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EFFICIENCY OF SOME ENTOMOPATHOGENIC FUNGI AS BIOCONTROL AGENTS AGAINST *Aphis fabae* SCOPOLI (HEMIPTERA: APHIDIDAE)

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This study evaluated the use of *Lecanicillium muscarium* and *Simplicillium lamellicola* isolates, the commercial bioinsecticide *Verticillium lecanii* and two different insecticides against *Aphis fabae* at 20°C and 25°C. Dead and live individuals were counted daily following treatment, and Lethal time $(LT)_{50}$ and LT_{90} values of entomopathogenic fungi (*L. muscarium*, *S. lamellicola* and *V. lecanii*) and insecticides (azadirachtin and imidacloprid) were calculated. LT_{50} values for *L. muscarium*, *S. lamellicola*, *V. lecanii*, azadirachtin and imidacloprid at 20°C were 1.77, 2.13, 2.33, 1.46 and 0.90/day, respectively. LT_{50} values at 25°C were 1.93, 1.96, 2.03, 1.28 and 0.86/day, respectively. LT_{90} values at 20°C were 4.49, 5.28, 5.13, 3.61 and 2.37, and LT_{90} values at 25°C were 4.46, 5.11, 5.03, 3.43 and 2.21, respectively. At the end of Day 7, death ratios were approximately 100% for all treatment groups at both temperatures. Comparisons of the fiducial limits showed that LT values did not vary significantly between temperatures.
Keywords: Entomopathogen, *Aphis fabae*, *Lecanicillium muscarium*, *Simplicillium lamellicola*, lethal time

INTRODUCTION

Approximately 4700 species of aphididae have been identified throughout the world. Of the close to 450 that have been recorded from crop plants, only about 100 have successfully exploited the agricultural environment to the extent that they are of economic significance (Emdem and Harrington, 2007). *Aphis fabae* Scopoli (Hemiptera: Aphididae), the black bean aphid, is one of the most widespread pests of cultivated crops around the world (Volkl and Stechmann, 1998). Aphid control is predominantly achieved through the use of chemical insecticides; however, this practice has caused environmental problems (Scorsetti *et al*., 2007). In view of their ability to increase rapidly and transmit plant viruses, farmers have applied high doses of pesticides to control these pests. Not only has the overuse of pesticides resulted in insect resistance, consumer demand for pesticide-free food and concerns over environmental residues have led many countries to try and reduce their use of pesticides, including biological control as an alternative method (Kim *et al*., 2001). Entomopathogenic fungi are natural enemies of arthropods that have attracted attention as potential biological control agents.

There are more than 700 species of entomopathogens in the fungal kingdom (Roy *et al*., 2006; Sandhu *et al*., 2012). Fungal entomopathogens such as *Lecanicillium* spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa* and *I. fumosorosea* play an important role in the regulation of insect populations (Gurulingappa *et al*., 2011, Zimmermann, 2008). *Lecanicillium* spp., formerly known as *Verticillium lecanii*, (Zimmermann, 2008; Zare and Gams, 2001) are opportunistic and widely distributed ascomycete fungi of the order Hypocreales. Following a critical taxonomic review using rDNA sequencing to assess diversity within the taxon (Zare and Gams, 2001), the species was divided into a number of new taxonomic entities, including *L. lecanii, L. longisporum*, *L. attenuatum*, *L. nodulosum* and *L. muscarium* (Brodeur, 2012).

Lecanicillium muscarium isolated from aphids, scales, whiteflies, thrips and other insects in various regions of the world have proven to be pathogenic against a number of different insects (Askary and Yarmand, 2007; Goettel *et al.,* 2008; Anand and Tiwary, 2009; Guclu *et al*., 2010). *L. muscarium* has also been shown to be an important natural enemy of *Ricania simulans* (Walker) (Ricaniidae), a widespread pest in the Black Sea region of Turkey whose extensive range of hosts includes fruits, vegetables and ornamentals (Guclu *et al*., 2010). *L. muscarium* is currently in the process of being made available as a commercial bioinsecticide, for example, Mycotal® (Koppert BV, Berkel en Rodenrijs, Netherlands), for use against whiteflies and thrips, and Verticilin® (Koppert BV, Berkel en Rodenrijs, Netherlands), for use against whiteflies and aphids (Goettel *et al*., 2008; Brodeur, 2012).

The genus *Simplicillium* presently consists of three species: *Simplicillium lanosoniveum*, *Simplicillium obclavatum* and *Simplicillium lamellicola* (Nonaka *et al*., 2013). Studies have reported on the use of *S. lamellicola* against ticks (Polar *et al*., 2005), *Heterodera glycines* Ichinohe cysts and *Meloidogyne arenaria* eggs (Gams, 1988).

The aim of this study was to determine the pathogenicity of entomopathogenic fungi (*L. muscarium* (TR-08) and *S. lamellicola* (TR-09) against *A. fabae* in the second nymphal stage under laboratory conditions at two different temperatures and compare this to results obtained with one commercially available biocontrol product [Bio-Catch WP (*V. lecanii*), an organic insecticide Nimbecidine EC (3% Azadirachtin)] and a synthetic insecticide [Conmirid SC 350 (Imidacloprid).

MATERIALS AND METHODS

Fungal cultures: Fungal cultures were isolated from infected *Palomena prasina* (Heteroptera: Pentatomidae) in hazelnuts orchards in the provinces of Duzce and Samsun, Turkey. Single-spore isolates were obtained by serial dilution (Dhingra and Sinclair, 1995) and identified as *L. muscarium* (isolate TR-08) and *S. lamellicola* (isolate TR-09). Isolates were maintained in tubes containing 6.5% Sabouraud dextrose agar (SDA) (Merck Ltd., Darmstadt, Germany) and deposited in the fungal culture collection of the Mycology Laboratory at the Ondokuz Mayis University Faculty of Agriculture's Department of Plant Protection in Samsun, Turkey and in the USDA-ARS Entomopathogenic Fungal Culture Collection in Ithaca, NY (ARSEF 11734 and 11735, respectively).

Conidial germination assessment: The viability of conidia of the two isolates (TR-08 and TR-09) was evaluated using a method modified from Lazreg *et al*. (2009). A conidial suspension was adjusted to 1×10^4 conidia/mL, and 0.2 mL was sprayed onto 9-cm-dia. Petri plates containing potato dextrose agar (PDA) (Oxoid Ltd, Basingstoke, UK). Petri plates were maintained at 25±1°C. After 24 h of incubation, percentages of germinated conidia were counted using an Olympus CX-31 compound microscope at 400x magnification. Conidia were regarded as germinated when they produced a germ tube at least half of the conidial length. Germination ratios for each fungus were calculated after examining a minimum of 200 conidia from each of 3 replicate plates.

Commercial products: The effects of *L. muscarium* and *S. lamellicola* isolates were compared with those of commercially available biocontrol product [Bio-Catch WP (*V. lecanii*)], organic insecticide [Nimbecidine EC (3% azadirachtin)] and a synthetic insecticide [Conmirid SC 350 (imidacloprid)] at the following dosages: 250 mL Bio-Catch/100 L water, 500 mL Nimbecidine/100 L water, 20 mL Conmirid SC 350/100 L water. The commercial products were diluted to recommended rates for used this study.

Inoculum of entomopathogen isolates: Isolates of *L. muscarium* (TR-08) and *S. lamellicola* (TR-09) were grown on SDA at 25±1°C for 15 days. Conidia were harvested with sterile distilled water containing 0.03% Tween 80. Mycelia were removed by filtering conidia suspensions through 4 layers of sterile cheesecloth. Conidia were counted under a compound microscope using a Neubauer hemocytometer to calibrate a suspension of 1×10^8 conidia/mL for each isolate.

Aphids: *Aphis fabae* were cultured on bean plants (*Phaseolus vulgaris* L.). Cultures were maintained at 18°C with a 16:8 h light:dark photoperiod (Douglas, 1997).

Experimental design: Second-stage *A. fabae* nymphs were placed on bean leaves in 9-cm Petri plates containing sterile water-soaked blotters (10 nymphs per plate). Conidial suspensions of entomopathogenic fungi (TR 08 and TR 09) and the 3 other treatments (Bio-Catch, Nimbecidine, Conmirid) were applied to the aphids (2 mL per Petri dish) using a Potter spray tower (Burkard, Rickmansworth, Hertz UK). Petri plates were loosely capped to prevent escape. Control leaves were treated with sterile distilled water (2-mL) containing 0.03% Tween 80. Plates were incubated at either 20±1°C or 25±1°C at 65±5% and a 16:8h light:dark photoperiod for 7 days. All plates were inspected daily. Dead nymphs were counted under a Leica EZ4 stereo dissecting scope at 40-70x magnification, and percent mortality was calculated per Petri plate. Evidence of *Lecanicillium and Simplicillium* on nymph cadavers was verified by microscopic inspection for the presence of diagnostic verticils of conidiogenous cells. The experiment was repeated twice, with four replicates per treatment.

Statistical analysis: Mortality data was corrected using Abbott's Formula (Abbott, 1925). The probit analysis program ***POLO***-PC (LeOra Software, 1994) was used to calculate 50% lethal time (LT_{50}) and 90% lethal time (LT_{90}). Comparisons of the fiducial limits of the LT_{50} and LT_{90} values were used to determine significant differences between entomopathogens and insecticides at 20ºC and 25ºC. In addition, comparisons of the fiducial limits values were used to determine significant differences among slopes.

RESULTS

Conidia viability was assessed before each application. Almost 100% of conidia of *L. muscarium* and *S. lamellicola* isolates germinated. LT_{50} values showed *L. muscarium* to be the most effective entomopathogenic fungus in eradicating *A. fabae* at both 20°C and 25°C (1.77 and 1.93/day), followed by *S. lamellicola* (2.12 and 1.96/day) and *V. lecanii* (2.33 and 2.03/day). Insecticides were nearly twice as effective as entomopathogens at eradicating *A. fabae* according to LT_{50} values, which were 1.46/day and 1.28/day for azadirachtin and 0.90/day and 0.86/day for imidacloprid at 20°C and 25°C, respectively. LT_{90} values also showed insecticides to be twice as effective as entomopathogens against *A. fabae*. According to fiducial limits, different temperature showed similar the performance of both entomopathogens and insecticides (Table 1, Fig. 2).

Table 1. Lethal time (LT_{50} and LT_{90}) for *Aphis fabae* treated with entomopathogenic fungi and insecticides at different temperatures.

Isolate and Insecticide	20°C			25°C		
	LT_{50} (95% fiducial limit)	LT_{90} (95% fiducial limit)	Relative potency (ratio)	LT_{50} (95% fiducial limit)	LT_{90} (95% fiducial limit)	Relative potency (ratio)
V. lecanii	2.33 (2.12-2.52) a* A**	5.13 (4.65-5.78) a A	1.000	2.03 (1.82-2.23) a A	5.02 (4.50-5.77) a A	1.000
L. muscarium	1.77 (1.57-1.95) a A	4.49 (4.02-5.13) a A	1.316	1.93 (1.74-2.11) a A	4.46 (4.02-5.06) a A	1.055
S. lamellicola	2.12 (1.91-2.33) a A	5.28 (4.72-6.06) a A	1.095	1.96 (1.74-2.17) a A	5.11 (4.54-5.92) a A	1.037
Azadirachtin	1.46 (1.28-1.62) a A	3.61 (3.25-4.11) a A	1.597	1.28 (1.08-1.46) b A	3.43 (3.04-3.95) b A	1.591
Imidacloprid	0.90 (0.72-1.05) b A	2.37 (2.10-2.73) b A	2.587	0.86 (0.67-1.03) c A	2.21 (1.93-2.58) c A	2.352

* The same small letters within columns indicates no significant differences between means

** The same capital letters within rows indicates no significant differences between means

Table 2. Slopes, Regression Equation, χ^2 and Heterogeneity of *Aphis fabae* treated with entomopathogenic fungi and insecticides at different temperatures

Isolate and Insecticide	20°C					25°C				
	Slope±SE	Regression Equation	χ^2	df	Hetero-geneity	Slope±SE	Regression Equation	χ^2	df	Hetero-geneity
V. lecanii	3.73±0.26a*	Y=-1.37+3.73x	25.68	40	0.64	3.26±0.24a	Y=-1.00+3.26x	27.86	40	0.70
L. muscarium	3.17±0.24b	Y=-0.78+3.17x	22.40	40	0.56	3.51±0.25a	Y=-1.00+3.51x	26.12	40	0.65
S. lamellicola	3.24±0.24ab	Y=-0.60+3.24x	36.30	40	0.91	3.08±0.24a	Y=-0.90+3.08x	30.81	40	0.77
Azadirachtin	3.25±0.25ab	Y=-0.53+3.25x	17.85	40	0.45	2.99±0.26a	Y=-0.32+2.99x	19.50	40	0.49
Imidacloprid	3.04±0.30b	Y=0.14+3.04x	23.39	40	0.59	3.15±0.35a	Y= 0.20+3.15x	40.55	40	1.01

* The same small letters within columns indicates no significant differences between means

Slopes, Regression Equations, χ^2 and Heterogeneity values for *A. fabae* treated with entomopathogenic fungi and insecticides at different temperatures are given in Table 2. When the distribution of deaths of *A. fabae* is examined, mortality was found to begin on Day 1 with all applications, whereas nearly 100% mortality was achieved on Day 3 with insecticides and on Day 6 with fungal entomopathogens (Fig. 1). Slopes values were compared by overlap. While *V. lecanii*, *S. lamellicola* and azadirachtin were same grup, *L. muscarium*, imidacloprid, *S. lamellicola* and azadirachtin were in the same group at 20°C. All applications are located in the same group at 25°C.

DISCUSSION

Our study compared LT_{50} and LT_{90} values of fungal entomopathogens and insecticides at two different temperatures. With the exception of *L. muscarium*, LT_{50} values decreased with increases in temperatures for all applications; however, comparisons of the fiducial limits showed no significant differences between LT_{50} values at 20°C or 25°C (Table 1). Similarly, while LT_{90} values were slightly lower at 25°C than at 20°C, the difference was not significant. This finding may be attributed to the selection of appropriate temperatures, in line with previous studies (Cuthbertson *et al*., 2005; Vu *et al*., 2007; Meyling and Eilenberg, 2007; Lawrence and Khan, 2009).

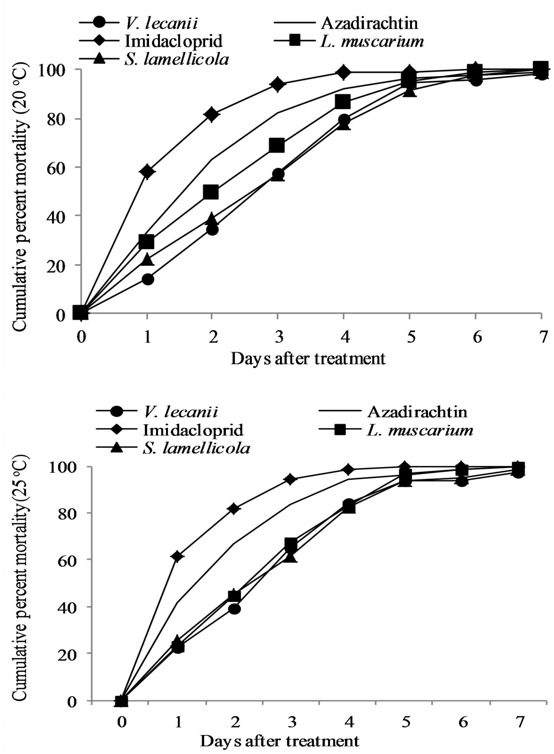

Figure 1. Mortality rates of *Aphis fabae* treated with entomopathogenic fungi and insecticides at 20°C and 25°C

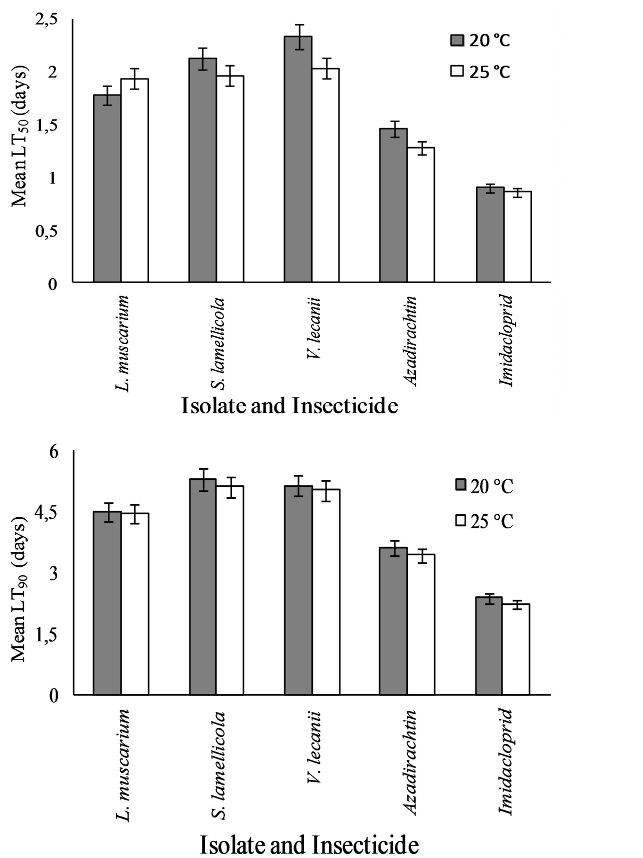

Figure 2. LT_{50} and LT_{90} of *Aphis fabae* treated with entomopathogenic fungi and insecticides at 20°C and 25°C

Among the all entomopathogens, *L. muscarium* performed the best effect in terms of LT_{50} values at 20°C and 25°C. But, the difference in performance among all entomopathogens was insignificant at both 20°C and 25°C (Table 1).

The virulence of an entomopathogenic fungus depends not only on the target aphid species, but also on the temperature and relative humidity (RH) of the environment (Yeo *et al.*, 2003; Vu *et al.*, 2007); thus, it is important to select an entomopathogenic fungus appropriate for climatic conditions (Vu *et al.*, 2007; Yeo *et al.*, 2003). LT_{50} values of the two entomopathogen isolates (*L. muscarium* and *S. lamellicola*) were similar to that of the commercial preparation (*V. lecanii*) at 20°C and 25°C.

The previous studies suggest that improvement in the performance of *V. lecanii* at lower temperatures was slower than that of the other pathogens (Vestergaard *et al.*, 1995; Barson, 2008). The other a study has shown 25°C to be the optimum temperature for *V. lecanii* growth (Sheng-yong *et al.*, 2007). But in our study, performance of *V. lecanii* found the same at both temperatures. In addition to, significant differences were also found between the commercial product and the synthetic insecticide at both temperatures.

When the performance of the two insecticide treatments were compared, azadirachtin was found to achieve 90% mortality of *A. fabae* in 3 days, compared to 2 days with imidacloprid at 25°C. In our study, the effect of azadirachtin on *A. fabae* found the same with the other entomopathogens (Table 1). In previous studies had been found azadirachtin to be affective against the brown citrus aphid (*Toxoptera citricida*) and the cotton aphid (*Aphis gossypii*) (Tang *et al.*, 2001; Dos Santos *et al.*, 2004).

The use of biological control agents against pests has recently raised in importance. A number of studies have identified entomopathogenic fungi as effective against *Aphis* spp. (Mesquita and Lacey, 2001; Steinkraus *et al.*, 2002; Yeo *et al.*, 2003; Kim *et al.*, 2005; Vu *et al.*, 2007; Scorsetti *et al.*, 2007; Gurulingappa *et al.*, 2011; Arıcı *et al.*, 2012).

In conclusion, the results of the present study showed entomopathogenic fungi (*L. muscarium*, *S. lamellicola*) and a commercially available biocontrol product (*V. lecanii*) to be similarly effective in eradicating of *A. fabae* as an organic insecticide (azadirachtin) and a synthetic insecticide (Imidacloprid) under laboratory conditions. To our knowledge, this is the first report to demonstrate the pathogenic effects of *L. muscarium* and *S. lamellicola* against *A. fabae* in our country. The findings suggest that *L. muscarium* and *S. lamellicola* may be developed to commercially acceptable levels; however, the effectiveness of both the entomopathogenic fungi under field conditions needs to be developed before these species can be used effectively in microbial control of *A. fabae.*

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