

Compatibility of Entomopathogenic fungus *Lecanicillium attenuatum* and Pesticides to control Cotton Aphid, *Aphis gossypii*

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Concerns of entomopathogenic fungi as alternative pest control agents are increasing even though chemical pesticides have been used as the main control agents for pests and diseases in crop production. This study was conducted to test the influence of fungicides and insecticides on an isolate of *Lecanicillium attenuatum* that was reported to have the pathogenicity against cotton aphid, because fungicides and/or insecticides can apply with mycopesticides simultaneous, before and/or after. Fungicides fenbuconazole+thiram and propineb inhibited the spore germination and mycelial growth of *L. attenuatum* CS625; dimethomorph and procymidone did not affect spore germination or mycelial growth. The insecticide abamectin, deltamethrin, imidachropride, and spinosad had no detrimental effects on spore germination or mycelial growth. Therefore, these results demonstrated that careful selection of pesticides and fungicides can be applied to the integrated pest and disease control with microbial pesticide.

Key words: Biological control, Cotton aphid, Entomopathogenic fungi, Fungicide, Insecticide, *Lecanicillium attenuatum*, *Verticillium lecanii*

Introduction

Many insect pests and diseases such as aphids and powdery mildew were known to infest cucumber (*Cucumis sativus* L.) in greenhouses. In particular, *Aphis gossypii*

Glover has long been recognized as a key pest of greenhouse crops in Korea (Kim *et al.*, 1986). The cotton aphid is highly difficult to control because of rapid population increase and mixed developmental stages of aphid population. A large amount of pesticides have been applied to control this serious pest and it evoked pesticide resistance and environmental pollution. Recently, the concerns of microbial pesticides are increasing as alternative control agents. Fungi such as *Verticillium lecanii* (Zimm.) Viegas, *Beauveria bassiana* (Bals.) Vuill, and *Paecilomyces fumosoroseus* (Wize) Brown & Smith have been reported the pathogenicity against various aphid species (Milner, 1997.) The *V. lecanii* species complex was recently reclassified as several *Lececanicillium* spp. (Zimm.) (Zare and Gams, 2001). Several mycopesticides have been developed and used in several countries including the United Kingdom and the United States. These include Vertalec[®] based on *Lecanicillium logisporum* (Petch), Zare & Gams (formerly known as *V. lecanii* against aphids and Botanigard[®] based on *B. bassiana* against aphids and whitefly (Goettel *et al.*, 2005).

Cucumber greatly suffers from fungal diseases such as powdery mildew and gray mould, both chemical and biological control agents may be used for the integrated control programs. Therefore, the utilization of entomopathogenic fungi in agricultural systems may be limited because of undesirable interference with agrochemicals applied to control plant diseases, especially fungal pathogen, insect pests and weeds. Fungicides, insecticides, and acaricides can influence spore germination and mycelial growth of fungal spores. Fungicides such as mancozeb inhibit spore germination and fungal growth (Hall, 1981; Smith and Hardee, 1996). In addition, pesticides may be applied with mycopesticide simultaneous, before and/or after. Kouassi *et al.* (2003) reported that when fungicide such as metalaxyl and mancozeb are simultaneously

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applied with a *B. bassiana* isolate, insect mortality decreased obviously. *Nomurea fresenii* prevalence in cotton aphids significantly declined in plots treated with carboxin and etridiazol (Smith and Hardee, 1996). Insects infected with entomopathogenic fungi may also be reduced in crops treated with granular fungicides (Smith and Hardee, 1996). On the contrary, fungicide, iprodione had no negative influence on conidiospore germination and mycelial growth of an isolate of *V. lecanii* (Hall, 1981). The nature of the effects varied with chemicals as well as with the strain and developmental stage of fungi. Therefore, the study of compatibility of chemicals and biocontrol agents are necessary.

Materials and Methods

Fungus

This study was conducted with *V. lecanii* CS625 that was kindly provided by Dr. C. S. Yoon, Research Institute of Engineering & Technology, Korea University, Seoul, Korea. This pathogen was reported to have the highest pathogenicity among the tested isolates against cotton aphid (Kim *et al.*, 2001). This isolate was re-identified as *Lecanicillium attenuatum* according to Zare and Gams's reclassification and ITS sequences (Dr. John Bissett, pers. comm.). The isolate was cultured for 14 days at $25 \pm 1^\circ\text{C}$ on potato dextrose agar (PDA, Difco Laboratories, Sparks, MD, USA) to obtain conidia. Alternatively, the isolate was cultured for 5 days on potato dextrose broth to obtain blastospores. Conidia were harvested from the cultured medium by adding 10 ml sterilized 0.001% aqueous Tween 80 and scrubbing the surface with a glass bar. The conidial suspension was filtered through sterilized cheesecloth and enumerated using a haemocytometer. Blastospores were separated from the mycelium by vacuum filtering through sterilized #1 Whatman filter paper. The blastospores were centrifuged at 1000 g for 20 min (Brushless D. C. Motor Centrifuge VS-15000CF, Vision Scientific Co., Buchon, Korea) and the pellet was re-suspended in 10 ml sterile 0.001% aqueous Tween 80 and enumerated using a haemocytometer.

Influence of pesticides on the spore germination and mycelial growth of *L. attenuatum*

To study the influence of fungicides and insecticides on spore germination and mycelial growth of *L. attenuatum* CS625, several fungicides and insecticides that generally used to control the fungal diseases and pests in cucumber in Korea were tested. The chemicals were added in agar medium at the recommended dosage by the manufacturers. The fungicide and insecticide was incorporated into

molten PDA at $45 - 55^\circ\text{C}$ and poured into Petri plates (Φ 3 cm) or onto glass slides. A drop of spore suspension (1×10^5 conidia/ml) was inoculated onto the Petri plates or slide surfaces and was incubated at $25 \pm 1^\circ\text{C}$ for 12 hrs. Lacophenol cotton blue dropped on the medium to stop and stain fungal growth. Percentage viability of conidia (germinated spores) was assessed under a microscope at 400x magnification. Spores were considered to have germinated once the length of germ tube was equal to, or exceeded, the width of spore. Two hundred spores were counted in the middle of each plate, and three agar plates used for each treatment. Three replicate tests were conducted on separate dates. Each test used different cultured spores.

To study the influence of pesticides on mycelial growth, a 10 mm plug of *L. attenuatum* CS625 cultured on PDA for 7 days was transferred onto the PDA plate containing pesticide and incubated at 25°C for 7 days. Five replicate plates were used for each pesticide. Three replicate tests were conducted on separate dates. Colony diameters were measured after 7 days. The influence of agrochemicals on fungi was calculated as follows: 1) Inhibition of spore germination = $1 - (\text{germination rate of treatment} / \text{germination rate of control})$; 2) Inhibition of mycelial growth = $1 - (\text{mycelial growth of treatment} / \text{mycelial growth of control})$. Zero means no influence and one means no germination and mycelial growth.

Statistical analysis

Inhibition index was averaged within replications for each pesticide. The results were analyzed using PROC GLM procedure (SAS Institute, 1989). Means were separated with Duncan's Multiple Range Test ($p=0.05$).

Results and Discussion

Because the control of the pests and diseases that infest crop can be achieved by both chemical and biological means, the influence of pesticides on the biological control agents must be considered. Significant differences in spore germination and mycelial growth rates of isolate CS625 were found as a function of exposures to differing fungicides (PROC GLM; spore germination of conidia, $F=974.74$; $df=9,50$; $Pr>F=<0.0001$, spore germination of blastospores, $F=934.5$; $df=9,50$; $Pr>F=<0.0001$, and mycelial growth, $F=73.47$; $df=8,18$; $Pr>F=<0.0001$) (Table 1). Fenbuconazole + thiram and propineb inhibited spore germination and mycelial growth of *L. attenuatum* CS625. Azoxystrobin and chlorothalonil strongly inhibited spore germination but did not affect on mycelial growth on nutrient medium. Dimethomorph and procymid-

Table 1. Inhibition by fungicides of spore germination and mycelial growth of *L. attenuatum* CS625 on agar

Fungicides	Recommended concentration (ppm) ¹	Inhibition of spore germination ²		Inhibition of mycelial growth ³
		Conidia	Blastospore	
Azoxystrobin	100	0.98 d	0.99 d	0.53 c
Benomyl	325	0.60 c	0.64 c	0.85 de
Carbendazim + Kasugamycin	434.5	0.14 b	0.07 b	0.84 de
Chlorothalonil	1237.5	0.97 d	1.00 d	0.29 b
Dimethomorph	250	0.00 a	0.01 a	0.16 a
Fenarimol	30	0.03 a	0.07 b	0.67 d
Fenbuconazole + Thiram	1430	0.97 d	1.00 d	0.93 e
Procymidone	500	0.02 a	0.02 a	0.21 ab
Propine	1400	0.98 d	1.00 d	0.83 de

¹Concentrations expressed in ppm as AI.

²Inhibition of spore germination: 1 - (germination rate of treatment/germination rate of control)

³Inhibition of mycelial growth: 1 - (mycelial growth of treatment/mycelial growth of control)

*Means within the same column followed by the same letter are not significantly different by Duncan's multiple range tests.

Table 2. Inhibition by insecticides of spore germination and mycelial growth of *L. attenuatum* CS625 on agar

Insecticides	Recommended concentration (ppm) ¹	Inhibition of conidia germination ²	Inhibition of mycelial growth ³
Abamectin	6.03	0.00 a	0.00 a
Deltamethrin	750	0.03 a	0.03 a
Fenitrothion	400	0.82 b	0.21 b
Imidacloprid	50	0.00 a	0.07 a
Spinosad	50	0.00 a	0.04 a

¹Concentrations expressed in ppm as AI.

²Inhibition of spore germination: 1 - (germination rate of treatment/germination rate of control)

³Inhibition of mycelial growth: 1 - (mycelial growth of treatment/ mycelial growth of control)

*Means within the same column followed by the same letter are not significantly different by Duncan's multiple range tests.

done affected neither spore germination nor mycelial growth. The toxicity of chlorothalonil to *V. lecanii* was similar with both isolate CS625 and an isolate tested by Hall (1981). Dimethomorph, fenarimol, and thiram were toxic to Hall's isolate (1981) but have no influence on *L. attenuatum* CS625. Fenarimol and carbendazim+kasugamycin strongly affected mycelial growth but did not impair spore germination. Benomyl inhibited mycelial growth of *V. lecanii* (Olmert and Kenneth, 1974; Wilding, 1972) and also resulted in inhibition of mycelial growth and spore germination of *L. attenuatum* CS625.

The insecticides abamectin, deltamethrin, imidacloprid, and spinosad had no effects on spore germination or mycelial growth, but fenitrothion significantly reduced both spore germination and mycelial growth (PROC GLM; spore germination of conidia, $F=129.10$; $df=4, 10$; $Pr>F<0.0001$ and mycelial growth, $F=6.31$; $df=4, 10$; $Pr>F=0.0084$) (Table 2). Hall (1981) reported that insecticide dicofol was harmless to the germination of *V. lecanii* strains C-3 and C-48, but inhibition of spore germination by malathion was 100% for strain C-3 and only

32% for strain C-48. Imidacloprid was harmless on spore germination of isolate CS625 but inhibited spore germination of an isolate of *L. muscarium* (Cuthbertson *et al.*, 2005). Mycelial growth in the presence of malathion was inhibited by 64% in strain C-3 and 40% in strain C-48 (Hall, 1981). Olmert and Kenneth (1974) reported that several insecticides including chlorpyrifos strongly inhibited the growth of *V. lecanii* but insecticides tested in our study had no effects on mycelial growth of the tested isolate. These results confirm that influence of fungicides on entomopathogenic fungi varied with fungicide, isolate and fungal developmental stage of fungi. On the basis of this study, careful selection of pesticides and fungicides might permit the combined use of *L. attenuatum* and chemical agents in integrated pest control programs.

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