also mycelium growth was inhibited at very low concentration such as 0.22-0.25 µg/ml in *in vitro* test. In spite of a great activity against mycelium growth, the curative effect on Phytophthora blight in pepper was not so clear as obtained in potatoes and vines, as shown in Fig. 2. In the case of potatoes and vines, the fungicide was applied on the upper part of host plants as leaves. When fluopicolide was applied 48 hrs after inoculation, it was effective in controlling *P. viticola* fungal ingress (Gouot et al., 2006). This suggested that fluopicolide was distributed into the leaf translaminarily, and inhibited downy mildew of vine following a curative application. In this study, however, fluopicolide was applied into soil 1 and 3 DAI. The efficacy of fluopicolide in soil-drenching application 1 DAI was not as efficient as that in leaf-spraying, which might result in the difference of penetration efficacy of the fungicide into the inside of pepper plant from roots to leaves.

As shown in the results of Toquin et al. (2006), fluopicolide showed no cross-resistance with other fungicide for Oomycetes and controlled fungal plant pathogen resistant to metalaxyl. Because of these results, fluopicolide may be used to prevent or delay the development of fungicide-resistant pathogens. Introducing fluopicolide in a fungicide-spraying program or using it as mixtures with other fungicides with different modes of action may reduce the development of fungicide-resistant populations.

In our study, fluopicolide was identified as a strong inhibitor of mycelial growth, zoospore release and zoosporangium germination. These traits of fluopicolide can be applied as an effective fungicide controlling Phytophthora blight. In the greenhouse test, fluopicolide provided the great control against Phytophthora blight in pepper in this study. Its performance in control of Phytophthora blight could make it a promising compound for practical use in a spray program with fungicides for pepper Phytophthora blight.

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