Saruhan *Egyptian Journal of Biological Pest Control* (2018) 28:89
https://doi.org/10.1186/s41938-018-0096-2

Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Efficacy of some entomopathogenic fungi against *Aphis fabae* Scopoli (Hemiptera: Aphididae)

I. Saruhan

Abstract

The present study was carried out to evaluate the efficacy of three different conidial concentrations (1×10^4, 1×10^5, and 1×10^6 conidia/ml) of five isolates (TR-04, TR-05, TR-07, TR-08, and TR-10) of *Lecanicillium muscarium*, one isolate (TR-01) of *Simplicillium lamellicola*, a commercial bioinsecticide *Verticillium lecanii*, and a synthetic insecticide (Imidacloprid) against the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) under laboratory conditions. Bioassays were conducted in Petri dishes, and insect mortality rate was recorded daily. The LT_{50} values (days) of the isolates at 1×10^6 conidia/ml were 2.96 (TR-05), 3.08 (TR-10), 3.21 (TR-07), 3.45 (TR-01), 3.73 (TR-04), and 3.83 (TR-08), while they were 4.37 and 0.73 for the commercial bioinsecticide and insecticide, respectively. The LT_{90} values (days) of the same conidial concentrations of the isolates attained 4.30 (TR-07), 4.35 (TR-05), 4.80 (TR-10), 5.15 (TR-04), 5.25 (TR-01), 6.06 (TR-08), 6.72 (commercial bioinsecticide), and 2.36 (insecticide). The 1×10^5 and 10^6 conidia/ml concentrations of all the entomopathogenic fungal isolates tested against *A. fabae* caused > 90% mortality by the end of the seventh day. It is concluded that both conidial concentrations of these isolates had significant potential to control black bean aphid.

Keywords: *Lecanicillium muscarium*, *Simplicillium lamellicola*, Entomopathogenic fungi, *Aphis fabae*

Background

Aphids (Hemiptera: Aphididae) are one of the most significant threats to agriculture and forests (Blackman and Eastop 2007). *Aphis fabae* Scopoli (Hemiptera: Aphididae), known as the black bean aphid, is one of the most important species causing yield losses in several cultivated crops, including broad bean and sugar beet (Volkl and Stechnann 1998). *A. fabae* is known to have > 200 host plants globally, and 50 plant species in Iran are vulnerable to this aphid species (Adabi et al. 2010).

The aphids are predominantly controlled by synthetic insecticides; however, their use has raised serious environmental problems (Scorsetti et al. 2007). The overuse of pesticides not only resulted in insect resistance but also forced many countries to reduce pesticide use through alternative methods, including biological control in wake of the increasing consumer tend to prefer pesticide-free food as well as environmental concerns (Kim et al. 2001). The biological control agents used against harmful insects have gained increased importance in recent years. A large number of entomopathogenic fungi (EPF) have been identified and used against aphids (Vu et al. 2007; Scorsetti et al. 2007 and Saruhan et al. 2014, 2015).

EPF such as *Beauveria bassiana*, *Isaria farinosa*, *I. fumosorosea*, *Lecanicillium lecanii*, *L. muscarium*, and *Metarhizium anisopliae* play an important role in the control of insect populations (Zimmermann 2008; Gurulingappa et al. 2011). *B. bassiana*, *L. muscarium*, *L. lecanii*, and *S. lamellicola* are known to be substantial entomopathogens of *A. gossypii* (Saruhan et al. 2015). *L. lecanii* recorded the highest virulent pathogenicity rate against *Myzus persicae* and *A. gossypii*, and their control values reached approximately 100% after 5 and 2 days, respectively, following the treatment (Vu et al. 2007). Yeo et al. (2003) investigated the implications of temperature on growth and germination of prospective bioinsecticides (*B. bassiana* and *V. lecanii*) and various isolates of *B. bassiana*, *V. lecanii*, *M. anisopliae*, and *I. fumosorosea*, formerly known as *Paecilomyces fumosoroseus* and their pathogenicity against aphids. Three

Correspondence: isaruhan@omu.edu.tr
Faculty of Agriculture, Department of Plant Protection, Ondokuz Mayis University, Atakum, 55139 Samsun, Turkey

isolates were tested against *A. fabae* and *M. persicae* at 10, 18, and 23 °C. *A. fabae* was generally found more vulnerable than *M. persicae* to infection by the tested fungal isolates. A meaningful interaction between aphid types and temperature signified that the pathogenic nature of an isolate was susceptible to both target aphid types as well as the temperature of the bioassay (Yeo et al. 2003). Saruhan et al. (2015) evaluated two isolates of *L. muscarium* and *S. lamellicola*, commercial bioinsecticide *V. lecanii*, and two different insecticides against *A. fabae* at 20 and 25 °C. At the end of the seventh day, mortality rates were approximately 100% at all treatments at both temperatures.

The objective of the present study was to evaluate the pathogenicity of five different EPF isolates of *L. muscarium* and one isolate of *S. lamellicola* against *A. fabae* and compare the results with one commercially bioinsecticide and a synthetic insecticide under laboratory conditions.

Materials and methods

Insect culture

A stock culture of *A. fabae*, originated from field collection of infesting broad bean (*Vicia faba* L.) plants in the experimental area of Ondokuz Mayis University, Turkey, during 2017 was established. The pest was reared in 30 × 20 × 40 cm cages at 22 ± 1 °C and 16 h photoperiod for several generations (Mohammed 2018).

Fungal cultures

The fungal isolates were obtained from the stock cultures at the Mycology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey. A total of six isolates of two EPF, i.e., *L. muscarium* (five isolates) and *S. lamellicola* (one isolate) were used for bioassays. The fungal isolates used in the present study were identified by Dr. Richard A. Humber, an insect mycologist (USDA-ARS). The isolates were cultured on potato dextrose agar (PDA; Merck, Darmstadt, Germany) for 5–7 days at 25 ± 1 °C. The fungi were stored at 4 °C on PDA dishes until the start of the bioassay experiments.

Conidial germination assessment

The conidium viability of the fungal isolates (TR-01, TR-04, TR-05, TR-07, TR-08, and TR-10) was determined following Saruhan et al. (2015). The conidial suspension was adjusted to 10^4 conidia/ml, and 100 μl was sprayed on Petri dishes (6 cm diameter) containing PDA. The dishes were then sealed by a parafilm (American National CanTM) and incubated at 25 ± 1 °C. The presence of germinating and non-germinating conidia was counted using an Olympus CX-31 compound microscope (Olympus America Inc., Lake Success, NY) at × 400 magnification after 24 h of incubation. Conidia were regarded as germinated when they produced a germ tube having at least half of the conidial length. The germination ratios of the fungi were determined by examining a minimum of 400 conidia from each of the replicate dishes.

Commercial products

One commercial bioinsecticide, *V. lecanii* (Nibortem SL, 250 ml/100 l of water), and a synthetic insecticide, Imidacloprid (Conmirid SC 350, 20 ml/100 l of water), were used in the study as a positive control.

Inoculum of isolates of EPF

The six fungal isolates belonging to *L. muscarium* and *S. lamellicola* were cultured on PDA at 25 ± 1 °C for 14 days before the initiation of the experiment. Conidial suspensions of the isolates were initially prepared in Tween 20 (0.02% in sterile distilled water) and then filtered through four layers of sterile cheesecloth to remove mycelium and agar pieces. The conidial suspensions were then vortexed for 3 min for homogenization. The concentration of conidial suspension was determined by a Neubauer hemocytometer and adjusted at 1×10^4, 1×10^5, and 1×10^6 conidia/ml.

Experimental design

Conidial suspensions of *L. muscarium* (five isolates: TR-04, TR-05, TR-07, TR-08, and TR-10) and *S. lamellicola* (TR-01), *V. lecanii*, and Imidacloprid were applied on fresh broad bean leaves, obtained from 3-week-old plants grown in pots, containing ten *A. fabae* (third nymphal instar) placed in Petri dishes (9 cm diameter) with sterile distilled water-soaked blotters. For eight treatments, a 2-ml solution was sprayed by a Potter spray tower (Burkard, Rickmansworth, Hertz, UK) on the nymphs of *A. fabae*. The Petri dishes were loosely capped to prevent the escape of insects. The same number of nymphs was used for control, where only sterile distilled water containing 0.02% Tween 20 was sprayed. All dishes were incubated at 25 ± 1 °C in 16 h light/8 h dark cycle and in 70 ± 5% RH for 7 days and inspected daily. Dead nymphs were counted, using a Leica EZ4 stereo dissecting scope at × 40–70 magnification, and the mortality rate was calculated per Petri dish. The experiment was repeated twice, with four replicates per treatment.

Measurement of mycelial growth and sporulation

Mycelial growth and sporulation of the isolates belonging to *L. muscarium* (TR-08) and *S. lamellicola* (TR-01) were assessed according to Cheng et al. (2016) with slight modifications. Mycelial disks (4 mm in diameter) from 10-day-old fungal cultures were placed in the centers of the Petri dishes (9 cm) containing PDA. Then, the dishes were sealed by a parafilm and incubated at 25

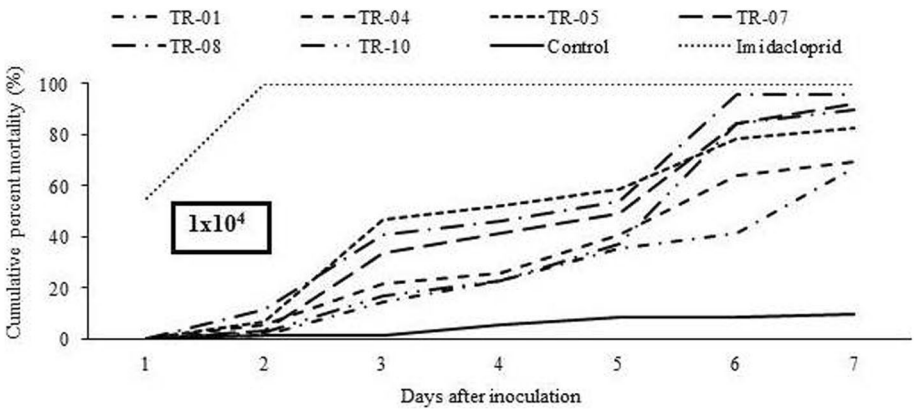

Fig. 1 Cumulative mortality of the third stage of the nymph of *Aphis fabae* after inoculation with different isolates of *Lecanicillium muscarium* and *Simplicillium lamellicola* at 1×10^4 conidia/ml suspension

± 1 °C. Mycelial growth was measured daily at two perpendicular colony diameters up to the point of nearly covering the Petri dishes, and their initial day of sporulation was recorded. At the end of the experiment, three agar pieces of 1 cm^2/fungus were cut from the Petri dishes in which fungal growth occurred, using a sterile scalpel and put into 50-ml sterile polypropylene tubes. The conidia produced on each of the PDA pieces were shaken and dispersed in 20 ml of 0.02% Tween 20 solution. Then, the conidia were counted under Olympus CX-31 compound microscope using a Neubauer hemocytometer, and the spore amount per unit area was calculated. The experiment had three replicates/isolate repeated at different times.

Statistical analysis

Mortality data was corrected using Abbott's formula (Abbott 1925). Serial time-mortality data from bioassays were analyzed by probit analysis using SPSS software (SPSS, version 21) to calculate the lethal times, 50% (LT_{50}) and 90% (LT_{90}). Mortality rates of *A. fabae* treated with EPF, mycelial growth rate and sporulation of six EPF isolates were compared by one-way analysis of variance (ANOVA), followed by Tukey student size post-hoc test where ANOVA indicated significance ($P < 0.05$).

Results and discussion

Among the different fungal isolates tested in the present study, TR-08 with 1×10^4 conidia/ml showed the highest efficacy (95.65%) against *A. fabae* at the end of the seventh day, followed by TR-07 (92.31%), TR-10 (90%), TR-05 (82.67%), TR-04 (69.57%), and TR-01 (67.14%) (Fig. 1). The TR-05, TR-07, TR-08, and TR-10 isolates showed 100% mortality with 1×10^5 conidia/ml, while TR-04 and TR-01 resulted in 89.33 and 71.83%, respectively, at the end of the seventh day. Similarly, TR-05, TR-7, TR-08, and TR-10 isolates at 1×10^6 conidia/ml caused 100% mortality rate, while TR-04 and TR-01 resulted in 98.68 and 95.45%, respectively (Figs. 2 and 3).

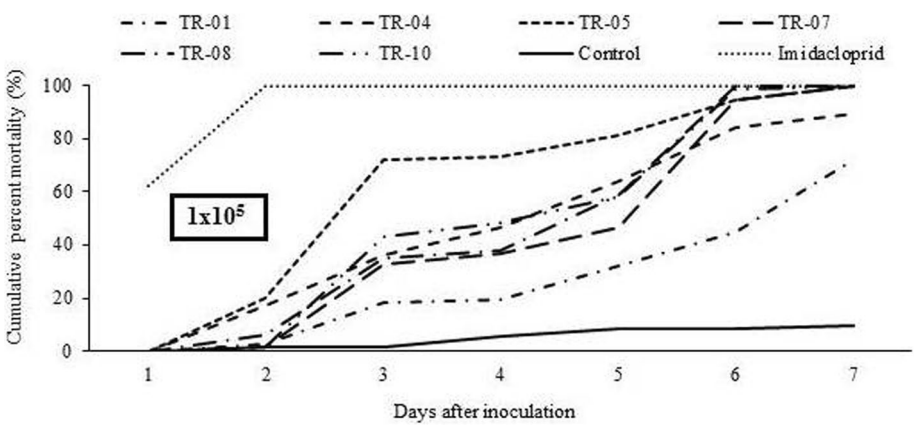

Fig. 2 Cumulative mortality of the third stage of the nymph of *Aphis fabae* after inoculation with different isolates of *Lecanicillium muscarium* and *Simplicillium lamellicola* at 1×10^5 conidia/ml suspension

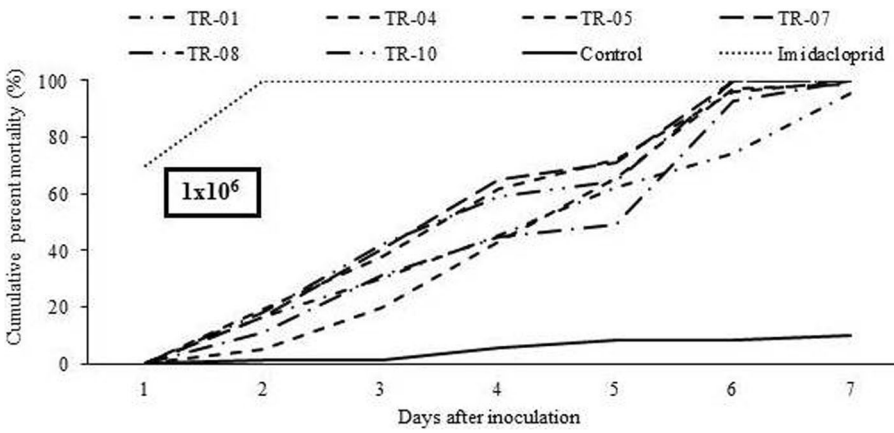

Fig. 3 Cumulative mortality of the third stage of the nymph of *Aphis fabae* after inoculation with different isolates of *Lecanicillium muscarium* and *Simplicillium lamellicola* at 1×10^6 conidia/ml suspension

The results of this study are in line with the results of previous studies. The cumulative mortality rates caused by the three isolates of EPF: *Conidiobolus obscurus*, *C. thromboides*, and *Basidiobolus ranarum* ranged from (51.2 to 91.7%) against *A. fabae* (Halımona and Jankevıca 2011). In another study, it was reported EPF, *B. bassiana*, caused 45% mortality of *A. fabae* at the end of the ninth day (Zamani et al. 2013). Guven et al. (2014) reported that at 1×10^8 conidia/ml of spore solution of *B. bassiana* isolates [BMAUM-A6-001 (90.78%), BMAUM-A6-002 (90.94%), BMAUM-005 (79.62%)], *M. anisopliae* (90.54%), and *Paecilomyces lilacinus* (84.15%) were the most effective against *A. fabae* on the third day based on the number of live individuals. In a study, to determine the biological effectiveness of the EPF, *Fusarium subglutinans* isolated from cotton aphid against *A. fabae*, applications of three different suspensions of two isolates of *F. subglutinans* resulted in meaningful differences in aphid mortality rate at 25 °C. Moreover, no differences in mortality rates were observed between 1×10^7 and 1×10^8 conidia/ml suspension (Arıcı et al. 2012).

The LT_{50} and LT_{90} values of six EPF isolates (*L. muscarium* and *S. lamellicola*) at 1×10^4, 1×10^5, and 1×10^6 conidia/ml suspensions; bioinsecticide (*V. lecanii*); and synthetic insecticide (Imidacloprid) against the third nymphal instar of *A. fabae* were also recorded in the present study. The LT_{50} values of TR-01, TR-04, TR-05, TR-07, TR-08, and TR-10 isolates at 1×10^6 conidia/ml were 3.45, 3.73, 2.96, 3.21, 3.83, and 3.08 days, respectively. Similarly, the LT_{50} values of the bioinsecticide and Imidacloprid were 4.37 and 0.73 days, respectively. The six fungal isolates and the commercial bioinsecticide were statistically similar, while the synthetic insecticide was different ($P < 0.05$) (Table 2). In a study, 12 EPF used against *A. fabae*, LT_{50} values ranged from 1.40 to 5.47 days (Vu et al. 2007). In another study, three EPF were used against *A. fabae*, and LT_{50} values ranged from 2.79 to 4.24 days (Yeo et al. 2003). Saruhan et al. (2015)

Table 1 Lethal times (LT_{50} and LT_{90}) for *Aphis fabae* treated with six entomopathogenic fungi (1×10^6 conidia/ml), commercial bioinsecticide, and synthetic insecticide

Treatments	LT_{50} (95% confidence limit)	LT_{90} (95% confidence limit)
TR-01	3.45 (2.98–3.92) a*	5.25 (4.65–6.28) ab
TR-04	3.73 (3.55–3.93) a	5.15 (4.86–5.55) ab
TR-05	2.96 (2.78–3.14) a	4.35 (4.08–4.68) ab
TR-07	3.21 (2.79–3.72) a	4.30 (3.79–5.28) ab
TR-08	3.83 (2.97–5.37) a	6.06 (4.81–11.40) a
TR-10	3.08 (2.25–3.88) a	4.80 (3.97–7.20) ab
Verticillium lecanii	4.37 (4.16–5.19) a	6.72 (6.09–7.01) a
Imidacloprid	0.73 (0.65–1.24) b	2.36 (2.01–2.98) b
F value	9.499	3.180
P value	< 0.001	< 0.026

*Within columns, means followed by the same small letter do not differ significantly

Table 2 Mycelial growth rate and sporulation of different entomopathogenic fungal isolates

Isolates	Colony diameter (cm)			Sporulation (conidia/cm^2)
	5 days	10 days	15 days	
TR-01	0.6 a*	1.4 a	1.9 a	4.7×10^7 b
TR-04	0.5 a	1.3 a	1.7 a	1.5×10^8 a
TR-05	0.7 a	1.5 a	1.9 a	1.4×10^8 a
TR-07	0.6 a	1.3 a	1.8 a	1.6×10^8 a
TR-08	0.6 a	1.4 a	1.8 a	1.5×10^8 a
TR-10	0.5 a	1.4 a	1.7 a	1.4×10^8 a

*Within columns, means followed by the same small letter do not differ significantly ($P < 0.05$) (initial sporulation time 3 days)

used EPF, *S. lamellicola* (TR-09) isolate, and the bioinsecticide, *V. lecanii*, against *A. fabae* at a suspension of 1×10^8 conidia/ml, and LT_{50} values were 2.12 and 2.33 days, respectively. Considering LT_{90} values of the present study, TR-07 proved to be the most effective isolate, with the lowest LT_{90} value (4.30 days), followed by TR-05 (4.35 days), TR-10 (4.80 days), TR-04 (5.15 days), TR-01 (5.25 days), and TR-08 (6.06 days). The LT_{90} values of the commercial bioinsecticide and synthetic insecticide were 6.72 and 2.36 days, respectively. Regarding the LT_{90} values, TR-08 isolate and the commercial bioinsecticide were found in different groups from the synthetic insecticide ($P < 0.05$) (Table 1).

The mycelial growth and sporulation rates of the EPF were determined on PDA at the end of 15 days. The colony diameter of *S. lamellicola* (TR-01) isolate was 1.9 cm, while it ranged from 1.7 to 1.9 cm in the five isolates of *L. muscarium*. The sporulation rate was 4.7×10^7 conidia/cm^2 for *S. lamellicola* (TR-01) isolate and changed to be between 1.4×10^8 and 1.6×10^8 conidia/cm^2 for *L. muscarium* isolates. Nonetheless, one isolate of *S. lamellicola* and five isolates of *L. muscarium* were statistically similar in terms of mycelial growth rate, but the conidial sporulation of *S. lamellicola* isolate was statistically different than all isolates of *L. muscarium* ($P < 0.05$) (Table 2). In addition, all the isolates of *L. muscarium* at 1×10^6 conidia/ml caused more than 95.45% mortality rates on the insect at the end of the seventh day (Fig. 3). According to these results, it was determined that there was no relationship between the number of conidia formed by the five *L. muscarium* isolates tested in the study and *A. fabae* mortality caused by them. In a similar study, two different isolates of *M. anisopliae* produced 1.2×10^8/cm^2 and 1.7×10^8/cm^2 conidia, while these isolates caused a mortality in *Curculio nucum* larvae at a similar rate after the seventh day (Cheng et al. 2016).

Conclusion

Different isolates belonging to the EPF: *L. muscarium* and *S. lamelicolla*, commercial bioinsecticide, and synthetic insecticide tested in this study showed similar pathogenicity against *A. fabae*. The isolates still need further evaluations under field conditions before recommending them in biological control of *A. fabae*.

Acknowledgements
I would like to thank Assoc. Prof. Dr. Ismail Erper for his help in collecting, reproducing, and applying the isolates.

Funding
No funding

Availability of data and materials
All data are available at the end of the article, and the materials used in this work are of high quality and grade.

Authors' contributions
IS designed the study, supervised the work, wrote the manuscript, carried out the experiments, and analyzed the data. The author read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The author declares that he has no competing interests.

Publisher's Note

Received: 9 July 2018 Accepted: 31 October 2018
Published online: 20 November 2018

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