

# Susceptibility of the Hymenopteran Parasitoid, *Habrobracon hebetor* (Say) (Braconidae) to the Entomopathogenic Fungi *Beauveria bassiana* Vuillemin and *Metarhizium anisopliae* Sorokin

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## Abstract

*Habrobracon hebetor* (Say) is an ectoparasitoid and has been studied as a biocontrol agent of various lepidopteran pests such as cotton bollworm *Helicoverpa armigera* (Hübner). With regards to the negative effects of common pesticides used in cotton fields on the parasitoids, in this study the effects of different isolates of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) were evaluated on *H. hebetor*. Bioassay experiments were performed by the immersion method. For each of the treatments 15 immature individuals of the parasitoid were used. After recording the results, data were analyzed using SAS software. Bioassay results of fungi isolates on larval stages showed that the value of LC<sub>50</sub> for IRAN187C isolate of *B. bassiana* was  $1.46 \times 10^9$  conidia/ml. Because of the low mortality caused by the other isolates, the value of LC<sub>50</sub> was not set for them. Also, bioassay of fungal isolates showed that none of the isolates had any effect on the parasitoids pupal stage. According to the obtained results, it can be concluded that various fungal isolates of *B. bassiana* and *M. anisopliae* had little adverse impact on the parasitoid wasp, thus after doing field tests, the microbial control agents may be used along with these parasitoids in integrated pest management programs (IPM) in cotton.

**Keywords:** *Habrobracon hebetor*, entomopathogenic fungi, IPM, bioassay, natural enemies..

## 1. Introduction

Cotton (*Gossypium hirsutum*) is the most important economic and fiber crop worldwide (Chen *et al.*, 2002). This crop is also a major agricultural product in Iran and the area cultivated with cotton is about 91019 hectare for the years 2009-2010 (Anonymous, 2011). Insect pests are limiting factors for healthy growth of cultivated plants (Ramzan Asi *et al.*, 2009). Among insect pests, *Helicoverpa armigera* (Hübner) (Lep.: Noctuidae) is one of the most important arthropod pests of cotton crop (Matthews, 1999). This pest is a polyphagous agricultural pest which attacks a wide variety of agricultural crops including cotton, corn, tomatoes, sorghum, soybeans and groundnuts (Fitt, 1989). Early instars are foliar feeders and later instars attack seeds, fruits and bolls leading to economic loss (Fitt, 1989) and their infestations cause severe economic losses as a result of crop yield reduction (Soomro *et al.*, 1992), and the pest causes economic losses up to 30% of the total production (Yazdanpanah *et al.*,

2009). Different strategies have been employed for control of this notorious pest (Ramzan Asi *et al.*, 2009). Farmers mostly prefer chemical pesticide application for its control because it is quicker, however, indiscriminate application of broad spectrum chemical pesticides exterminates these susceptible natural enemies and leaves behind the pests that are more resistant to pesticides (Feng *et al.*, 1994) as well as these compounds can cause serious problems such as pest outbreaks (Luck *et al.*, 1977; Metcalf, 1986). In order to reduce crop losses, the use of microbial control agents which have a lower risk on the environment and humans is recommended (Hull and Beers, 1985). Among the microbial control agents are entomopathogenic fungi. Entomopathogenic fungi have a considerable potential for efficacious suppression of a variety of arthropod pests. *Beauveria bassiana* (Balsamo) Vuillemin is one of the most important entomopathogenic fungi (Leland *et al.*, 2005; Quesada-Moraga *et al.*, 2006; Al-maza *et al.*, 2006). This fungus is widely distributed in the world (St.-Leger *et al.*, 1986) and has the potential to control over 70 insect pest species (Hung and Boucias, 1992; Alizadeh *et al.*, 2007).

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Another fungus effective in controlling insect pests is *Metarhizium anisopliae* Sorokin that is able to control a wide range of pests (Zimmermann, 1993).

On the other hand, one of the important methods to control pests is the use of natural enemies. Among these natural enemies is the parasitoid wasp *Habrobracon hebetor* (Say) (= *Bracon hebetor*) (Haeselbarth, 1983; Amir-Maafi and Chi, 2006) (Hymenoptera: Braconidae). *H. hebetor* is a valuable biocontrol agent of lepidopteran pests attacking crop plants and stored products, including *H. armigera* (Magro and Para, 2001). In Iran, mass rearing of *H. hebetor* is done on Mediterranean flour moth, *Ephestia (Anagasta) kuehniella* Zeller (Mudd and Corbet, 1982) and the adult wasps are released to parasitize *H. armigera* larvae in cotton fields in Ardabil and Golestan provinces in the northern parts of the country (Attaran, 1996; Navaei *et al.*, 2002).

Since the strategy of IPM includes the simultaneous use of different methods of control, different methods of control must be examined together to finally be able to utilize them for pest control. Potential effects of microbial control agents on the parasitoids must be studied (Hajek and St. Leger, 1994). In particular, *Metarhizium anisopliae* Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin have been isolated from diverse species of parasitoids (Thungrabeab and Tongma, 2007).

In the present study, the effects of different isolates of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* were evaluated on the immature stages of *H. hebetor* in the laboratory, in order to evaluate the possibility of simultaneous application of entomopathogenic fungi and the parasitoid *H. hebetor* in the field.

## 2. Materials and Methods

### 2.1. Insect Rearing

The *H. hebetor* colony was obtained from an insectarium maintained by the Plant Protection Bureau of Kaleibar, Iran in 2010. The colony was maintained in the laboratory at  $26 \pm 1^\circ\text{C}$ ,  $60 \pm 5\%$  RH and a photoperiod of 16:8 (L: D) on larval *E. kuehniella*, that was reared on flour in a growth chamber at the above mentioned environmental conditions. Parasitoid wasps were reared on 5<sup>th</sup> instar larvae of *E. kuehniella* for five generations and used for all experiments. Honey was provided as food for the adult parasitoids on  $5 \times 30$  mm strips of paper (Sarmadi, 2008).

### 2.2. Fungal Isolates

Fungi isolates used in this study are shown in Table 1.

**Table 1.** Isolates of fungi used in this study and origin within Iran

Fungi	Isolates	Host	Location area
<i>Beauveria bassiana</i>	IRAN 187C	<i>Leptinotarsa decemlineata</i>	Ardabil
<i>Beauveria bassiana</i>	EUT116	Lepidopteran larvae	Tehran
<i>Beauveria bassiana</i>	EUT105	Soil	Fashand-Karaj
<i>Metarhizium anisopliae</i>	M-115	<i>Parandra caspica</i>	Sari
<i>Metarhizium anisopliae</i>	M-396	<i>Parandra caspica</i>	Sari

### 2.3. Culture of Fungi

Fungi were cultured on Saboraud's Dextrose Agar Yeast extract (SDAY) in Petri dishes at  $25 \pm 1^\circ\text{C}$ ,  $80 \pm 5\%$  RH and a photoperiod 16:8 h (L:D). After preparing the medium, piece of the culture medium containing conidial fungi to be removed by a sterile scalpel were transferred to Petri dishes containing fresh medium. After 15 days the stages were full of germinated fungi. Petri dishes containing conidial of entomopathogenic fungi were used for experiments. Since strains maintained in the laboratory, after preparing them for about 2-3 weeks.

### 2.4. Production of Suspension

For producing fungi suspension, conidia were transferred into tubes with lid consist of sterile distilled water. For screening mycelium and medium, this suspension was passed through mesh fabric. A haemocytometer (Paul Marienfeld GmbH and Co. KG, Germany) was used to determine the concentration of conidia in the initial suspension. The hemocytometer is a device originally designed for the counting of blood cells. It is now also used to count other types of cells as well as other microscopic particles for example the entomopathogenic fungi conidia. After counting conidia using haemocytometer, the main concentration was determined using the formula  $Y = 5X \times 10^4$  ( $X$  = number of conidia in five squares) (Erwin, 2002). Subsequent concentrations were determined using the logarithmic distant.

### 2.5. Bioassays

The immature stages of the parasitoid were dipped in fungi solutions at the 4<sup>th</sup> or 8<sup>th</sup> day for 10 s. These days correspond to larval (without cocoon) and pupal (with cocoon) stages of the parasitoid, respectively (Rafiee-Dastjerdi *et al.*, 2008). Initial dose-setting experiments were carried out to determine the highest and lowest concentrations causing 80% and 20% mortality for both isolates (Robertson *et al.*, 2007). Concentration ranges were  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$  and  $10^{11}$  conidia/ml. Tween 80 (Merck, Darmstadt, Germany) was used at a concentration of 200 ppm in all dilutions as a spreader (Rosenheim and Hoy, 1988). Our previous experiments showed that Tween 80 (200 ppm) has no effects on bioassays. The control plates were sprayed with distilled water plus Tween 80. After immersion, Petri dishes containing filter paper and immature stages were transferred to growth chamber with  $26 \pm 1^\circ\text{C}$  and 80% RH. Data analysis was performed by SAS program (SAS Institute, 2002).

## 3. Results and Discussion

Effects of *B. bassiana* and *M. anisopliae* isolates showed that the isolates EUT105 (*B. bassiana*) and M-396 (*M. anisopliae*) didn't cause mortality in the *H. hebetor* larval stage at all concentrations tested. The EUT116 and IRAN187C isolates of *B. bassiana* at the concentration  $10^{10}$  conidia/ml had 6.67 and 51.11% mortality on parasitoid larvae, respectively. Also, M-115 isolates of *M. anisopliae* at  $10^{10}$  conidia/ml/ml caused 2.22% mortality on larval stage of *H. hebetor* (Table 3). The above results showed that,  $LC_{50}$  values didn't apply for the listed

isolates. The only isolate for which the value of  $LC_{50}$  was determined was IRAN187C isolate of *B. bassiana* (Table 2).

**Table 2.** Probit analysis of the fungal isolates IRAN187C (*B. bassiana*) tested on parasitoid larval stage

Isolate	*N	Slope ± SE	<sup>2</sup> χ	LC <sub>50</sub> Conidia/ml	**FL (95%)
IRAN187C	270	0.6	3.4	$1.46 \times 10^9$	$(6.5 \times 10^8$ $- 3.2 \times 10^9)$
<i>B. bassiana</i>		±0.08			

\* Number of used insect

\*\* Fiducial limits

According to our results, the isolate of IRAN187C (*B. bassiana*) had an adverse influence on the larval stage of the parasitoid followed by EUT116 (*B. bassiana*) and M-115 (*M. anisopliae*). Different isolates of *Beauveria* and *Metarhizium* did not show any effect on the pupal stage of the parasitoid (Table 3). Probably, the cocoon around the parasitoid pupa was responsible for the lack of effectiveness of the fungal treatments on this developmental stage. The results showed that in the control treatment (normal conditions) in the larval and pupal stages, no losses were observed (Table 3).

**Table 3.** Mortality (± SE) of immature stages parasitoid treated with concentration of  $10^{10}$  conidia/ml of fungal isolates tested and control treatment

Isolates of fungi	Stages of parasitoid	
	Larval	Pupal
IRAN187C ( <i>B. bassiana</i> )	51.11 ± 4.01	0 ± 0
EUT116 ( <i>B. bassiana</i> )	6.67 ± 3.85	0 ± 0
EUT105 ( <i>B. bassiana</i> )	0 ± 0	0 ± 0
M-115 ( <i>M. anisopliae</i> )	2.22 ± 2.22	0 ± 0
M-396 ( <i>M. anisopliae</i> )	0 ± 0	0 ± 0
Control	0 ± 0	0 ± 0

Means in column followed by different small letters are significantly different. ANOVA with Tukey post hoc test ( $\alpha < 0.05$ )

Before this study, no investigation has been conducted on the impact of entomopathogenic fungi on *H. hebetor*, but the impact of *B. bassiana* and *M. anisopliae* on other parasitoids was studied. Rashki *et al.* (2009), in studying the effect of *B. bassiana* on *Aphidius matricariae* and its host *Myzus persicae*, showed that this pathogen had no effect on biological parameters of the parasitoid and concluded that *B. bassiana* and the parasitoid *A. matricariae* can be successfully combined for biological control of *M. persicae*. These reports are in line with the results of this study. The results of this study indicate very little effect of the entomopathogenic fungi on the parasitoid which is consistent with the results of Stolz *et al.* (2002). In evaluating the susceptibility of the parasitoids *Apoanagyrus lopezi* and *Phanerotoma sp.* to the entomopathogenic fungus *M. anisopliae*, they reported that different isolates of this fungus had very little risk on parasitoids. Also, Rosa *et al.* (2000) studying the effect of *Beauveria* and *Metarhizium* on the parasitoid *Prorops nasuta* reported that various isolates of the fungus have little negative impact on the parasitoid and can be used as a component compatible with natural enemies. Also, the

effects of fungal isolates on the host field (*Helicoverpa armigera* Hübner) showed that mentioned isolates had a good control on the *H. armigera* (Vojoudi, 2011). According to the obtained results, it can be concluded that different isolates of the fungi *B. bassiana* and *M. anisopliae* had few adverse effects on *H. hebetor*, and therefore these microbial control agents can be used of along with the parasitoid in integrated pest management (IPM) programs.

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