

The effect of entomopathogenic fungus, *Beauveria bassiana* on life table parameters and behavioural response of *Aphis gossypii*

Maryam RASHKI¹, Asghar SHIRVANI²

¹Department of Ecology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

²Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

The melon aphid, *Aphis gossypii* Glover is a severe phytophagous pest with worldwide distribution. This study examined effects of *Beauveria bassiana* strain DEBI008 (Balsamo) Vuillemin on fitness of the melon aphid using leaf disc as experimental unit and its ability to detect and transmit the fungus to uninfected colonies in microcosm conditions. The results of the bioassay revealed that higher fungal concentrations of *B. bassiana* strain DEBI008 significantly influenced all life table parameters. In addition, by increasing conidial concentration, the l_x and m_x values decreased. Choice experiment indicated that *B. bassiana* strain DEBI008 had effect on host-plant selection of *A. gossypii*. The mean number of the melon aphid regained from damaged cucumber plant was significantly lower than undamaged one. It subsequently caused higher mean number of aphid on undamaged plant in presence of *B. bassiana*-sporulating cadavers (7.75 ± 1.11 aphids) compared to damaged plant with *B. bassiana*-sporulating cadavers. The present study demonstrated that the melon aphid avoided cucumber plants containing *B. bassiana* and preferred intact plants without the fungus. The foraging *A. gossypii* slightly resulted in disseminating *B. bassiana* among the melon aphid colonies. During foraging and colonization, the cucumber plant conditions (damaged or undamaged plants) did not significantly influenced the number of sporulating cadavers. Our results clarified the high potential of the pathogenic fungus, *B. bassiana* strain DEBI008, to use in IPM programs as an efficient biocontrol agent against the important aphid pest, *A. gossypii* and its progeny, either by reducing the melon aphid fitness or by repelling the aphid.

Key words: *Aphis gossypii*, behavioural response, entomopathogenic fungus, life table parameters.

Introduction

The melon aphid, *Aphis gossypii* Glover (Rhynchotha Aphididae) is a severe phytophagous pest with worldwide distribution. The aphid pest causes two kinds of damage: direct damage by sucking sap of its host plant including Cucurbitaceae, Rutaceae and Malvaceae and producing honeydew; indirect damage by transmitting more than 50 plant viruses such as Cucumber Mosaic Virus (CMV) in fields and greenhouses (Ebert and Cartwright, 1997; Blackman and Eastop, 2000). There exist several findings of resistance to chemical insecticides in *A. gossypii* (Li and Han, 2004; Herron and Wilson, 2011; Shi *et al.*, 2012). By growing indiscriminate resistance in the aphid, application of the entomopathogenic fungi promotes biological control programs.

Entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota Hypocreales), has a wide host range and is being commercially used to control aphids (Inglis *et al.*, 2001). All isolates of entomopathogens examined in the laboratory triggered high mortality on *A. gossypii* Glover (Herlinda, 2010) and also it was shown that the aphid was more susceptible to *B. bassiana* strain J57 than *Myzus persicae* (Sulzer) (Rhynchotha Aphididae) according to LT_{50} values (Vu *et al.*, 2007). Both studies indicated effectiveness of the fungus for controlling the melon aphid population. Other studies showed that *B. bassiana* (Gurulingappa *et al.*, 2011) and *Lecanicillium attenuatum* CS625 (Zimmermann) Zare *et al.* W. Gams, directly and indirectly had negative impacts on *A. gossypii* and decreased its fitness (Kim, 2007). To study interactions between entomopathogens and in-

sects, behavioural response of host insects should be taken into account (Baverstock *et al.*, 2010). Few reports about the effects of *B. bassiana* on life table data and behaviour of target pest exist (e.g. Baverstock *et al.*, 2006; Chouvenec *et al.*, 2008).

Because the melon aphid is a common and economically important pest in Iran, more research on the native entomopathogen *B. bassiana* strain DEBI008 as a biocontrol agent is essential, especially in context of its effects on fitness of the aphid pest. Therefore, the impact of fungal infection on the life table parameters of *A. gossypii* was investigated. Also, this study examined the ability of melon aphid to detect the fungus, because the aphid was anticipated to reduce its contact with conidia. In addition, we assessed if *A. gossypii* was able to transmit the fungus to uninfected colonies.

Materials and methods

Plant and insect rearing

The colonies of *A. gossypii*, were reared on cucumber plants var. Dominus in plastic pots (15 cm diameter, 12 cm height) in a controlled environment room (25 ± 1 °C, $70 \pm 10\%$ RH, 16L: 8D). *A. gossypii* were collected from a field of cucurbit cultures in Kerman.

Fungus culture and sporulating aphid cadaver preparation

The native fungus, *B. bassiana* strain DEBI008 was isolated from *Chorthippus brunneus* Tunberg (Orthoptera Acrididae). Before culturing the fungus on Sa-

bouraud dextrose agar with yeast extract (SDAY), it was passed through *A. gossypii* and then incubated 14 days at 25 ± 1 °C. According to Hansen and Steenberg (2007), the harvested conidia were kept at 4 °C. To ascertain the viability of the conidia, four plates of SDAY with conidial suspension were preserved for 15 h at 25 ± 1 °C. The fungal conidia were completely germinated (100%). To provide sporulating aphid cadavers, third-instar *A. gossypii* were dipped for 6 seconds in fungal suspension then transferred on cucumber leaves fitted in 2% water agar and were incubated at 25 ± 1 °C, 85% RH (16L: 8D) till they died. Aphid cadavers were put on wet filter paper in Petri dishes (58 mm diameter) until the fungus sporulated.

Effect of *B. bassiana* on life table parameters of *A. gossypii*

To assess the effect of fungal infection on life table parameters of the melon aphid, thirty day-1 adult *A. gossypii* were sprayed with 0.02% Tween 80 (a non-ionic surfactant and emulsifier) as control, sub-lethal concentration (LC_{10} equal to 5.6×10^2 conidia/ml), 1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 and 1×10^8 conidia/ml of *B. bassiana* in 0.02% Tween 80 (1 ml per spray application). A fine mist held above the aphids with 90° angle was used (Groszek, Kwazar, Jaktorow, Poland, <http://www.kwazar.com.pl>). The infected day-1 adults were retained individually in reversed Petri dish (58 mm diameter) containing a thirty-day-old cucumber leaf that matched the Petri dish and fixed with the abaxial side up in 2% water-agar. After 24 h, one melon aphid nymph dishes was put in a new inverted Petri dish with a hole (2 cm diameter) on its lid covered with a fine mesh screen for ventilation and maintained at 25 ± 1 °C, 85% RH (16L: 8D). The nymphs were daily monitored till they developed to adulthood. Any infected nymph with *B. bassiana* was removed. The new adults initiated to produce offspring and the number of nymphs was registered every day until the adults died. The original infected adults of *A. gossypii* were checked to confirm fungus infection with *B. bassiana* when died. The uninfected replicates were not calculated.

Choice experiment

To conduct the test, plastic boxes (40 × 40 × 50 cm) were used containing two 30-day old cucumber plants (whole plants), separately planted in pots (7 cm diameter, 8 cm height) and put with 20 cm distance. A plastic sheet connected the base of one plant to the other in a surface. To determine whether aphid-induced cucumber plant volatiles influenced host-plant selection, forty aphids of different instars were established on each cucumber plants for three days and then removed prior to experiments (Girling *et al.*, 2006) in each box. These plants were in 'damaged' condition. The experiment was repeated with undamaged plants. *B. bassiana*-sporulating cadavers on water-agar discs (1 cm diameter) were exploited when fungus was needed. Five water-agar discs, each containing two fungus-sporulating cadavers, were randomly placed on plant surface of either left-hand or right hand 30-day old cucumber plants to avoid spatial influence in each box.

Then, 30, third-instar aphids were settled on the plastic sheet between two cucumber plants. After 24 h, the number of melon aphids on each plant was counted. The test was repeated four times at 25 ± 1 °C, 85% RH (16L: 8D).

Assessment of *B. bassiana* transmission during plant colonization by *A. gossypii*

To estimate the fungus dissemination during cucumber plant colonization, two plastic boxes (40 × 40 × 50 cm) including four 30-day old cucumber plants were arranged. The experiment was carried out with damaged, prepared according to previous test, and undamaged plants. Five water-agar discs described in preceding test were applied and added to four plants in both boxes. Fifteen third-instar *A. gossypii* were positioned at the base of each cucumber plant. After 5 days, the number of *B. bassiana*-sporulating cadavers and alive aphids were recorded. Each treatment was done four times.

Experiment analysis

The intrinsic rate of increase (r_m) and other life table parameters of the melon aphid were computed using the following equations (Carey, 1993):

$$\begin{aligned} r_m &= \ln R_0/T, \\ R_0 &= \sum l_x m_x, \\ \lambda &= \exp(r_m), \\ T &= \ln(R_0)/r_m, \\ r_w &= (e^{r_m})^7, \end{aligned}$$

where l_x is the number of survivorship at the age time x , and m_x is the number of aphids produced per aphid per day. The R_0 (net reproductive rate) is the mean number of aphid produced by an aphid during one generation. T , λ , r_w and DT parameters are mean generation time, finite rate of increase, increase rate in one week and doubling time, respectively. All life table parameters data were subjected to analyses of variance (ANOVA) and the averages were compared with Tukey test at the 0.05 level. The Statistical Analysis System (SAS, 1989) was used for calculation.

To analyze the data related to the effect of *B. bassiana* on host-plant selection and fungus transmission by *A. gossypii*, χ^2 test was used.

Results

Effect of *B. bassiana* on life table parameters of *A. gossypii*

The results of the bioassay revealed that higher fungal concentrations of *B. bassiana* strain DEBI008 influenced the intrinsic rate of increase of the melon aphid (r_m) ($F = 59.79$; $df = 6, 203$; $P = 0.0001$) (table 1). There was no significant difference in the intrinsic rate of increase values among control (0.02% Tween 80 only) and lower concentrations containing sub-lethal concentration (5.6×10^2 conidia/ml), 1×10^4 and 1×10^5 conidia/ml ($P > 0.05$). The r_m values significantly varied among control and higher concentrations including 10^6 , 10^7 and 10^8 conidia/ml but the difference in the r_m values between 10^7 and 10^8 conidia/ml was not significant ($P > 0.05$).

The results showed the same process for the r_w values in different conidial concentrations and control ($F = 118.25$;

Table 1. The effect of different treatments of *B. bassiana* on the life table parameters (Mean \pm SEM) of *A. gossypii*.

Treatments (conidia/ml)	r_m (♀/♀/day)	R_0 (♀/♀/generation)	λ (day)	T (day)	DT (day)	r_w (♀/♀/week)
Tween-80	0.469 (\pm 0.015 a)	78.75 (\pm 2.56 a)	1.608 (\pm 0.024 a)	9.29 (\pm 0.30 c)	1.45 (\pm 0.05 c)	11.18 (\pm 0.17 a)
5.6×10^2	0.462 (\pm 0.005 a)	77.56 (\pm 0.76 a)	1.576 (\pm 0.008 a)	9.43 (\pm 0.10 c)	1.50 (\pm 0.02 c)	11.10 (\pm 0.06 a)
1×10^4	0.455 (\pm 0.010 a)	74.94 (\pm 0.38 a)	1.584 (\pm 0.014 a)	9.48 (\pm 0.19 c)	1.50 (\pm 0.04 c)	11.00 (\pm 0.11 a)
1×10^5	0.446 (\pm 0.005 a)	69.96 (\pm 0.35 b)	1.560 (\pm 0.008 a)	9.57 (\pm 0.31 c)	1.56 (\pm 0.05 c)	10.92 (\pm 0.18 a)
1×10^6	0.400 (\pm 0.004 b)	68.15 (\pm 0.30 b)	1.482 (\pm 0.005 b)	10.58 (\pm 0.12 b)	1.73 (\pm 0.02 b)	10.42 (\pm 0.05 b)
1×10^7	0.341 (\pm 0.005 c)	54.14 (\pm 0.32 c)	1.418 (\pm 0.007 c)	11.74 (\pm 0.16 a)	2.04 (\pm 0.03 a)	9.82 (\pm 0.03 c)
1×10^8	0.323 (\pm 0.004 c)	50.03 (\pm 0.26 c)	1.383 (\pm 0.006 c)	12.20 (\pm 0.17 a)	2.03 (\pm 0.26 a)	9.64 (\pm 0.03 c)

Means followed by different letters in the same column are significantly different (Tukey test, $P < 0.05$). r_m : intrinsic rate of increase; R_0 : net reproductive rate; λ : finite rate of increase; T : mean generation time; DT : doubling time; r_w : increase rate in one week.

df = 6, 203; $P = 0.0001$). The fungal treatment differently influenced the net reproductive rate (R_0) ($F = 728.11$; df = 6, 203; $P = 0.0001$) (table 1). By increasing conidial concentration, the R_0 value significantly decreased.

The mean generation time (T) ($F = 793.45$; df = 6, 203; $P = 0.0001$) and the doubling time (DT) ($F = 829.76$; df = 6, 203; $P = 0.0001$) raised when the conidial concentration escalated (table 1). Nevertheless, calculated values of the T and DT were not significantly different between 5.6×10^2 , 1×10^4 , 1×10^5 and control ($P > 0.05$). The finite rate of increase (λ) ($F = 743.13$; df = 6, 203; $P = 0.0001$) conversely changed in relation to conidial concentration. The number of aphids surviving at time x (l_x) and number of offspring produced per aphid per day (m_x) affected by different treatments and 0.02 % Tween 80 as control are shown in figure 1. The fluctuations of the l_x and the m_x were almost similar between control, sub-lethal concentration and 1×10^4 conidia/ml of *B. bassiana* and the l_x and m_x values of 1×10^5 and 1×10^6 conidia/ml nearly had similar changes. The high conidial concentrations containing 1×10^7 and 1×10^8 conidia/ml led to decline the l_x and the m_x values. As the results declared elevation of conidial concentration made the l_x and m_x values decrease.

Choice experiment

The mean number of melon aphid regained from damaged cucumber plant was significantly lower than undamaged plant ($\chi^2 = 5.10$; df = 1; $P < 0.05$) (figure 2A). The mean number of 17.50 ± 1.04 and 24.50 ± 0.50 melon aphids regained from damaged and undamaged cucumber plant, respectively. It subsequently caused higher mean number of aphid on undamaged plant in presence of *B. bassiana*-sporulating cadavers (7.75 ± 1.11 aphids) compared to damaged one with *B. bassiana*-sporulating cadavers (4.50 ± 0.50 aphids) ($\chi^2 = 4.41$; df = 1; $P < 0.05$). The melon aphids were considerably attracted to undamaged plant without the entomopathogenic fungus with a mean number of 16.75 ± 1.10 in compared to undamaged plant containing the entomopa-

thogenic fungus with a mean number of 7.75 ± 1.11 ($\chi^2 = 15.48$; df = 1; $P < 0.05$) (figure 2B). The mean number of melon aphid regained from damaged cucumber plants significantly varied between treatment including *B. bassiana*-sporulating cadavers (4.50 ± 0.50 aphids) and treatment excluding *B. bassiana*-sporulating cadavers (13.00 ± 1.00 aphids) ($\chi^2 = 17.30$; df = 1; $P < 0.05$).

Assessment of *B. bassiana* transmission during plant colonization by *A. gossypii*

The foraging *A. gossypii* slightly resulted in disseminating *B. bassiana* among the melon aphid colonies with a mean number of 3.37 ± 0.8 sporulating cadavers regained during colonization (figure 3). During foraging and colonization, the cucumber plant conditions (damaged or undamaged plants) did not significantly influenced the number of sporulating cadavers ($\chi^2 = 0.02$; df = 1; $P = 0.906$). The mean number of the sporulating cadavers regained from damaged and undamaged plants was 2.50 ± 0.28 and 3.50 ± 0.60 cadavers, respectively.

Discussion

There is not enough information on sub-lethal and chronic impacts of entomopathogenic fungi on the life table parameters of aphid. In addition, several evidences stated the efficiency of entomopathogenic fungi that affected the melon aphid's reproduction numerically not physiologically (Kim, 2007; Pampapathy *et al.*, 2010; Gurulingappa *et al.*, 2011).

Our results indicated that *B. bassiana* strain DEBI008 had no effects on life table parameters of *A. gossypii* when the conidial concentration was less than 1×10^6 conidia/ml. On the other hand, the entomopathogenic fungal strain had no sub-lethal influences on the melon aphid as well. If the aphids were exposed continuously to sub-lethal concentration of the fungus, the progeny would be possibly affected. Similar to Fatiha *et al.* (2008)

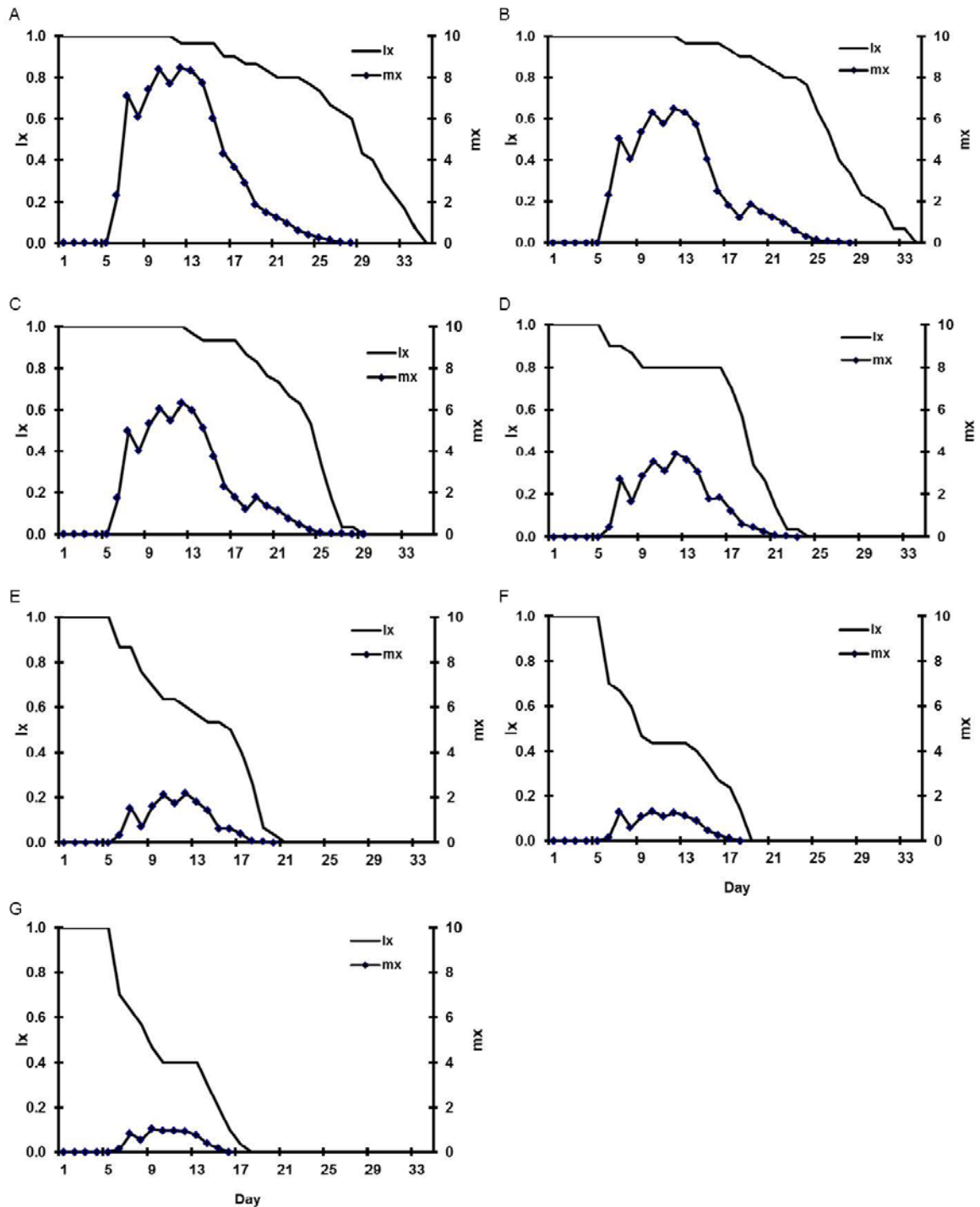


Figure 1. Survival rate (l_x) and number of females produced per female per day (m_x) of *A. gossypii* treated with different concentrations of *B. bassiana* compared to control. (A) 0.02% Tween 80; (B) 5.6×10^2 conidia/ml; (C) 10^4 conidia/ml; (D) 10^5 conidia/ml; (E) 10^6 conidia/ml; (F) 10^7 conidia/ml; (G) 10^8 conidia/ml.

concluded that of *L. lecanii*, did not impact the biological characteristics of the *Serangium japonicum* Chapin (Coleoptera Coccinellidae), a whiteflies predator.

The high concentrations significantly changed the r_m values with respect to control but the r_m values did not differ between 1×10^7 and 1×10^8 conidia/ml. The melon aphid somehow showed resistance to increased fungal inoculums, especially up to 1×10^6 conidia/ml. Scarborough *et al.* (2005) discovered that symbionts of aphids protected them against entomopathogenic fungi

and reduced fungus sporulation and transmission. Hence, the presence of such endosymbionts and their function against pathogens in *A. gossypii* need to be investigated. Despite our results, Baverstock *et al.* (2006) reported that the r_m value of pea aphid, *Acyrtosiphon pisum* (Harris) (Rhynchota Aphididae) was not affected by either *Pandora neoaphidis* (Remaudiere and Hennebert) Humber or *B. bassiana*, however, the number of nymphs produced was lower in treated pea aphids than untreated ones.

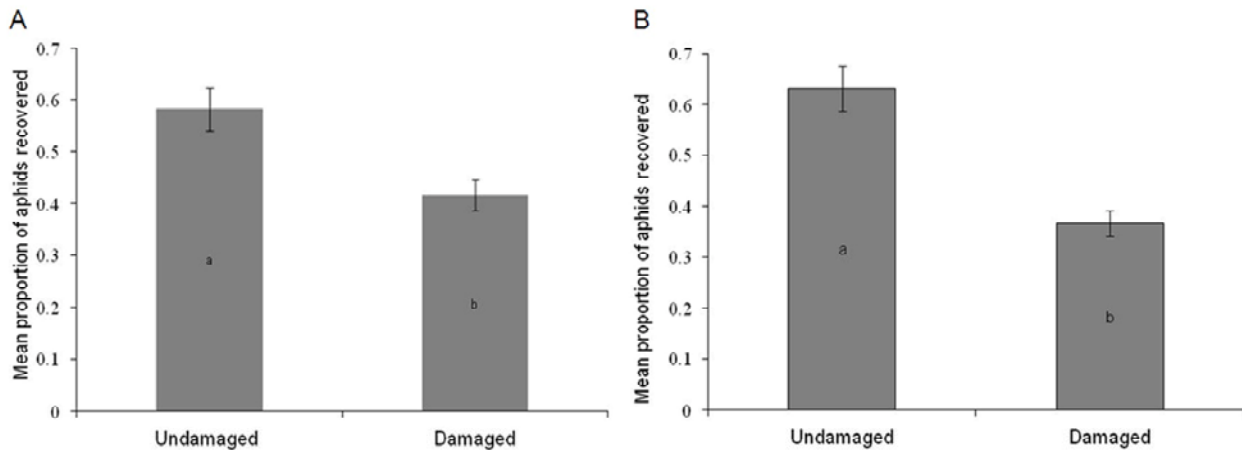


Figure 2. Proportion of *A. gossypii* regained from (A) the all cucumber plants and (B) the cucumber plants containing *B. bassiana*-sporulating cadavers only. The plants were either undamaged or previously infested with *A. gossypii*.

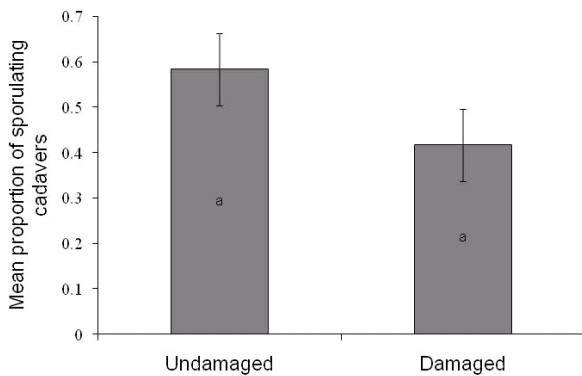


Figure 3. Proportion of *B. bassiana*-sporulating cadavers regained after colonization on undamaged or damaged cucumber plants previously infested with *A. gossypii*.

The net reproductive rate (R_0) of the melon aphid varied over the different conidial concentrations as well as the intrinsic rate of increase. Hence, the lower values of the R_0 belonged to 1×10^7 and 1×10^8 conidia/ml. Vu *et al.* (2007) found that infection with different conidial concentrations of *L. lecanii* strain 41185 had indirect effects on the fitness of the *M. persicae* and the R_0 values reduced when the conidial concentration elevated. Also, because of no difference between 1×10^7 and 1×10^8 conidia/ml in the R_0 values, they recommended to apply 1×10^7 conidia/ml for suppression the peach aphid. Those findings are also similar to data were achieved for treated *A. gossypii* with different concentrations of *B. bassiana* in present study. Accordingly, the melon aphids treated with 1×10^7 and 1×10^8 conidia/ml produced the lower r_m , R_0 , λ and the higher T and DT values without significant difference. Thus, it is suggested to spray 1×10^7 conidia/ml of *B. bassiana* strain DEBI008 to control *A. gossypii*.

Determining responses of insect pests and non-target arthropods including recognition and avoidance of fungal pathogens has a key role in success of biocontrol program. As previously described by Baverstock *et al.* (2005), *A. pisum* moved towards and colonized bean plants with *P. neoaphidis* and aphid-induced plant vola-

tiles had no effect on aphid infection. The present study demonstrated that the melon aphid avoided cucumber plants containing *B. bassiana* and preferred intact plants without the fungus. It has been proved that some filamentous fungi such as *B. bassiana* release volatiles (Sunesson *et al.*, 1995; Sunesson *et al.*, 1996; Crespoa *et al.*, 2008). Thus, it was concluded that *A. gossypii* was able to distinguish the volatile released from *B. bassiana*-sporulating cadavers. Roy *et al.* (2006) mentioned to the relationship existed between the fungus, *B. bassiana*, wide host array and host inhibition of the pathogen. Previously, Mburu *et al.* (2009) examined the repellency effect of different isolates of *Metarhizium anisopliae* and *B. bassiana* on the termite *Macrotermes michaelseni* Sjolstedt (Isoptera Macrotermes) and reported the termite avoidance of virulent isolates.

There are evidences of predators and parasitoids avoiding entomopathogenic fungi such as foraging predator *Anthocoris nemorum* L. (Rhynchota Anthocoridae) recognized *B. bassiana* and prevented contact with the fungal inoculants (Meyling and Pell, 2006), also the frequency of parasitoid attacks reduced when parasitisation of host aphids occurred 3 days after fungal infection (Brobyn *et al.*, 1988). Similarly, the whitefly parasitoid, *Encarsia formosa* Gahan (Hymenoptera Aphelinidae) had the ability of distinguishing the pathogenic fungus at late stage of fungal host infection (Fransen and van Lenteren, 1993).

Moreover, our results are in coincidence with findings which imply that semiochemicals produced by infested plants inhibited herbivores from host plant colonization (Khan *et al.*, 2008). Besides, presence of the fungus volatiles did not mask the plant semiochemicals, consequently the melon aphid chose intact plant to colonize. In spite of the repellency of *B. bassiana*, interestingly this response can be effective on the melon aphid control, as it was elucidated by other studies to control crucial pests (Villani *et al.*, 1994; Thompson and Brandenburg, 2005; Sun *et al.*, 2008).

It is also apparent from the analyzed data that dispersal or transmission of the pathogenic fungus, *B. bassiana*, by the foraging *A. gossypii* happened. Because the

fungus repelled the melon aphid, the transmission was partly occurred and the cucumber plant conditions had no impact on distribution of the entomopathogenic fungus. Although, fungal traits like virulence, spore production and temperature can also change the fungal transmission (Sun *et al.*, 2003). As depicted by Hussain *et al.* (2010), *Coptotermes formosanus* Shiraki (Isoptera Rhinotermitidae) was able to respond to the entomopathogenic fungi, while promotion of the fungal transmission depended on the conidial concentration acquired by the vector termites. It can be hypothesized that our result may therefore be due to, first, *B. bassiana* strain DEBI008 sufficiently sporulating and second, taking some times to detect the fungus by foraging aphids and obtaining even a few conidial inoculums from their surroundings at their first entrance that consequently resulting in transmitting the fungus into the aphid colony.

Very little research has explored the influence of entomopathogenic fungi on fitness of aphid pest. Our results clarified the high potential of the pathogenic fungus, *B. bassiana* strain DEBI008, to use in IPM programs as an efficient biocontrol agent against the important aphid pest, *A. gossypii* and its progeny, either by reducing the melon aphid fitness or by repelling the aphid. The effect and activity of the strain under greenhouse and field conditions require clarification.

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Authors' addresses: Maryam RASHKI (corresponding author: ma_rashkigh@yahoo.com), Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman 76315-117, Iran; Asghar SHIRVANI, Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

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