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Sublethal effects of *Metarhizium anisopliae* on life table parameters of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* larvae at different time intervals

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Abstract The ectoparasitoid wasp, *Habrobracon hebetor* Say and the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin are biocontrol agents attacking larval stages of *Helicoverpa armigera* (Hübner). Life table parameters of *H. hebetor* were studied on *H. armigera* third instar larvae previously (0, 24, 48, and 72 h) infected with a sublethal concentration (LC_{30}) of *M. anisopliae* (isolate M14). Fungal infection adversely affected life table parameters of *H. hebetor* depending on the host post-inoculation time for parasitoid release. The entropy values showed the age-specific survivorship (l_x) curves of type 1 at ≤24 h treatments. The highest and lowest intrinsic rates of increase (r_m) were 0.223 and 0.109 for control and 72 h treatment, respectively. Statistically different variations were observed for r_m values when post-exposure time was longer than 24 h. Our findings highlight appropriate introduction times of *H. hebetor* in combination with *M. anisopliae* (isolate M14) for successful integrated management of *H. armigera*.

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Introduction

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a highly polyphagous pest, annually causing economically important crop losses (Asokan et al. 2014). It has a wide range of potential host plants, reaching about 200 species including cotton, chickpea, pigeon pea, tomato, corn and sorghum (Surekha Devi et al. 2011). *Helicoverpa armigera* can severely reduce crop yields, mainly because it feeds on flowering and fruiting structures of the host plants (Avilla and González-Zamora 2010).

Chemical insecticides have been widely used to control this pest, but the repeated use of these insecticides has resulted in development of resistance, elimination of existing natural enemies in addition to causing environmental problems (Baskar and Ignacimuthu 2012). Therefore, considerable interest has been devoted to implementation of environmentally safe methods for integrated management of *H. armigera* with special emphasis on biological control (Sedaratian et al. 2013).

The entomopathogenic fungus, *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) and the braconid wasp, *Habrobracon hebetor* Say (Hymenoptera: Braconidae) are biocontrol agents

attacking *H. armigera* larvae (Fathipour and Sedaratian 2013). *Metarhizium anisopliae* is applied for biological control of pest insects with well documented efficacy (Nguyen et al. 2007) and commercial products are widely available (Zimmermann 2007). Besides, *H. hebetor* is a valuable ectoparasitoid of many lepidopteran pests and is mainly used in biocontrol programs of *H. armigera* (Chen et al. 2013).

The ecological balance in agriculture is very sensitive and may have been disrupted by different factors (Husberg and Hokkanen 2001). An increase in the richness of natural enemy species could culminate in the host population increase if interference between natural enemies is sufficient and as such would constitute an antagonistic interaction. The successful augmentation of these natural enemies may be impeded by this antagonism (Roy and Pell 2000). It has even been suggested that entomopathogenic fungi could be a reason for drastic reductions in the number of insect natural enemies (Husberg and Hokkanen 2001). It is therefore important to study the risks associated with the compatibility of biocontrol agents to screen for an isolate that would be aggressive towards the target pest, but reasonably safe to parasitoids.

Mortality is the most commonly measured parameter to determine the coexistence of natural enemies, whereas lethal and sublethal effects of entomopathogens on beneficial insects (predators and parasitoids) are worth evaluating (Fatiha et al. 2008). Although sublethal effects on life table parameters of parasitoid insects are poorly studied, these parameters are important for the success of an Integrated Pest Management (IPM) program (Abedi et al. 2014). Sublethal effects may be expressed as changes in lifespan of the insect such as in developmental rates, fecundity, sex ratio and behavior that can be measured by estimating the intrinsic rate of increase (r_m) value (Desneux et al. 2007).

Limited information is available on lethal and sublethal effects of entomopathogenic fungi against non-target organisms. Stolz et al. (2002) indicated that mycopesticides based on three isolates of *M. anisopliae* posed a low risk to parasitic hymenopterans *Apoanagyrus lopezi* (Hymenoptera: Encyrtidae) and *Phanerotoma* sp. (Hymenoptera: Braconidae) under field conditions. Rosa et al. (2000) studied the lethal effect of entomopathogenic fungi, *M. anisopliae* and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae), on the parasitoid wasp *Prorops nasuta* (Hymenoptera: Bethylidae). They reported that various isolates of these fungi have little negative impact on *P. nasuta* and can be used as a component, concurrently with this parasitoid. Nielsen et al. (2005) revealed that *M. anisopliae* and *Spalangia cameroni* (Hymenoptera: Pteromalidae) could be compatible under field conditions by direct contact methods. Rashki et al. (2009) demonstrated that *B. bassiana* had no adverse effect on biological parameters of the parasitoid wasp *Aphidius matricariae* (Hymenoptera: Braconidae) and can be successfully combined for biological control of *Myzus persicae* (Hemiptera: Aphididae). Tounou et al. (2003) studied the potential side effects of entomopathogenic fungi, *M. anisopliae* and *Paecilomyces fumosoroseus* (=*Isaria fumosorosea*) (Hypocreales: Trichocomaceae) on *Anagrus atomus* (Hymenoptera: Mymaridae). Husberg and Hokkanen (2001) reported direct and indirect effects of *M. anisopliae* on pollen beetle, *Meligethes aeneus* (F.) (Coleoptera: Nitidulidae) and its two larval endoparasitoids, *Phradis morionellus* (Holm.) (Hymenoptera: Ichneumonidae) and *Diospilus capito* Nees (Hymenoptera: Braconidae).

To our knowledge, there is no information on sublethal effects of entomopathogenic fungi against any ectoparasitoid, including *H. hebetor*. Furthermore, the compatible application of *M. anisopliae* and *H. hebetor* is essential for integrated management of *H. armigera*. Accordingly, the objective of this study was to assess the host suitability of *H. armigera* larvae infected with *M. anisopliae* (isolate M14) for growth and development of *H. hebetor*.

Materials and methods

Experimental conditions and insect rearing

Insect cultures and all experiments were carried out at 26 ± 1 °C, RH 70 ± 5 % and a photoperiod of L:D 8:16 h, under laboratory conditions. *Helicoverpa armigera* larvae were originally collected from cotton fields in Gorgan region of northeastern Iran in July 2013. The stock culture was initiated on an artificial diet described by Naseri et al. (2009) with following modifications: removal of Nipagin M and

formaldehyde. The insects were reared for three generations before the experiments were conducted. In order to prevent cannibalism, third instar larvae were kept individually in transparent plastic containers (3.5 cm diameter and 6 cm height) and maintained until pupation. Emerged adults (15 pairs) were transferred to oviposition jars (12 cm diameter and 21 cm height) with a 1:1 sex ratio and fed on 10 % honey solution. The open end of jars was covered with fine mesh net. Net pieces containing *H. armigera* eggs were collected and replaced daily.

Adults of *H. hebetor* were obtained from an insectarium maintained by Plant Protection Bureau of Mazandaran Province, Iran, in 2013. The wasps were reared on fifth instar larvae of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). Honey solution (10 %) was provided as food for adult wasps on strips of paper (5 × 30 mm). One-day-old *H. hebetor* females were used for all experiments.

Fungal pathogen

Metarhizium anisopliae isolate M14 (from soil: Garmsar-Iran) was used in this experiment and is preserved at Department of Agricultural Entomology, Iranian Research Institute of Plant Protection, Tehran, Iran. This isolate proved to be more effective than some others in preliminary bioassays (unpublished data). Subsequent to fungal passage through *H. armigera* larvae, it was cultured on Sabouraud Dextrose Agar (BBL, USA) with 1 % yeast extract (SDAY). After two weeks, conidia were scraped to make aqueous suspension with 0.02 % Tween-80. Conidia concentration was determined using a Neubauer hemocytometer (Weber Scientific International Ltd, UK).

Conidial viability was determined by inoculating plates of SDAY with stock suspension, which was then incubated for 24 h at 26 °C. Germination was considered positive when the length of germ tube was as long as the width of conidia (Inglis et al. 2012). The percentage germination of conidia was 96 %.

Host bioassay

Bioassays were also conducted to assess absolute virulence of M14. However, the focus in this research was on LC_{30} value, as a sublethal concentration. Third instar larvae of *H. armigera* were inoculated individually in fungal suspension (10^5, 10^6, 10^7, 10^8 and 10^9 conidia ml^{-1}) for 10 s. Control insects were immersed in sterile distilled water containing 0.02 % Tween-80. Treated larvae were allowed to crawl on a filter paper to remove excess moisture. To maintain humidity, insects were placed individually on filter paper (Whatman No. 1) which was moistened on the first and every other day with 1 ml distilled water, in a plastic Petri dish (6 cm diameter). A piece of artificial diet (1 cm^3) was added to each Petri dish as food source for the larva and replaced by a fresh one daily. Insect mortalities were assessed daily for ten days and dead insects were transferred to Petri dishes containing a piece of moistened filter paper to promote the growth of respective fungus. For each conidial concentration, 50 larvae were used and the entire experiment was repeated three times.

Life table parameters

Based on mortality data from bioassays, third instar larvae of *H. armigera* were exposed to sublethal concentration (LC_{30}) of *M. anisopliae* (the same as bioassays). Subsequent to time intervals 0, 24, 48 and 72 h post-exposure, treated larvae were individually transferred to transparent plastic containers (3.5 cm diameter and 6 cm height). One pair of parasitoids (male and female) were introduced to each container and allowed to parasitize the infected larva for 24 h. Subsequently, parasitoids were removed from containers and one *H. hebetor* egg was left on body surface of each larva. Fifty-four wasp eggs were used for each treatment and untreated control. Duration of different life stages and survival of each individual *H. hebetor* were recorded daily. The emerged parasitoids of F1 generation (varying from 41 adults in control to 26 in 72 h treatment) were used to evaluate the effect of fungus on life table parameters of *H. hebetor*. Adult wasps (male and female) were paired in containers and two untreated third instar larvae of *H. armigera* were supplied daily for wasp oviposition. The survival of individuals and fecundity of each female wasp were recorded daily for their whole lifespan. Parasitized larvae were kept under rearing conditions described above and the sex of emerged wasps (F2) was recorded.

Statistical analysis

Daily schedules of mortality and fecundity were integrated into a life table format (Carey 1993) and

used to calculate net reproductive rate (R_0), intrinsic rate of increase (r_m), finite rate of increase (λ), mean generation time (T) and doubling time (DT) values. For estimation of pseudo-values of these parameters jackknife procedure was used (Maia et al. 2000). The obtained data were subjected to an analysis of variance (ANOVA). In addition, sublethal effects of *M. anisopliae* on duration of different pre-imaginal stages, adult fecundity and longevity were analyzed with one-way ANOVA and mean separation was performed at a 5 % level of significance by Student Newman-Keuls (SNK) test (SAS 2003).

Results

The LC_{50} and LC_{30} values of *M. anisopliae* (isolate M14) on third instar larvae of *H. armigera* were 1×10^7 ($CI_{95\ \%} = 5.24 \times 10^6$–$2.57 \times 10^7$) conidia ml^{-1} and 2×10^6 ($CI_{95\ \%} = 7.41 \times 10^5$–$4.70 \times 10^6$) conidia ml^{-1}, respectively, whereas no mortality was recorded in control insects.

Developmental time for different life stages and fecundity

Developmental durations for life stages and fecundity of *H. hebetor* on *H. armigera* treated larvae at different time intervals are shown in Tables 1 and 2. The shortest developmental period for immature life stages was observed in the control, whereas the longest development occurred in parasitoids exposed to larvae, 72 h post-infection.

There were significant differences in duration of incubation period ($F = 2.59$; $df = 4, 158$; $P = 0.04$), larval stages ($F = 5.59$; $df = 4, 158$; $P < 0.0001$), pupal period ($F = 13.99$; $df = 4, 158$; $P < 0.0001$) and total immature stages ($F = 23.42$; $df = 4, 158$; $P < 0.0001$). Furthermore, significant differences were observed in the longevity of males ($F = 6.83$; $df = 4, 83$; $P < 0.0001$) and females ($F = 9.32$; $df = 4, 70$; $P < 0.0001$). Pre-oviposition ($F = 14.31$; $df = 4, 70$; $P < 0.0001$) and oviposition ($F = 15.01$; $df = 4, 70$; $P < 0.0001$) periods, daily ($F = 103.53$; $df = 4, 70$; $P < 0.0001$) and total ($F = 125.82$; $df = 4, 70$; $P < 0.0001$) fecundity of *H. hebetor* were also significantly affected. However, sublethal concentration of fungal isolate had no significant effect on post-oviposition period ($F = 0.31$; $df = 4, 70$; $P = 0.87$).

Life table parameters

Population growth parameters of *H. hebetor* in different treatments are listed in Table 3. Net reproductive rate (R_0) of *H. hebetor* was significantly affected by different treatments ($F = 344.30$; $df = 4, 70$; $P < 0.0001$). In comparison with control insects, no significant differences were observed in intrinsic rate of increase (r_m) ($F = 289.63$; $df = 4, 70$; $P < 0.0001$) and finite rate of increase (λ) ($F = 281.42$; $df = 4, 70$; $P < 0.0001$), when larvae were exposed to parasitoids immediately after fungal infection. However, there were statistically considerable variations among r_m of three other treatments (24, 48 and 72 h). Also, this was the case for λ.

Table 1 Mean (±SE) different life stages, total immature and adult longevity durations of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* third instar larvae treated with sublethal concentration (LC_{30}) of *Metarhizium anisopliae* at different time intervals (0, 24, 48 and 72 h)

Parameter	Stage or sex	Treatments				
		Control	0 h	24 h	48 h	72 h
Developmental time (day)	Egg	1.49 ± 0.08b	1.52 ± 0.08ab	1.59 ± 0.09ab	1.75 ± 0.11ab	1.86 ± 0.12a
	Larva	2.54 ± 0.09b	2.62 ± 0.09b	2.78 ± 0.11b	2.96 ± 0.15ab	3.27 ± 0.16a
	Pupa	8.32 ± 0.08c	8.50 ± 0.11c	8.69 ± 0.17bc	9.04 ± 0.17b	9.68 ± 0.15a
Total immature (day)		12.34 ± 0.13d	12.65 ± 0.15 cd	13.06 ± 0.19c	13.75 ± 0.23b	14.82 ± 0.31a
Longevity (day)	Female	18.85 ± 0.30a	18.11 ± 0.32ab	17.27 ± 0.42bc	16.33 ± 0.54 cd	15.67 ± 0.58d
	Male	17.52 ± 0.27a	17.05 ± 0.33ab	16.06 ± 0.41bc	15.69 ± 0.51bc	14.77 ± 0.62c

Means followed by the same letter in the same row are not significantly different (SNK test, $P < 0.05$)

Table 2 Pre-oviposition, oviposition and post-oviposition periods and fecundity (mean ± SE) of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* third instar larvae treated with sublethal concentration (LC_{30}) of *Metarhizium anisopliae* at different time intervals (0, 24, 48 and 72 h)

Parameter	Treatments				
	Control	0 h	24 h	48 h	72 h
Pre-oviposition (day)	0.00 ± 0.00c	0.11 ± 0.07c	0.27 ± 0.12c	0.67 ± 0.14b	1.11 ± 0.26a
Oviposition (day)	17.55 ± 0.34a	16.74 ± 0.42ab	15.53 ± 0.58b	14.08 ± 0.45c	12.89 ± 0.51c
Post-oviposition (day)	1.30 ± 0.21a	1.32 ± 0.25a	1.47 ± 0.27a	1.58 ± 0.31a	1.67 ± 0.41a
Fecundity-daily (eggs)	7.01 ± 0.13a	6.85 ± 0.15a	5.80 ± 0.17b	3.90 ± 0.19c	2.90 ± 0.23d
Fecundity-total (eggs)	131.75 ± 2.23a	123.58 ± 2.73a	100.13 ± 3.47b	63.67 ± 3.66c	45.67 ± 4.39d

Means followed by the same letter in the same row are not significantly different (SNK test, $P < 0.05$)

Table 3 Life table parameters (mean ± SE) of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* third instar larvae treated with sublethal concentration (LC_{30}) of *Metarhizium anisopliae* at different time intervals (0, 24, 48 and 72 h)

Parameter	Treatments				
	Control	0 h	24 h	48 h	72 h
Net reproductive rate (R_0)	49.063 ± 0.831a	43.895 ± 0.969b	27.767 ± 0.963c	14.235 ± 0.818d	7.677 ± 0.734e
Intrinsic rate of increase (r_m) (day^{-1})	0.223 ± 0.002a	0.221 ± 0.002a	0.187 ± 0.001b	0.150 ± 0.003c	0.109 ± 0.004d
Finite rate of increase (λ) (day^{-1})	1.250 ± 0.003a	1.248 ± 0.003a	1.205 ± 0.002b	1.161 ± 0.004c	1.115 ± 0.005d
Mean generation time (T) (day)	17.098 ± 0.197b	17.483 ± 0.214b	17.748 ± 0.156b	17.817 ± 0.293b	18.767 ± 0.567a
Doubling time (DT) (day)	3.113 ± 0.033d	3.134 ± 0.034d	3.715 ± 0.028c	4.628 ± 0.099b	6.358 ± 0.256a

Means followed by the same letter in the same row are not significantly different (SNK test, $P < 0.05$)

Sublethal concentration of *M. anisopliae* (isolate M14) had no significant effect on mean generation time (T) ($F = 4.52$; $df = 4, 70$; $P = 0.003$) except when the larvae were exposed to parasitoids 72 h after fungal infection. Furthermore, doubling time (DT) ($F = 212.44$; $df = 4, 70$; $P < 0.0001$) of the population showed significant increases after sublethal fungal inoculation at different treatments (24, 48 and 72 h). No difference was observed in DT when parasitoids were introduced to host larvae immediately after fungal infection, compared with the control.

Age-specific survivorship (l_x) and fecundity (m_x)

The l_x and m_x curves are shown in Fig. 1. The highest and lowest survivorships of immature stages were 0.76 and 0.41 % for control and 72 h treatment, respectively. In order to determine the type of survivorship curves, entropy was used as a criterion. The entropy values were 0.27, 0.30, 0.47, 0.57 and 0.69 for control and other treatments (0, 24, 48 and 72 h), respectively. The entropy value less and more than 0.5 shows the survivorship curves near to type 1 and 3, respectively (Carey 2001).

Life expectancy (e_x)

The life expectancy (e_x) values of a newborn female wasp were 24.09, 23.52, 18.70, 16.04 and 13.55 days for control and other treatments (0, 24, 48 and 72 h), respectively. The e_x trend of *H. hebetor* tends to decay over time at different treatments (Fig. 2). The peaks could be observed during the first five days of *H. hebetor* lifespan which could be due to the passage of individuals through crucial life stages.

Discussion

Insects can become co-infected by a combination of parasitoid and pathogen, e.g. parasitoids may prey on pathogen infected hosts (Goettel et al. 2010).

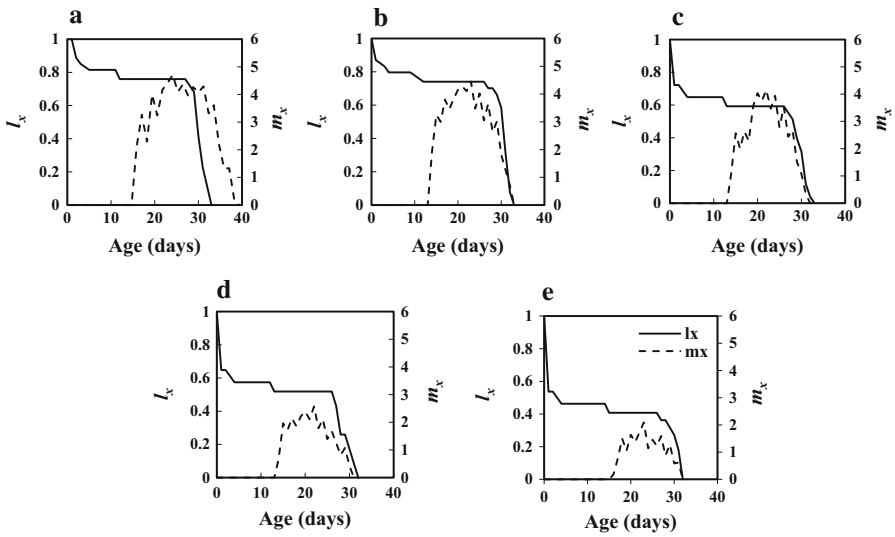

Fig. 1 Age-specific survivorship (l_x) and fecundity (m_x) of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* larvae treated with sublethal concentration (LC_{30}) of *Metarhizium anisopliae* at different time intervals. **a** Control, **b** 0 h, **c** 24 h, **d** 48 h and **e** 72 h

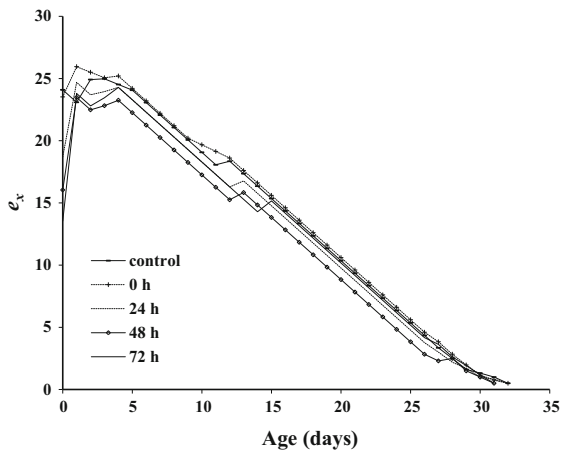

Fig. 2 Life expectancy (e_x) of *Habrobracon hebetor* parasitizing *Helicoverpa armigera* larvae treated with sublethal concentration (LC_{30}) of *Metarhizium anisopliae* at different time intervals (0, 24, 48 and 72 h)

Although, for the most part, this interaction is positive (Roy and Pell 2000), the outcome can be either nil, antagonistic, additive or synergistic (Goettel et al. 2010), depending on the competing conditions (especially precedence) for host resources. In addition to mortality as a direct effect of entomopathogenic fungus, decreased fecundity of the parasitoids may occur as a sublethal effect (Nielsen et al. 2005).

In the present study, we investigated biological parameters of *H. hebetor* parasitizing *H. armigera* larvae infected with a sublethal concentration (LC_{30}) of *M. anisopliae* (isolate M14) at different time intervals. Moreover, we have studied demographic parameters for the first time to evaluate sublethal effects of a fungal entomopathogen on the biological performance of *H. hebetor*. Such information could be helpful to determine the compatibility of this ectoparasitoid with *M. anisopliae* in IPM programs of *H. armigera*.

The results revealed that infection of *H. armigera* larvae to LC_{30} of *M. anisopliae* affected the biological performance of its parasitoid, *H. hebetor*. Additionally, the total fecundity and survival of the parasitoid wasp was affected by different treatments. At the longest post-exposure time (72 h), daily and total fecundity of female parasitoids were significantly less than in other treatments.

Additionally, the age-specific survivorship (l_x) of *H. hebetor* was negatively affected by sublethal concentration of *M. anisopliae* (isolate M14). Based on survival curves, the parasitoid showed higher mortality rates at early stages of extended post-inoculation times (48 and 72 h). Similar to our results, survival of *S. cameroni* was significantly reduced by the highest concentration of *M. anisopliae* (Nielsen et al. 2005). Conversely, *Verticillium* (=*Lecanicillium*) *lecanii* (Zimm.) Viegas (Hypocreales: Clavicipitaceae) was found to have no adverse effect on survival of *Serangium japonicum* (Coleoptera: Coccinellidae) larvae (Fatiha et al. 2008). The decrease in the survivorship of wasp F1 generation suggested that

M. anisopliae outcompetes parasitoid population growth as time intervals between fungal inoculation and parasitism increased. A possible reason is that fungal pathogen may reduce the host quality for parasitoid larvae (Rashki et al. 2009). Previously, competition within the host have necessitated parasitoid oviposition immediately after fungal infection in order to outcompete the pathogen (Baverstock et al. 2009). In this study, fecundity of female wasps decreased with increasing time interval between host fungal treatment and parasitoid encounter. The observed decrease in fertilization rate may be due to the negative changes in physiological status of female parasitoid related to fungal infection (Roy and Pell 2000). Moreover, female parasitoid preference for treated or untreated larvae is another possible reason for lower fecundity of *H. hebetor* (Mesquita and Lacey 2001).

Additionally, sublethal concentration of *M. anisopliae* (isolate M14) prolonged developmental durations of *H. hebetor* (immature stages) depending on time interval between fungal inoculation and parasitoid release. Similarly, Rashki et al. (2009) reported that pupal stage of *A. matricariae* was extended especially in female parasitoids. In contrast, Fatiha et al. (2008) revealed that *V. lecanii* had no sublethal effects on developmental biology of *S. japonicum* surviving after direct inoculation by entomopathogenic fungus.

Above all, demographic toxicology has been suggested to evaluate all effects of a toxicant on a population (Stark et al. 2004). Our study showed that life table parameters of *H. hebetor* were significantly affected while parasitizing *H. armigera* larvae treated with *M. anisopliae* (isolate M14). The R_0 values of *H. hebetor* attacking fungal infected host larvae were significantly lower than control wasps. Certainly, the r_m value is the most important parameter to evaluate population growth (Carey 1993). The higher r_m in control wasps compared with sublethal treatments clearly indicated that *M. anisopliae* (isolate M14) had adverse effects on population increase of *H. hebetor* at extended post-infection timescales (24, 48 and 72 h). Furthermore, the reduction in r_m values was the result of a longer mean generation time (T) of parasitoid wasps. Undoubtedly, the lower T for parasitoids compared with their host is an advantage, because it leads to more generations in a given period. Thus, if an insecticide causes an increase in mean generation time (T), it has a harmful effect on the parasitoid (Mahdavi et al. 2011). Based on time intervals between host fungal infection and female wasp introduction, sublethal treatment of *H. armigera* larvae with *M. anisopliae* not only increased T value of *H. hebetor*, but also doubling time (DT) of the parasitoid was significantly affected by longer timescales (24, 48 and 72 h).

In brief, time intervals between fungal inoculation and parasitoid encounter were responsible for significant differences among treatments. Likewise, Rashki et al. (2009) demonstrated that the number of mummies produced per aphid differed significantly depending on timescales between exposure to *A. matricariae* and application of *B. bassiana*. These researchers showed that *B. bassiana* isolate EUT116 did not influence the R_0 values of parasitoid offspring but reduced the r_m value. On the contrary, Fatiha et al. (2008) showed that direct application of *V. lecanii* decreased the R_0 value of *S. japonicum* and different concentrations of fungus resulted in similar intrinsic rates of increase (r_m). Also, they indicated that mean generation time (T) was not significantly different among fungal treatment and control.

Since our study was based on population growth parameters, additional studies including foraging and mating behavior of males and females or physiological systems such as phenoloxidase activity of *H. hebetor* may be profitable. In addition, it would be interesting to investigate the interaction between *M. anisopliae* and *H. hebetor* on different host life stages (small vs. large) and treated/untreated larvae. As our results were based on laboratory studies, further attention should be devoted to semi-field and field experiments to obtain more applicable results.

In conclusion, this study demonstrated that sublethal treatments of *H. armigera* larvae with *M. anisopliae* (isolate M14) had potential for deleterious effects on life table parameters of *H. hebetor*: effects increased with time interval between fungal infection and wasp encounter of host larvae. In order to avoid antagonistic interactions between the studied biocontrol agents, the timing of introductions is imperative. Therefore, appropriate parasitoid introduction time (<24 h) after host inoculation with fungal entomopathogen is the most important factor in combining *H. hebetor* with *M. anisopliae* for successful biological control of *H. armigera*. To put it simply, precise time management is necessary to

avoid antagonistic interactions between two mentioned biocontrol agents.

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