Preliminary Evaluation of *Paecilomyces lilacinus* HY-4 to Control *Tetranychus urticae*

Tae-Young Shin¹, Won-Woo Lee¹, Seung-Hyun Ko¹, Zhen Ji², Dong-Ha Shin², Kwang-Hee Son³,
Ho-Yong Park², and Soo-Dong Woo^{1,*}

¹Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea

²Industrial Bio-materials Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Korea

³Insect Biotech Co. Ltd., Daejeon 305-811, Korea

(Received 07 February 2011; Accepted 05 March 2011)

The virulence against the two-spotted spider mite, *Tetranychus urticae* Koch, was evaluated with entomopathogenic fungus *Paecilomyces lilacinus* (Thom) Samson HY-4 which is isolated from coleopteran insect and registered to Korean and US patents. Virulence tests were conducted with conidial suspensions (1.5×10^6 conidia/ml) of *P. lilacinus* HY-4 against *T. urticae* adults and positive results were recorded in laboratory conditions. The spraying device was also developed for the efficient and exact evaluation of treatment. The developed spraying device was named as SD-tower sprayer and its efficacy of spraying conidia was evaluated. The accumulated mortality caused by *P. lilacinus* HY-4 using SD-tower spray was about 73% at 6 days after inoculation. This suggest that the isolate *P. lilacinus* HY-4 may be considered as promising for a new approach to prevent adult infestations by *T. urticae*.

Key words: *Tetranychus urticae*, *Paecilomyces lilacinus*, Entomopathogenic fungi, Tower spray

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch

(Acari: Tetranychidae) has been recorded on more than 150 hosts of some economic value throughout the world (Irigaray *et al.*, 2003). It is probably the most important pest species in the family Tetranychidae. This pest has become a serious problem because of the large scale use of the chemical insecticides. One explanation for this is that insecticides reduce natural enemies, causing a reduction in predation pressure, which may allow the two-spotted spider mite numbers to increase (Gatarayihia *et al.*, 2010). Chemical acaricides have since been relied upon. However, these are not always effective and their continuous use has resulted in resistance among two-spotted spider mite populations. Like other mites, this pest is capable of rapidly developing extensive resistance to many acaricides (Dagli and Tunc, 2001; Nauen *et al.*, 2001). Thus, there is a need to find alternative control methods to suppress two-spotted spider mites populations.

Entomopathogenic fungi can play an important role in the regulation of arthropod pest populations. Several fungal species have been isolated from their respective hosts and used as pest control agents in many countries (Wraight *et al.*, 1998; Ansari *et al.*, 2004; Vu *et al.*, 2007; Patricia *et al.*, 2008). Recently, some fungi such as *Beauveria bassiana* (Bals.) Vuill, *Metarhizium anisopliae* (Metsch.) Sorokin, *Hirsutella thompsonii* Fisher and *Lecanicillium lecanii* (Zimm.) (Viegas) Zare & Gams have been screened against two-spotted spider mite and hold potential for their development as bioacaricides (Tamai *et al.*, 2002a; Chandler *et al.*, 2005; Gatarayihia *et al.*, 2010). In Korea, it is also necessary to develop biological control mechanisms as a part of integrated pest management programs against the two-spotted spider mite.

*To whom the correspondence addressed

Department of Agricultural Biology, College of Agriculture, Life & Environment Science, Chungbuk National University, Cheongju 361-763, Korea.

Tel: +82-043-261-2553; Fax: +82-043-271-4414;

E-mail: sdwoo@cbnu.ac.kr

Dose-response evaluations of entomopathogenic fungus conducted under controlled conditions typically involve application of known droplet dose onto certain body parts of individual insects and spraying of surfaces with known amounts of fungus (Nansen *et al.*, 2010). Of the two, spraying method is more suitable for small-size insects and practical.

Paecilomyces lilacinus (Thom) Samson HY-4 was isolated from unidentified coleopteran insects in Korea and registered to Korean and US patents with accession number 0035708 and 7435411, respectively. The present study aimed to evaluate the potential of an entomopathogenic fungus *P. lilacinus* HY-4 as a candidate for control of two-spotted spider mite. For this, novel spraying device is also developed and evaluated its efficacy.

Materials and Methods

Two-spotted spider mite

Two-spotted spider mites were obtained from a colony of a continuous culture in the Insect Ecotoxicology Laboratory, Chungbuk National University, Korea. The initial culture originated from two-spotted mite collected from Kongju, Korea in 2010. The two-spotted mite population was reared and maintained on garden pea (*Pisum sativum* L.) at 25°C, 70% relative humidity, and 12:12 (L:D) photoperiod. To obtain fixed-age females for the bioassays, quiescent deutonymphs were collected from the two-spotted spider mite cultures and put on leaf discs. The newly emerged females were used for the experiments 1-2 day later (Wekesa *et al.*, 2005).

Preparation of conidial suspension

One gram of dry conidia suspended in 10 ml sterile distilled water with 0.05% Tween-80. The conidial suspension was vortexed for 10 min to produce a homogenous suspension. Conidial viability was determined by serial dilution plating onto SDYA, with colony forming unit counted 3 days after incubation at 25°C (Glare *et al.*, 2008), and results used for adjusting the concentration to 1.5×10^6 viable conidia/ml.

SD- tower sprayer

To evaluate the virulence of *P. lilacinus* HY-4 against two-spotted spider mite, a SD-tower sprayer was constructed by partially modified bottle sprayer method (Nansen *et al.*, 2010). The SD-tower sprayer consists of the following materials: a dual action airbrush (BD-186: Fenghua Bida Machinery Manufacture Co., China) with a 0.3 mm needle, an acrylic cylinder (diameter: 90 mm, height: 200 mm), two of cuboid acrylic supports (area: 4900 mm², height:

Fig. 1. SD-tower sprayer. Air compressor hose is connected to an airbrush, which is sprayed inside an acrylic cylinder. Scale bars: 100 mm.

100 mm) and air compressor set (working pressure of 1 kg/cm²) (Fig. 1).

Two-spotted spider mite bioassay

A garden pea without two-spotted spider mites was cut into a 25 mm diameter circle and placed on moist cotton wool in a 60 mm petri dish. Fifteen two-spotted spider mite adults were transferred into each dish using a single-hair brush pen. One milliliter of a fungal suspension (1.5×10^6 conidia/ml) was sprayed into a dish using a SD-tower sprayer and placed in an incubator at 25°C, 70% relative humidity, and 12:12 (L:D) photoperiod. Each treatment was repeated 3 times and included control sprayed only with 0.05% Tween-80. Under each of the sprays, a hemocytometer was placed on side of the leaf to collect the deposited conidia. Two-spotted spider mites were checked daily for mortality, and all cadavers were placed at >90% relative humidity in an incubator at 25°C until conidiation was observed. Cadavers with visible fungal growth on their body surface were considered to have died as a result of fungal infection.

Table 1. Comparison of deposited conidia between SD-tower sprayer and various potter tower sprayers in bioassays.

Type of tower sprayer	Amount of applied conidia	Deposited conidia per cm ²	Rate of Deposited conidia per cm ² (%)	Reference
SD-tower sprayer	1.5×10^6 conidia	8.7×10^3 conidia	0.58	-
Potter tower sprayer	2×10^8 conidia	1.49×10^4 conidia	0.00745	Shapiro-Ila <i>et al.</i> , 2008
	1×10^8 conidia	1.2×10^5 conidia	0.01179	Shan <i>et al.</i> , 2010
	2×10^8 conidia	6.9×10^4 conidia	0.0347	Shi <i>et al.</i> , 2008
	1×10^8 conidia	9.7×10^4 conidia	0.097	Tian <i>et al.</i> , 2006
	8×10^6 conidia	1.2×10^4 conidia	0.15	Vicentini <i>et al.</i> , 2001

Results and Discussion

Evaluation of SD-tower sprayer

The mean ($\pm$ SD) concentration of dry conidial *P. lilacinus* HY-4 deposited onto the leaves was $8.7 \pm 1.4 \times 10^3$ conidia/cm² for the each sprayed and no detective dead of two-spotted spider mite during one day after inoculation with 0.05% Tween-80. To evaluate efficacy of SD-tower sprayer, we compared the deposited conidia rates with previously reported results using potter tower sprayer in bioassay (Vicentini *et al.*, 2001; Tian *et al.*, 2006; Shapiro-Ila *et al.*, 2008; Shi *et al.*, 2008; Shan *et al.*, 2010;) (Table 1). The result showed that SD-tower sprayer was superior to other sprayers with 0.58% rate of deposited conidia per cm². This may be related with the difference of used nozzle of air brush, velocity of air and length of spray tube. Anyway, this suggested that SD-tower sprayer could be used efficiently to bioassay of small-size invertebrate including insects.

Virulence of *P. lilacinus* HY-4 against two-spotted spider mite

Paecilomyces lilacinus HY-4 caused substantial mortalities of two-spotted spider mite adults on day 6 after spraying compared with those observed in the controls and 73.3% mortality occurred at 6 days post inoculation (Fig. 2a). Mycosis began to appear on the second day after death (Fig. 2b) and could be seen by naked eye when mite was incubated at high humidity. Generally, 1×10^8 or upper concentration of conidia is used to bioassay, but we achieved high mortality with 1.5×10^6 conidia in this experiment. Therefore, this result suggest that *P. lilacinus* HY-4 may be utilized effectively to control two-spotted spider mite.

Recently, entomopathogenic fungi including *B. bassiana*, *M. anisopliae*, *P. farinosus* (Holmsk) and *Hirsutella* sp. was considered as promising fungi for two-spotted spider mite control (Tamai *et al.*, 2002a). *Paecilomyces lilacinus* is a commonly isolated form soil and as one of

Fig. 2. (a) The mean ($\pm$ SD) mortality of two-spotted spider mite adults during 6 days after treatment with 4.4×10^5 conidia of *P. lilacinus* HY-4 in 0.05% Tween-80. (b) The two-spotted spider mite adults infected with *P. lilacinus* HY-4 at 2 days after death. Arrows indicate infected mites. Scale bars: (b) 0.5 mm. (Online figure in color).

the effective biological control agents against plant-pathogenic nematodes and pest (Marti *et al.*, 2006; Fiedler and Sosnowska, 2007; Demirci and Denizhan, 2010). Although our isolate *P. lilacinus* HY-4 was isolated from

coleopteran insect, this study showed that *P. lilacinus* HY-4 is also effective pathogen to two-spotted spider mite other than beetles. Although we have not confirmed the efficacy of *P. lilacinus* HY-4 in field condition, we believe that *P. lilacinus* HY-4 have potential for management of two-spotted spider mite.

Acknowledgement

This work was supported by the grant from the Technology Development Program for Agriculture and Forestry (AGC0911011) of the Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

References

- Ansari MA, Vestergaard S, Tirry L, Moens M (2004) Selection of a highly virulent fungal isolate, *Metarhizium anisopliae* CLO 53, for controlling *Hoplia philanthus*. *J Invertebr Pathol* 85(2), 89-96.
- Chandler D, Davidson G, Jacobson R J (2005) Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology* 15, 37-54.
- Dagli F, Tunc I (2001) Dicofol resistance in *Tetranychus cinnabarinus*: resistance and stability of resistance in populations from Antalya, Turkey. *Pest Manag Sci* 57, 609-614.
- Demirci F, Denizhan E (2010) *Paecilomyces lilacinus*, a potential biocontrol agent on apple rust mite *Aculus schlechtendali* and interactions with some fungicides *in vitro*. *Phytoparasitica* 38, 125-132.
- Fiedler Z, Sosnowska D (2007) Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson is also a biological agent for control of greenhouse insects and mite pests. *BioControl* 52, 547-558.
- Gatarayih MC, Laing MD, Miller RM (2010) Effects of adjuvant and conidial concentration on the efficacy of *Beauveria bassiana* for the control of the two spotted spider mite, *Tetranychus urticae*. *Exp Appl Acarol* 50, 217-229.
- Glare TR, Reay SD, Nelson TL, Moore R (2008) *Beauveria caledonica* is a naturally occurring pathogen of forest beetles. *Mycological Research* 112, 352-360.
- Irigaray FJS, Mear-Mancebón V, Pérez-Moreno I (2003) The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the two spotted spider mite *Tetranychus urticae*. *Biological Control* 26, 168-173.
- Marti GA, Lastra CC, Pelizza SA, García JJ (2006) Isolation of *Paecilomyces lilacinus* (Thom) Samson (Ascomycota: Hypocreales) from the Chagas disease vector, *Triatoma infestans* Klug (Hemiptera: Reduviidae) in an endemic area in Argentina. *Mycopathologia* 162(5), 369-372.
- Nansen C, Hinson B, Davidson D, Vaughn K, Gharalari AH (2010) Novel Approaches to Application and Performance Assessment of Insecticide Applications to Crop Leaves. *J. Econ. Entomol* 103(2), 219-227.
- Nauen R, Stumpf N, Elbert A, Zebitz CP, Kraus W (2001) Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). *Pest Manag Sci* 57, 253-61.
- Patricia HS, Pedro MOJN, Talita MA, Daniele S, Luis FAA, Maria HPF (2008) Selection of *Beauveria bassiana* isolates to control *Alphitobius diaperinus*. *J Invertebr Pathol* 97(2), 83-90.
- Shan LT, and Feng MG (2010) Evaluation of the biocontrol potential of various *Metarhizium* isolates against green peach aphid *Myzus persicae* (Homoptera: Aphididae). *Pest Manag Sci* 66, 669-675.
- Shapiro-Ilan DI, Cottrell TE, Jackson MA, Wood BW (2008) Virulence of Hypocreales fungi to pecan aphids (Hemiptera: Aphididae) in the laboratory. *J Invertebr Pathol* 99(3), 312-317.
- Shi WB, Feng MG, Liu SS (2008) Sprays of emulsifiable *Beauveria bassiana* formulation are ovicidal towards *Tetranychus urticae* (Acari: Tetranychidae) at various regimes of temperature and humidity. *Exp Appl Acarol* 46, 247-257.
- Tamai MA, Alves SB, de Almeida JEM, Faion M (2002a) Evaluation of entomopathogenic fungi for control of *Tetranychus urticae* Koch (Acari: Tetranychidae). *Arq Inst Biol* 69, 77-84.
- Tian L, Feng MG (2006) Evaluation of the time-concentration-mortality responses of *Plutella xylostella* larvae to the interaction of *Beauveria bassiana* with a nereistoxin analogue insecticide. *Pest Manag Sci* 62, 69-76.
- Vicentini S, Faria M, Oliveira MRV (2001) Screening of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolates against nymphs of *Bemisia tabaci* (Genn.) biotype B (Hemiptera: Aleyrodidae) with description of a new bioassay method. *Neotropical Entomology* 30(1), 97-103.
- Vu VH, Hong SI, Kim K (2007) Selection of Entomopathogenic Fungi for Aphid Control. *J Biosci Bioeng* 104(6), 498-505.
- Wekesa VW, Maniania NK, Knapp M, Boga HI (2005) Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi*. *Exp Appl Acarol* 36(1-2), 41-50.
- Wraight SP, Carruthers RI, Bradley CA, Jaronski ST, Lacey LA, Wood P, Galaini-Wraight S (1998) Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. *J Invertebr. Pathol* 71(3), 217-226.