J. Crop Prot. **2018, 7 (3): 283-291**

Research Article

Side-effects of azadirakhtin (NeemAzal) and flubendiamide (Takumi) on functional response of *Habrobracon hebetor* (Hymenoptera: Braconidae)

Fatemeh Rostami[1], Nooshin Zandi-Sohani[1*], Fatemeh Yarahmadi[1], Leila Ramezani[1] and Karim Avalin Chaharsoghi[2]

1. Department of Plant Protection, Faculty of Agriculture, Khuzestan Agricultural Sciences and Natural Resources University, Mollasani, Ahvaz, Iran.
2. Jahad Agricultural Organization of Khuzestan, Iran.

Abstract: *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is one of the major parasitoids which is used against various lepidopteran larvae in Iran. Due to extensive application of chemical pesticides in farms, study of their side effects on natural enemies is necessary. Therefore, in this study, side-effects of two prevalent insecticides, NeemAzal and Takumi (at dosage 2000 mg/l and 437.5mg/l, respectively), on functional response of *H. hebetor* to different densities of 5th instar larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were evaluated in laboratory conditions. Host densities of 2, 4, 8, 16, 32 and 64 were randomly exposed to selected treated females of *H. hebetor* in 8 cm Petri dishes. Ten replications were conducted for each host density. The control was treated with water. The data were analyzed using logistic regression to find functional response type and non-linear regression to estimate functional response parameters. The results revealed a type II response for all treatments. This study showed that *H. hebetor* had the shortest (0.269 h) and longest (1.822 h) handling times in Takumi and NeemAzal treatments, respectively. Also, the highest and lowest searching efficiency of *H. hebetor*were recorded for the Takumi (0.188 h^{-1}) and NeemAzal (0.0396 h^{-1}) treatments, respectively. According to the results of the study, Takumi may be more compatible with biological control agent in IPM programs.

Keywords: Behavioral Response, Botanical insecticide, Ectoparasitoid, Integrated Pest Management

Introduction

Parasitoid wasps are generally considered as beneficial natural enemies. They help in control of insect pest population (Hentz *et al*., 1998). These wasps have a great economic importance in agricultural ecosystems, as they attack a wide range of insect pests that feed on important crops (Hentz *et al*., 1998; Mahdavi and Saber, 2013). The number of attacked hosts by parasitoids may change the as a function of host density which is called functional response (Solomon, 1949). Holling (1959, 1966) considered three types of functional responses. The early functional response researches conducted by Holling (1959), resulted in the formulation of mathematical models (type I, type II, and type III) describing parasitism responses that were influenced by changes in parasitoid behavior. Most arthropod

Handling Editor: Ali Asghar Talebi

* **Corresponding author**, e-mail: zandi@ramin.ac.ir
Received: 6 January 2018 Accepted: 10 May 2018
Published online: 16 July 2018

parasitoids possess a type II response (Holling, 1961) with some exceptions (Tostowaryk, 1972; Hassell, 1978).Studies of functional response which determine the predation/parasitism of a natural enemy can be helpful in integrated pest management programs (Abedi *et al*., 2012).

Habrobracon hebetor is a valuable biocontrol agent of lepidopteran pests which attacks crop plants and stored product pests, including *Helicoverpa armigera* Hub. (Magro and Parra, 2001), *Plodia interpunctella* (Hübner) (Milonas, 2005), *Ephestia cautella* (Walker) (Press *et al*., 1982), *Ephestia kuehniella* Zeller (Darwish *et al*., 2003), and *Galleria mellonella* L. (Kryukova *et al*., 2011). It has been widely used in various studies related to host-parasitoid interactions because of its high reproductive rate, short generation time, and wide range of host species (Yu *et al*., 2002). In Iran, it is reared commercially in mass rearing programs and released widely against *H. armigera* on cotton, *Gossypium herbaceum* L. and tomato, *Solanum lycopersicum* L.in fields (Faal-Mohammad-Ali *et al*., 2015).

Selective pesticides application and biological control are the two important strategies used in integrated pest management (IPM) programs (Zhao, 2000). Pesticides application is often unavoidable; however, they should be used whenever they have the least adverse effects on biological control agents (Guedes *et al*., 2016). Integrating application of biocontrol agents and pesticides for pest management requires knowledge about the interactions and compatibility of the pesticides and natural enemies (Desneux *et al*., 2007; Mahdavi, 2013). Chemical products may affect physiology and behavioral characteristics of natural enemies (Desneux *et al*., 2007). In order to combine both biological and chemical methods for control of target pest, it is essential to investigate sub lethal effects of pesticides on natural enemies (Biondi *et al*., 2013, Abbes *et al*., 2015).

The study of parasitoids' and predators 'behavior is an important key towards understanding about the insects' life, their influence on the population dynamics of their hosts or preys, and their impact on the structure of the insect communities in which they live. So, it is a necessary prerequisite for selection of natural enemies in biological control programs, and evaluation of their efficiency after release (Jervis and Kids, 1996).

Previous research has been conducted to show the effects of various insecticides on functional responses of *H. hebetor* on different laboratory hosts (Rafiee-Dastjerdi *et al*., 2009; Abedi *et al*., 2012; Mahdavi *et al*., 2013; Mahdavi and Saber, 2013; Faal-Mohammad-Ali *et al*., 2015). Different insecticides showed various effects on the searching efficiency of the parasitoid. For example, the highest and lowest effects on searching efficiency of *H. hebetor* were observed in spinosad and hexaflumuron (Rafiee-Dastjerdi *et al*., 2009), pyridalyl and cypermethrin (Abedi *et al*., 2012), abamectin and chlorpyrifos (Mahdavi *et al*., 2013), diazinon and Malathion (Mahdavi and Saber, 2013), respectively. However, according to Faal-Mohammad-Ali *et al*. (2015) the searching efficiency of *H. hebetor* was not affected significantly by chlorpyrifos and fenpropatrin treatments compared with control. NeemAzal and Takumi are principal the principal insecticides used in tomato fields in Khuzestan province for control of lepidopteran pests. At the same time, *H. hebetor* is released against lepidopteran pests. Hence, the aim of this study is to investigate sub lethal effects of NeemAzal and Takumi on functional response of the ectoparasitoid, *H. hebetor* in the laboratory.

Materials and Methods

Insects rearing

A stock culture of *E. kuehniella* was obtained from a colony at insectarium of Plant Protection Organization in Ahvaz, Iran, and reared at 26 ± 1 °C, 65 ± 5% RH and dark condition. The stock culture was maintained in plastic containers on 1.5kg of wheat (*Triticum aestivum* L.) flour, which provided the moth larvae with excess food throughout their development.

The larvae of *Heliothis armigera* Hubner parasitized by *H. hebetor* were collected from tomato fields near Ahvaz, Iran that had not been sprayed with any insecticide, previously. Then, they were maintained in plastic cups covered with a fine mesh until adult emergence. The colony was maintained in the laboratory at 26 ± 1 °C, 65 ± 5% RH and a photoperiod of 16: 8 (L: D) h on larval stages of *E. kuehniella*. Then parasitoid wasps were reared on the fifth instar larvae of *E. kuehniella* for five generations under the same conditions as above. Cotton soaked in honey diluted (10%) with water was provided as food with the adult parasitoids (Sarmadi, 2008).

Insecticides

The insecticides applied in the assays were commercial formulations including NeemAzal® (azadirachtin EC 1%) and Takumi® (flubendiamide WG 20%).

Experiments

Petri dishes (8 cm diameter) were used as experimental units. The inner surface of each Petri dish and its cover were sprayed with field recommended lethal dosage of NeemAzal (2 per thousand equivalent to 2000 mg/l) and Takumi (175 g/ha equivalent to 437.5 mg/l). The control pates were sprayed with distilled water. A hand sprayer was used to make a uniform, sufficient coverage. The amount of insecticides in Petri dishes was 2×10^{-4} mg a.i./cm^2 for NeemAzal and 1.75×10^{-4} mg a.i./cm^2 for Takumi. Then the Petri dishes were maintained at room temperature to become completely dry. Twenty mated young females (< 24 h old) were anesthetized by putting them in freezer for less than 30 seconds, then they were placed in Petri dishes. After 24h, individual mated females were randomly selected and transferred to new Petri dishes (8 cm diameter) with 2, 4, 8, 16, 32 and 64 5th instar larvae of *E. kuehniella.* Ten replications were conducted for each host density. Petri dishes were transferred to a growth chamber(26 ± 1 °C, 65 ± 5% RH) and a photoperiod of 16: 8 h (L: D) for 24 h. Tiny droplets of honey was supplied as food for the adult parasitoids. The number of parasitized larvae was recorded after 24 h. Parasitized larvae were identified by checking the parasitoid eggs laid externally on them.

Statistical analysis

The data analysis included two distinct steps. At the first step, the type of functional response (type II or III) was found using logistic regression of the proportion of host parasitized versus the number of initial host. This is done by fitting a polynomial function:

$$\frac{N_a}{N_0} = \frac{\exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)} \qquad (1)$$

Where N_a is the number of parasitized hosts, N_0 is the initial number of hosts offered. Then, the parameters P_0, P_1, P2 and P_3 were estimated by Maximum Likelihood Analysis (CATMODE) procedure in SAS software (Juliano, 1993). The value of P_1 (Linear parameter) indicates the type of functional response. If negative, it indicates the type II functional response, while a positive linear parameter means type III functional response. At the second step, The non-linear least square regression (NLIN) procedure was used to fit Roger's random attack model, to estimate the parameters of attack rate and handling time by SAS software (SAS Institute, 2005).Since our data fit a type II functional response we used the Rogers type II equation as follows:

$$N_a = N_0\{1 - \exp[a(T_h N_a - T)]\} \qquad (2)$$

Where N_a is the number of parasitized host, N_0 is the initial number of host, a is the instantaneous searching efficiency (attack rate), T is the total amount time available for searching (in this experiment T = 24 h), P_t the number of parasitoids, and T_h is the handling time.

Results

The negative values for the linear parameters obtained from logistic regression analysis in the present study indicated a type II functional response for *H. hebetor* females against fifth instar larvae of *E. kuehniella* in insecticides and control treatments (Table 1). The number of parasitized hosts increased with increasing the host densities in all treatments; however, the proportion of host parasitization decreased as host density increased (Fig. 1). The Rogers' type II model showed an acceptable fit to the data at all treatments examined. The handling time and searching efficiency values are shown in Table 2. According to the results, parasitoid females treated with Takumi showed the lowest value of handling time (0.269 ± 0.56 h) and those treated with NeemAzal showed the highest value (1.822 ± 0.14 h), respectively. The value of handling time in NeemAzal was significantly higher than Takumi and control treatments at confidence intervals (CI 95%). The highest and lowest values of searching efficiency were observed in Takumi (0.1884 ± 0.14 h^{-1}) and NeemAzal (0.0396 ± 0.006 h^{-1}) treatments, respectively. According to confidence intervals, the searching efficiency of *H. hebetor* in NeemAzal treatment was significantly lower than control and Takumi treatments (Table 2).

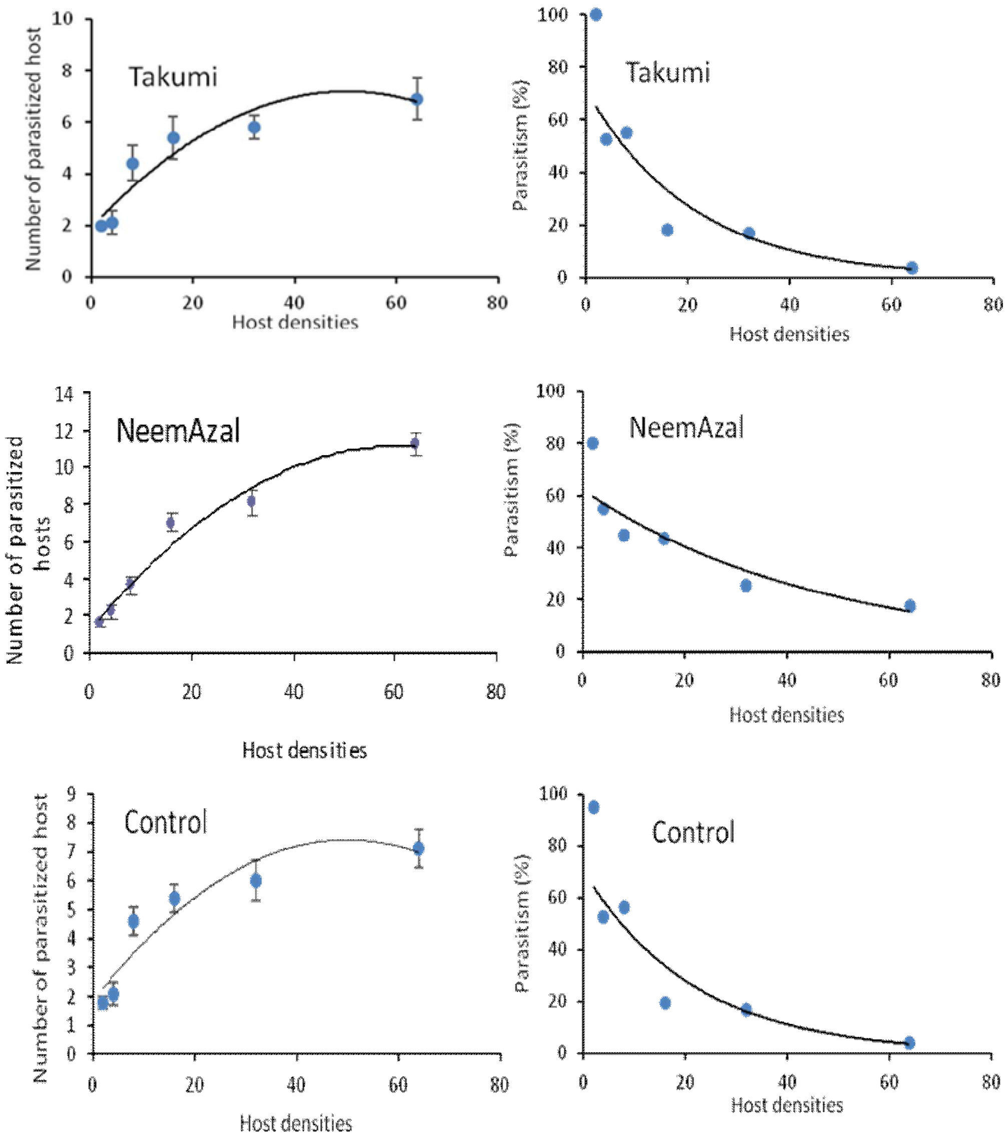

Figure 1 Functional response of the ectoparasitoid *Habrobracon hebetor*, exposed to field concentrations of Takumi and NeemAzal, and in the control.

Table 1 Results of the logistic regression analysis of the proportion of *Ephestia kuehniella* larvae parasitized by *Habrobracon hebetor*, against the initial density.

Treatment	Parameters	Estimate	SE	P-value
Control	P_0 (constant)	1.992	0.382	0.024
	P_1 (linear)	-0.014	0.058	0.043
	P_2 (quadratic)	1×10^{-4}	0.002	0.123
	P_3 (cubic)	-3×10^{-5}	2×10^{-5}	0.193
NeemAzal	P_0 (constant)	2.621	0.379	2×10^{-4}
	P_1 (linear)	-0.069	0.058	0.009
	P_2 (quadratic)	4×10^{-4}	2×10^{-4}	0.339
	P_3 (cubic)	-1×10^{-5}	2×10^{-5}	0.958
Takumi	P_0 (constant)	1.934	0.442	$< 1 \times 10^{-4}$
	P_1 (linear)	-0.301	0.071	$< 1 \times 10^{-4}$
	P_2 (quadratic)	0.008	0.002	0.002
	P_3 (cubic)	-8×10^{-5}	2×10^{-5}	0.004

Table 2 Estimated functional response parameters of *Habrobracon hebetor* exposed to field concentrations of NeemAzal and Takumi and control.

Treatment	Functional response	Searching efficiency ± SE (h) (lower - upper)	Handling time (h) (lower - upper)	T/T_h
Control	II	0.193 ± 0.005 (0.046 - 0.256)	0.252 ± 0.049 (0.142 - 0.321)	95.01
NeemAzal	II	0.032 ± 0.006 (0.026 - 0.052)	1.822 ± 0.143 (1.490 - 2.061)	13.17
Takumi	II	0.188 ± 0.143 (- 0.094 - 0.471)	0.269 ± 0.565 (0.151 - 0.387)	89.16

Values in parentheses are asymptote 95% confidence interval.

Discussion

The present study indicated that treatments of Takumi and NeemAzal in their field recommended doses did not change the type of parasitoid functional response compared to the control treatment. *Habrobracon hebetor* showed type II functional response in both insecticide treatments and in control. In a study Abedi *et al.* (2012) *H. hebetor* showed a type II functional response for all the insecticide treatments (NeemAzal, cypermethrin, methoxyfenozide, and pyridalyl) and control which is consistent with the results of current study. Faal-Mohammad-Ali *et al.* (2015) also reported type II functional response for adults of *H. hebetor* when they were exposed to sublethal concentrations of chlorpyrifos and fenpropathrin. However, our results are different from Mahdavi *et al.* (2013) who found type III functional response for *H. hebetor* exposed to chlorpyrifos, carbaryl, abamectin and spinosad. The studies have indicated that type II functional response is more frequent among parasitoids. Previous researchers believed that among all natural enemies, species that show type III functional response are more likely to be able to regulate their host density (Hassell, 1978). It has also been mentioned that parasitoids with type III functional response are able to determine their host density and adjust their searching efficiency (O'Neil, 1990). However, another study proved that the form of functional response curves, on its own, cannot be attributed to the success or failure of parasitoids in biological control (Fernandez-Arhex and Corely, 2003). Although the functional response is one of the features which is considered to be related to parasitoid success (Hassell, 1978; Berryman, 1999), some other factors such as time and rate of release, quality and quantity of parasitoids, climatic conditions, intrinsic rate of increase, host patchiness, predation and competition and agricultural practices such as application of insecticide are considered as important factors for success of a parasitoid (Fernandez-Arhex and Corley, 2003; Pervez, 2005).

The searching efficiency and handling time values obtained in this experiment were not significantly different in control and Takumi treatment. This shows that Takumi had lower hazardous effects on the searching efficiency of *H. hebetor* than NeemAzal. The lower rate of searching efficiency in NeemAzal compared to Takumi treatment may be due to the changes made in parasitoid behavior by the insecticide (Abedi et al., 2012). In other studies the effect of insecticides on searching efficiency and handing time of *H. hebetor* compared to control has been reported (Rafiee-Dastgerdi *et al.*, 2009; Abedi *et al.*, 2012; Mahdavi and Saber, 2013; Mahdavi *et al.*, 2013; Faal-Mohammad-Ali *et al.*, 2015). According to Abedi *et al.* (2012) *H. hebetor* showed the highest searching efficiency when it was exposed to pyridalyl, compared to the values calculated for treatments of NeemAzal, cypermethrin, methoxyfenozide and control. Their results are similar to ours regarding to Takumi. According to Mahdavi *et al.* (2013) chlorpyrifos-and carbaryl-treated *H. hebetor* showed the highest and lowest searching efficiency, respectively. However, Mahdavi and Saber (2013) found that Diazinon and Malathion-treated wasps showed lower searching efficiency compared to control. *Habrobracon hebetor* also had the lowest and highest searching efficiency when exposed to LC_{25} of hexaflumuron and spinosad, respectively. In contrast, chlorpyrifos and fenpropathrin had no significant effect on the searching efficiency of *H. hebetor* compared with control (Faal-Mohammad-Ali, 2015).

In conclusion, our results demonstrated the effect of NeemAzal and Takumi on functional response of *H. hebetor*. According to the results, Takumi did not show adverse effects on searching efficiency of *H. hebetor* and it can be recommended as a compatible insecticide for simultaneous use with the parasitoid in integrated pest management systems. However, several other studies like field surveys, parasitoid ecology and other behavioral interactions under more natural conditions are needed for conclusive decision on the success of the concomitant use of parasitoids and insecticides in IPM programs.

Acknowledgments

The authors are grateful for the financial support by the research deputy of Khuzestan Agricultural Sciences and Natural Resources University, Iran.

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اثرات جانبی نیم‌آزال و تاکومی روی واکنش تابعی *Habrobracon hebetor* (Hymenoptera: Braconidae)

فاطمه رستمی[1]، نوشین زندی سوهانی[1*]، فاطمه یاراحمدی[1]، لیلا رمضانی[1] و کریم اولین چهارسوقی[2]

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، اهواز، ایران.
۲- جهاد کشاورزی استان خوزستان، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: zandi@ramin.ac.ir
دریافت: ۱۶ دی ۱۳۹۶؛ پذیرش: ۲۰ اردیبهشت ۱۳۹۷

چکیده: زنبور پارازیتوئید *Habrobracon hebetor* Say (Hymenoptera: Braconidae) یکی از پارازیتوئیدهای مهم است که علیه لارو حشرات آفت راسته Lepidoptera در ایران مورد استفاده قرار می‌گیرد. به‌دلیل کاربرد گسترده حشره‌کش‌های شیمیایی در مزارع، مطالعه اثرات جانبی آنها روی دشمنان طبیعی ضروری است. در این مطالعه، اثرات جانبی دو حشره‌کش رایج نیم‌آزال و تاکومی (به‌ترتیب در دوزهای ۲۰۰۰ میلی‌گرم بر لیتر و ۴۳۷/۵ میلی‌گرم بر لیتر) روی واکنش تابعی *H. hebetor* نسبت به تراکم‌های مختلف لارو سن پنجم *Ephestia kuehniella* Zeller در شرایط آزمایشگاهی بررسی شد. تراکم‌های ۲، ۴، ۸، ۱۶، ۳۲ و ۶۴ میزبان در پتری‌دیش‌های ۸ سانتی‌متری در معرض ماده‌های تیمار شده با حشره‌کش‌ها که به‌صورت تصادفی انتخاب شده بودند قرار گرفتند. آزمایشات در ۱۰ تکرار انجام شد. در تیمارشاهد به‌جای حشره‌کش، آب استفاده شد. برای تعیین نوع واکنش تابعی، داده ها با استفاده از رگرسیون لجستیک تجریه تحلیل شدند و برای تخمین پارامترهای واکنش تابعی از رگرسیون غیرخطی استفاده شد. نتایج نشان‌دهنده نوع دوم واکنش تابعی برای همه تیمارها بود. در این بررسی سموم تاکومی و نیم‌آزال به ترتیب کم‌ترین (۰/۲۶۹ ساعت) و بیش‌ترین (۱/۸۲۲ ساعت) مقدار زمان دستیابی را به خود اختصاص دادند. هم‌چنین، بیش‌ترین و کم‌ترین مقادیر کارایی جستجوگری به‌ترتیب برای زنبورهای تیمار شده با تاکومی (۰/۱۸۸ بر ساعت) و نیم‌آزال (۰/۰۳۹۶ بر ساعت) مشاهده شد. براساس این نتایج، حشره‌کش تاکومی احتمالاً سازگاری بیش‌تری با این عامل کنترل بیولوژیکی در برنامه‌های مدیریت تلفیقی آفات دارد.

واژگان کلیدی: واکنش رفتاری، حشره‌کش گیاهی، پارازیتوئید خارجی، مدیریت تلفیقی آفات