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Evaluation of Some Commercial Products of Entomopathogenic Fungi as Biocontrol Agents for *Aphis fabae* Scopoli (Hemiptera: Aphididae)

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ABSTRACT

Pathogenicity of five commercial bioinsecticides; Bio-Catch (*Verticillium lecanii*), Priority (*Paecilomyces fumosoroseus*), Bio-Nematon (*Paecilomyces lilacinus*), Bio-Magic (*Metarhizium anisopliae*) and Bio-Power (*Beauveria bassiana*) was evaluated against *Aphis fabae* under laboratory conditions at 20 and 25ºC. LT_{50} values for *V. lecanii, P. fumosoroseus, P. lilacinus, M. anisopliae, B. bassiana* at 20 ºC were 2.33, 2.49, 2.67, 2.24 and 2.60/days, respectively while at 25ºC they were 2.03, 2.18, 2.28, 2.04 and 2.77/days, respectively. LT_{90} values at 20ºC were 4.49, 5.94, 6.46, 5.01 and 6.64, while at 25ºC, they were 5.03, 5.40, 6.68, 5.18 and 7.42, respectively. On the 7th day, mortality rates were approximately 100% at all treatments. Insignificant differences were found among the tested products as well as between the two temperatures studied (P=0.989, P>0.05).

Key words: Bioinsecticides, Entomopathogenic fungi, *Aphis fabae*, Evaluation.

INTRODUCTION

Aphids have a world wide distribution, although the greatest number of species occur in temperate regions, and represent a huge pest problem in agriculture (Yeo, 2000). Close to 450 species have been recorded from crop plants and only about 100 have successfully exploited the agricultural environment to the extent that they are of economic significance (Tuncer *et al.*, 2004 and Emdem and Harrington, 2007). It is not surprising that with such high use of chemical insecticides, there has been increasing concern over public health and the environment. Additionally, problems of insecticide resistance and secondary pest outbreaks have also become more apparent (Yeo, 2000).

Aphis fabae Scopoli (Hemiptera: Aphididae), the black bean aphid, is one of the most widespread pests of cultivated crops around the world (Völkl and Stechmann, 1998). *A. fabae* has been recorded on more than 200 host plant species throughout the world; in Iran, about 50 plant species have been found to be susceptible to attack by this aphid (Adabi *et al.*, 2010).

Aphid control is predominantly achieved through the use of chemical insecticides; however, this practice has caused environmental problems (Scorsettı *et al.*, 2007). Entomopathogenic fungi are promising alternatives to chemical insecticides (Strasser *et al.*, 2000).

Fungal entomopathogens such as *Lecanicillium* (formerly *Verticillium*) spp., *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa* (formerly *Paecilomyces farinosus*) and *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*) play an important role in the regulation of insect populations (Zimmermann, 2008 and Gurulingappa *et al.*, 2011). 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; Vu *et al.*, 2007; Scorsettı *et al.*, 2007; Gurulingappa *et al.*, 2011 and Arıcı *et al.*, 2012).

Lecanicillium muscarium isolated from aphids, scales, whiteflies, thrips and other insects in various regions of the world has proven to be pathogenic against a number of different insects including whiteflies, aphids and thrips (Goettel *et al.*, 2008). *P. lilacinus*, the nematophagous fungus, has showed greatest efficacy in controlling wingless forms of *Aphis gossypii*. The aphid's population was almost totally eliminated under laboratory conditions (Fiedler and Sosnowska, 2007). *L. muscarium* has been shown to be an important natural enemy of *Ricania simulans* (Walker) (Ricaniidae), a widespread pest in the Black Sea region of Turkey which has an extensive range of hosts includes fruits, vegetables and ornamentals (Güçlü *et al.*, 2010).

The aim of this study was to evaluate the effectiveness of five fungal commercial bioinsecticides against *A. fabae* under laboratory conditions.

MATERIALS AND METHODS

Commercial products

Efficacy of the five commercial bioinsecticides; Bio-Catch (*Verticillium lecanii*), Priority (*Paecilomyces fumosoroseus*), Bio-Nematon (*Paecilomyces lilacinus*), Bio-Magic (*Metarhizium anisopliae*) and Bio-Power (*Beauveria bassiana*) at

Table (1): Compounds tested against the *Aphis fabae*

Commercial Product	Entomopathogenic Fungi	Concentration (conidia ml^{-1})	Manufacturer
Bio Power	*Bauveria bassiana*	1 x 10^8	T. Stanes & Company Ltd., India
Bio Magic	*Metarhizum anisopilae*	1 x 10^8	
Bio Catch	*Verticillium lecanii*	1 x 10^8	
Priority	*Paecilomyces fumosoroseus*	1 x 10^8	
Bio Nematon	*Paecilomyces lilacinus*	1 x 10^8	

250 mL/100 L water was evaluated against second instar nymphs of *A. fabae* at 20 and 25ºC. The commercial products were diluted at the manufactory recommended rates for using in this study (Table 1).

Aphid

Aphis fabae was cultured on bean plants (*Phaseolus vulgaris* L.). Cultures were maintained at the laboratory conditions of 18°C, 65±5% R.H. and 16:8h light:dark photoperiod (Douglas, 1997).

Experimental design

Individuals from the second-nymphal instar of *A. fabae* were placed on bean leaves in 9-cm Petri dishes containing sterile water-soaked blotters (10 nymphs per plate). Five treatments were applied to the aphids (2 mL per Petri dish), using a Potter spray tower (Burkard, Rickmansworth, Hertz UK). Petri dishes were loosely capped to prevent escape. Control leaves were treated with sterile distilled water (2-mL). Dishes were incubated at either 20±1°C or 25±1°C at 65±5% R.H. and a 16:8h light:dark photoperiod for 7 days. All dishes were inspected daily. Dead nymphs were counted under a Leica EZ4 stereo dissecting microscope at 40-70X magnification, and percent mortality was calculated per Petri dish. The experiment was repeated twice, with four replicates per treatment.

Statistical analysis

The probit analysis program POLO-PC (LeOra Software, 1994) was used to calculate 50% lethal time (LT_{50}) and 90% lethal time (LT_{90}). Effects of different temperatures and entomopathogenic fungal species on aphid mortality were analyzed using two-way analysis of variance (ANOVA) (P<0,05), followed by a comparison of means using Duncan's multiple range test (from SPSS, v21).

RESULTS AND DISCUSSION

LT_{50} values (Fig. 1 and Table 2) showed that at 20ºC, *M. anisopliae* (2.24 days) was the most effective entomopathogenic fungus against *A. fabae*, followed by *V. lecanii* (2.33 days), *P. fumosoroserus* (2.49 days), *B. bassiana* (2.60 days) and then *P. lilacinus* (2.67 days). *V. lecanii* (2.03 days) was the most effective species at 25ºC, followed by *M. anisopliae* (2.04 days), *P. fumosoroseus* (2.18 days), *P. lilacinus* (2.28 days) and *B. bassiana* (2.77 days) (Table 2). Some isolates of *M. anisopliae* used against *Tetranychus kanzawai* and LT_{50} values was determined as 3.00 - 4.23 days. In the same study, *P. lilacinus* LT_{50} value was determined as 6.33 days at 27ºC (Sanjaya *et al.*, 2013).

The LT_{50} values of the entomopathogenic fungi used in this study decreased as the temperature increased. An increase for only the LT_{50} value of *B. bassiana* was observed. This is due to the fact that, the optimum growth temperature of *B. bassiana* is 25ºC and higher temperature values. In a previous study, *B. bassiana* was used in an efficacy trial against peach aphid, *Myzus persicae* and the LT_{50} value was 4.40 days at 20ºC, while this value has dropped to 1.55 days at 30ºC (Vu *et al.*, 2007).

LT_{90} values (Fig. 1 and Table 2) showed that *V. lecanii* was the most effective entomopathogenic fungus against *A. fabae* at both 20 and 25ºC. It recorded (4.49 days), followed by *M. anisopliae* (5.01 days), *P. fumosoroseus* (5.94/ days), *P. lilacinus (*6.46 days) and then *B. bassiana (*6.64 days). Respective results at 25ºC were (5.03 days), followed by *M. anisopliae* (5.18/ days), *P. fumosoroseus* (5.40 days), *P. lilacinus* (6.68 days) and *B. bassiana* (7.42 days). The reason for such effect of *P. lilacinus* against the black bean aphid may be because the fungus is much specific to nematodes.

Data revealed that there was insignificant difference among the LT_{50} and LT_{90} of the five products tested as well between the two temperatures used (P=0.989 P>0.05) (Table 2 and Figure 1).

Yeo *et al.*, (2003) reported that the pathogenicity of *V. lecanii* increased as the temperature increased. However, at the two different studied temperatures, the LT_{50} and LT_{90} values of fungus species showed non statistically significant. Commercial preparations of *B. bassiana*, *V. lecanii* and *M. anisopliae* were tested against *A. fabae* and *M. persicae* at different temperatures (Yeo *et al.*, 2003). The LT_{50} values at 23ºC were in agreement with those recorded in the present study. Some isolates of *B. bassiana* and *M. anisopliae* were tested against *Collosobruchus maculatus* at 27 ºC and the LT_{50} values ranged between 2.38 to 6.77 (Cherry *et al.*, 2005). Some isolates of *B. bassiana* and *M. anisopliae* were tried against *A. gossypii* at 25-27ºC and the LT_{50} values ranged between 2.54 to 3.66 (Herlinda *et al.*, 2010).

Table (2): Lethal time (LT50 and LT90) for *Aphis fabae* treated with entomopathogenic fungi at different temperatures.

Entomopathogenic fungi	20 ºC			25 ºC		
	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 (2.16-2.49) ^{ab*A**}	4,49 (4.09-5.01) ^{cA***}	1,07	2,03 (1.82-2.23) $^{b\ A**}$	5,03 (4.50-5,77) ^{cA***}	1,08
P. fumosoroseus	2,49 (2,28-2,71) ^{abA}	5,94 (5,32-6,80) $^{ab\ A}$	1,00	2,18 (1.97-2.39) $^{b\ A}$	5,40 (4.84-6,19) $^{bc\ A}$	1,00
P. lilacinus	2,67 (2,46-2,89) $^{a\ A}$	6,46 (5,79-7,40) $^{a\ A}$	0,93	2,28 (2,03-2.52) $^{b\ A}$	6,68 (5,81-8,00) ^{abA}	0,96
M. anisopliae	2,24 (2,04-2,43) ^{bA}	5,01 (4,53-5,66) $^{bc\ A}$	1,12	2,04 (1.82-2.24) $^{b\ A}$	5,18 (4.62-5,95) $^{bc\ A}$	1,07
B. bassiana	2,60 (2,36-2,84) $^{ab\ A}$	6,64 (5,88-7,72) $^{a\ A}$	0,96	2,77 (2,53-3,02) $^{a\ A}$	7,42 (6,51-8,77) ^{aA}	0,79

* The same small letters within columns indicates no significant differences between means
** The same capital letters within rows indicates no significant differences between means (LT_{50})
*** The same capital letters within rows indicates no significant differences between means (LT_{90})

Table (3): Slopes, regression equation, $\chi 2$ and heterogeneity of *Aphis fabae* treated with entomopathogenic fungi at two different temperatures, 20 and 25 ºC

Entomopathogenic fungi	20 ºC					25 ºC				
	Slope	Regression Equation	χ^2	df	Heterogeneity	Slope	Regression Equation	χ^2	df	Heterogeneity
V. lecanii	3.73	Y= -1.37+3.73x	25.68	40	0.64	3.26	Y= -1.00+3.26x	27.86	40	0.70
P. fumosoroseus	3.40	Y= -1,35+3.40x	23,90	40	0.60	3,26	Y= -1.11+3.26x	31,33	40	0.78
P. lilacinus	3.34	Y= -1,43+3.34x	31,83	40	0.80	2,74	Y= -0.98+2,74x	29,10	40	0.73
M. anisopliae	3.65	Y= -1,28+3.65x	32,90	40	0.82	3,17	Y= -0,98+3,17x	34,66	40	0.87
B. bassiana	3.15	Y= 1,31+3.15x	27,67	40	0.69	3.00	Y= 1,33+3.00x	21,83	40	0,55

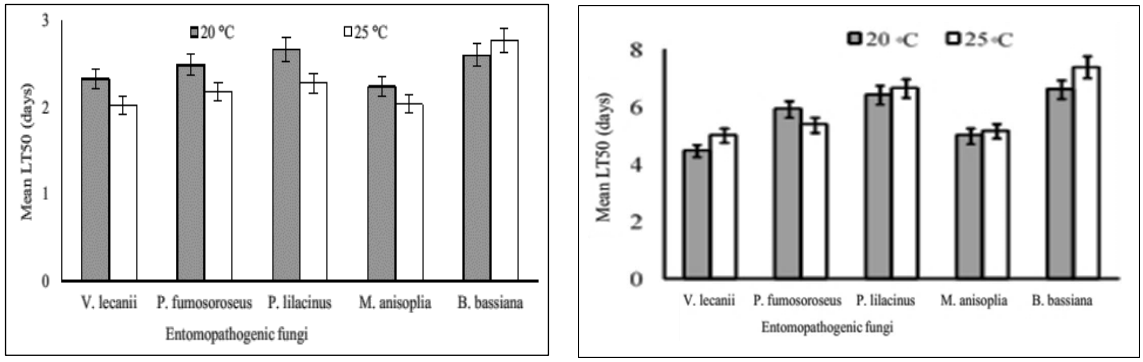

Figure (1): LT_{50} and LT_{90} of *Aphis fabae* treated with entomopathogenic fungi at 20 and 25ºC.

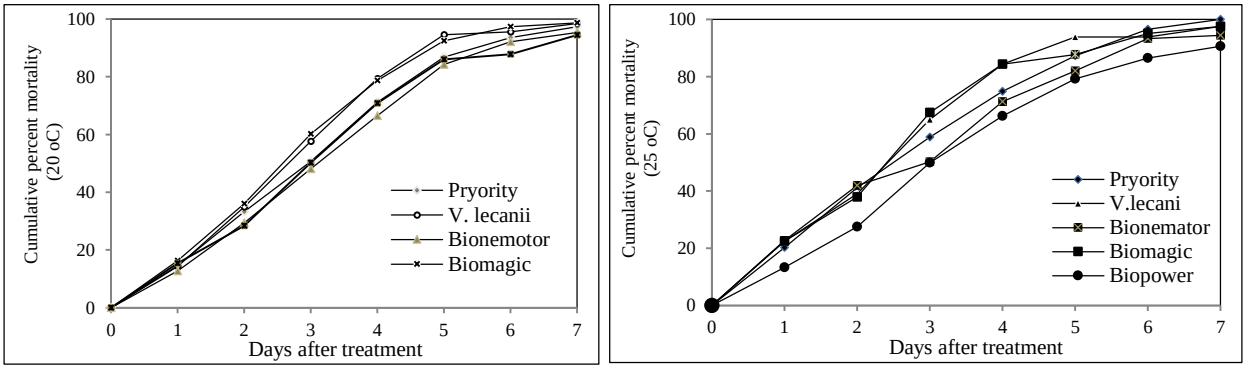

Figure (2): Mortality rates of *Aphis fabae* treated with entomopathogenic fungi at 20 and 25ºC

Slopes, regression equations, χ^2 and heterogeneity values for *A. fabae* treated with the entomopathogenic fungi at different temperatures are given in Table (3). Mortality of *A. fabae* was recoded from day 1 at all applications and achieved nearly 100% mortality on day 7, with all fungal entomopathogen species (Fig. 2).

At 20 and 25ºC and in all the entomopathogenic fungi that used against *A. fabae*, a rate of 50% mortality was attained on the 3rd day and approximately 90% mortality was on the 6th day.

It would be appropriate to test the biocontrol agents against different pest species, as *P. lilacinus* which is a nematophagous fungus when tested against *A. gossypii* in laboratory, it caused a mortality rate of 96% on 7th day (Fiedler and Sosnowska, 2007). These results suggest that commercial bioinsecticides may be very successful in biological control of the *A.*

fabae and may be an alternative for chemical pest management.

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