EFFECTS OF AZADIRACHTIN, CYPERMETHRIN, METHOXYFENOZIDE AND PYRIDALIL ON FUNCTIONAL RESPONSE OF *HABROBRACON HEBETOR* SAY (HYM.: BRACONIDAE)

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Abstract: *Habrobracon hebetor* Say is one of the most important natural enemies of the *Helicoverpa armigera* Hübner (Lep.: Noctuidae). In this study, the sublethal effects of LC_{30} concentration of azadirachtin, cypermethrin, methoxyfenozide and pyridalil on the functional response of *H. hebetor* to different densities of 5th instars larvae of *Ephestia kuehniella* Zeller were evaluated. Young females were exposed to LC_{30} of the insecticides for an appropriate time of exposure. Then, six randomly selected alive females were transferred individually to plastic Petri dishes (10 cm in diameter) and supplied with the following densities: 2, 4, 8, 16, 32 and 64 of 5th instars of *E. kuehniella* for 24 h. Eight replicates were considered for each insecticide treatment and the control. The results revealed a type II functional response in the control, and all of the insecticide treatments. This study showed that the control and the cypermethrin treatment had the shortest (0.4143 h) and longest (0.624 h) handling time, respectively. The highest (0.0035 per hour) and lowest (0.0029 per hour) attack rate was observed in pyridalil and cypermethrin treatments, respectively. Based on values of handling time, cypermethrin had the highest adverse effect on host-finding in *H. hebetor*.

Key words: *Habrobracon hebetor*, functional response, conventional and biorational insecticides

INTRODUCTION

Habrobracon hebetor Say is a gregarious, idiobiont and larval ectoparasitoid of pyralid and noctuid moths (Magro and Parra 2001; Dweck *et al*. 2008). This parasitoid can easily be mass reared and it has been released in the field for effective control of *Heliothis* and *Helicoverpa* spp. (Radhika and Chitra 1996; Heimpel *et al.* 1997). Correlation between values obtained from laboratory testing and field performance is important to be able to select a limited set of laboratory criteria that give meaningful information about performance after release (van Lenteren *et al*. 2003). Parasitoids and predators may respond to changes in host or prey densities in two ways. Firstly, parasitoid numbers may change with changes in host density. Secondly, the number of hosts attacked by a parasitoid may change with changes in host density. These changes in parasitoid numbers and numbers of hosts attacked are referred to as the numerical and functional responses, respectively (Solomon 1949). One biological parameter, the functional response of a parasitoid to host density, can indicate the probable success of a parasitoid as a biocontrol agent (Kfir 1983; Rohlfs and Mack 1984; Isenhour 1985; Hopper and King 1986). The functional response is an essential element of host-parasitoid association dynamics and is an important determinant of the system's stability (Oaten and Murdoch 1975). Furthermore, functional response tests show the potential of parasitoid/predator ability to suppress the different density of prey/host (Moezipour *et al.* 2008). Holing (1959a, b, 1965, 1966) has worked extensively on the mathematical modeling of functional responses. Holing (1961, 1965) described four types of functional responses all of which have been observed in experimentation. In type I, the response curve increases linearly to a plateau. In type II, the response curve rises in a negatively accelerating manner to a plateau. In type III, the response curve rises in an S-shaped manner to a plateau. In the fourth type, the response curve is dome-shaped. Studies on the interaction of a natural enemy and its host, as the functional response, for instance, can be helpful in integrated pest management programs, once this helps determine the predation/parasitism dynamics of a natural enemy (Carneiro *et al.* 2010). On the other hand, the success of the Integrated Pest Management (IPM) programs simultaneous use of biological control agents and chemical compounds are recommended (Hull and Beers 1985). Furthermore, given that chemical compounds may affect host-finding behavior and behavioral responses of natural enemies,

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the effect of pesticides on the functional response of the natural enemies is evaluated. Rafiee-Dastjerdi *et al.* (2009) showed that profenofos, thiodicarb, hexaflumuron and spinosad had a negative effect on the functional response of *H. hebetor.*

The purpose of this study was to assess the sublethal effects of conventional and biorational insecticides such as azadirachtin, cypermethrin, methoxyfenozide and pyridalil on the functional response of ectoparasitoid *H. hebetor*. The reason for such an assessment was to enable selection of soft insecticides in order to protect beneficials and thereby improve the IPM.

MATERIALS AND METHODS

Insects

Adults of *H. hebetor* were obtained from an insectarium maintained by the Plant Protection Bureau of Mazandaran Province, Iran in 2011. Parasitoid wasps were reared on 5th instars larvae of *Ephestia kuehniella* Zeller at 26±1°C, 70±5% relative humidity (RH) with a photoperiod of 16:8 h (L:D). Fifth instars of *E. kuehniella* were used for all the experiments as the host. Honey was presented as food for the adult parasitoids on a stripe of paper (Rafiee-Dastjerdi *et al*. 2009).

Insecticides

The insecticides used in the experiments were two formulations of azadirachtin (BioNeem® 0.09% EC, Safer Co., USA and NeemGuard® 1% EC, Shalimar International L.L.C Co., Germany), cypermethrin (Patron® 40% EC Ariashimi Co., Iran), methoxyfenozide (Runner 2F® 21–24% EC, Dow AgroSciences Limited Co., England) and pyridalil (Sumipleo® 50% EC, Sumitomo Co., Japan).

Functional Response bioassay

Newly mated, young (< 24 h old) *H. hebetor* adult females were exposed to an LC_{30} (sublethal concentration) of azadirachtin (Neem Guard® and Bio neem®), cypermethrin, methoxyfenozide and pyridalil insecticides that were 2,508, 6,139, 9.7, 4,38.98 and 1,437 ppm, respectively. Exposure cages (130x130 mm) were used (Saber *et al*. 2005) for bioassay experiments. The glass surfaces of the cages were sprayed with aqueous solutions of the LC_{30} concentration of the insecticides. A hand sprayer was used to make a uniform, sufficient coverage. The control plates were sprayed with distilled water plus Tween 80. Tween 80 (Merck Darmstadt, Germany) was used in all dilutions as a spreader (Rosenheim and Hoy 1988). The exposure cages were assembled after drying the plates. Before completely assembling the cages, 60 mated young female adults (< 24 h old) were introduced in each cage. The cages were then transferred to the growth chamber under the above mentioned conditions. The exposure time was 24, 72, 72, 72, 96 h for cypermethrin, two formulations of azadirachtin, methoxyfenozide and pyridalil treatments, respectively, because LC_{30} of these insecticides were calculated in the mentioned period of time. Then six randomly selected, live females were transferred individually to plastic Petri dishes (10 cm in diameter) with the following densities: 2, 4, 8, 16, 32 and 64 of 5th instars of *E. kuehniella,* for 24 h, and the wasps were provided with honey as food. The numbers of parasitized and remaining alive larvae were recorded after 24 h. There were eight replications for each insecticide treatment.

Statistical analysis

The analysis of functional responses were comprised of two distinct steps (Juliano 1993; Messina and Hanks 1998; De Clercq *et al*. 2000; Juliano 2001; Mohaghegh *et al*. 2001; Allahyari *et al*. 2004). In the first step, the curve shape or type of functional response was established, typically by determining if the data fit a type II or III functional response. The parameters to be estimated were P0, P1, P2 and P3. These parameters were estimated using the Maximum Likelihood Analysis (CATMOD) procedure in SAS software (Juliano 2001). In the second step, a nonlinear least square regression was used [Nonlinear (NLIN) procedure with Multivariate Secant or False Position (DUD) method in SAS (2002)] to estimate the functional response parameters of the Holling's disc equation (Williams and Juliano 1985) Then, the obtained parameters were compared [T_h and either *a* (for type II)]. The coefficient of determination was calculated as $r^2 = 1$ – residual sum of squares/corrected total sum of squares (Allahyari *et al*. 2004; Farrokhi *et al*. 2010).

RESULTS

The outcome of the logistic regression indicated a type II functional response for all treatments (Fig. 1, 2). The logistic regression for all treatments had a significant linear parameter (Table 1). The study of basic aspects of

Table 1. Results of the logistic regression analysis of the proportion of *E. kuehniella* larvae parasitized by *H. hebetor*

Treatment	Coefficient	Estimate	Standard error (SE)	Chi – square value (χ^2)	P-value
1	2	3	4	5	6
The control	P0 (constant)	8.8027	2.5284	12.12	0.0005
	P1 (linear)	–0.2487	0.1207	4.25	0.0394
	P2 (quadratic)	0.0003	0.0013	0.06	0.7999
	P3 (cubic)	0.00003	–	–	–
Azadirachtin (Neem Guard)	P0 (constant)	8.1394	2.8896	7.93	0.0049
	P1 (linear)	–0.5987	0.3310	3.27	0.0705
	P2 (quadratic)	0.0167	0.0107	2.46	0.1170
	P3 (cubic)	–0.00014	0.0001	2.19	0.1389
Azadirachtin (Bio neem)	P0 (constant)	6.4222	1.2290	27.31	< 0.001
	P1 (linear)	–0.1232	0.0462	7.10	0.0077
	P2 (quadratic)	–0.0024	–	–	–
	P3 (cubic)	0.00005	6.705E-6	47.51	< 0.0001

1	2	3	4	5	6
Cypermethrin	P0 (constant)	6.2986	1.6540	14.50	0.0001
	P1 (linear)	–0.4661	0.1955	5.69	0.0171
	P2 (quadratic)	0.0124	0.0064	3.73	0.0534
	P3 (cubic)	–0.0001	0.00006	3.08	0.0792
Methoxyfenozide	P0 (constant)	31.40	0.0757	172,351.4	< 0.0001
	P1 (linear)	–3.1297	–	–	–
	P2 (quadratic)	0.0952	–	–	–
	P3 (cubic)	–0.00084	–	–	–
Pyridalil	P0 (constant)	23.79	0.0835	81,106.53	< 0.0001
	P1 (linear)	–2.0250	–	–	–
	P2 (quadratic)	0.0577	–	–	–
	P3 (cubic)	–0.0005	–	–	–

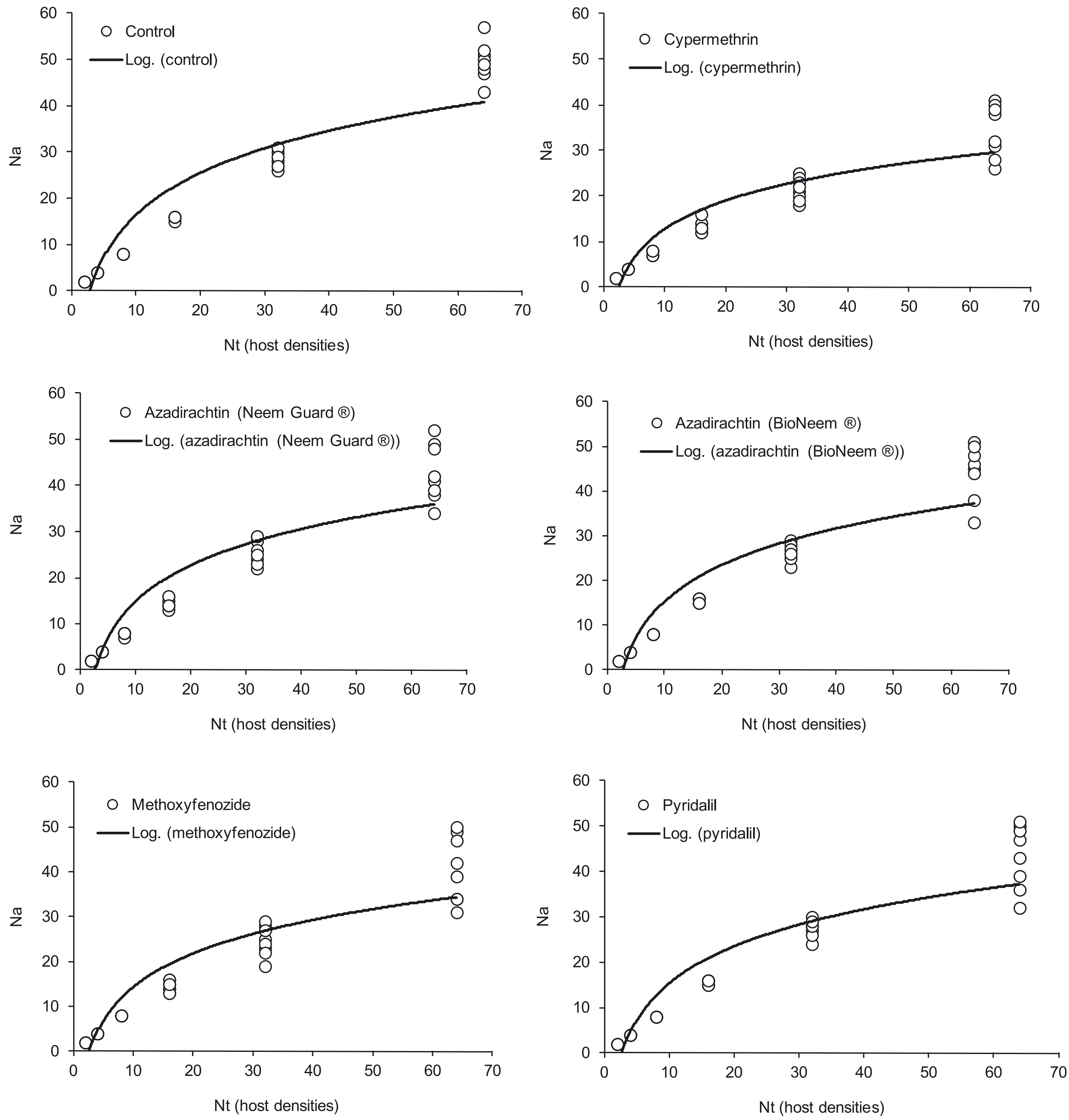

Fig. 1. Functional response of the *H. hebetor* exposed to the LC_{30} of different insecticides and in control to densities of 5th instars of *E. kuehniella* larvae. A type II cure was fitted by least squares, following Holling (1959) equation in all treatments. Overlapping values are shown as a single dot, n = 8 for each density

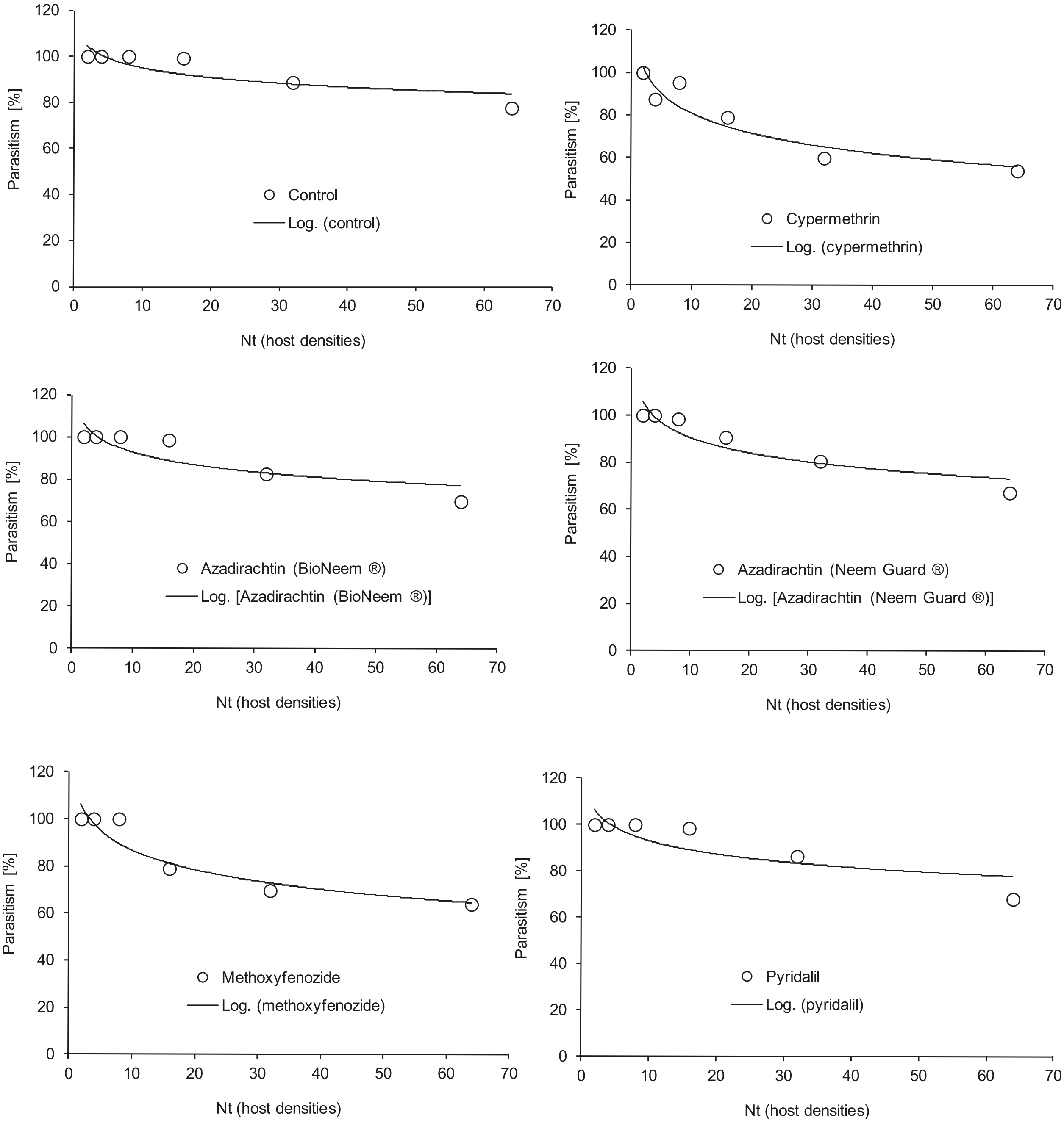

Fig. 2. The percentage of parasitized host by *H. hebetor* exposed to LC_{30} of different insecticides and in control were declined with increasing host density. Relationship between the larval densities of *E. kuehniella* larvae and the parasitization rate by *H. hebetor*

Table 2. Functional response parameters estimated for *H. hebetor* which had been exposed to LC_{30} insecticides

Treatment	Functional response type	Attack rate [h] a ±SE (Lower Upper)	Handling time [h] T_h±SE (Lower Upper)	(r^2)	Maximum attack rates [(24 hour/ handling time (T_h)]
The control	II	0.00292±0.000242 (0.00243–0.00341)	0.4143±0.0155 (0.3830–0.4455)	0.96	57.92
Azadirachtin (Neem Guard)	II	0.00298±0.000322 (0.00233–0.00362)	0.4975±0.0225 (0.4522–0.5428)	0.93	48.24
Azadirachtin (Bio neem)	II	0.00313±0.000324 (0.00248–0.00378)	0.4839±0.0207 (0.4421–0.5256)	0.94	49.59
Cypermethrin	II	0.00259±0.000359 (0.00187–0.00332)	0.6240±0.0349 (0.5538–0.6942)	0.89	38.46
Methoxyfenozide	II	0.00309±0.000395 (0.00230–0.00389)	0.5353±0.0274 (0.4802–0.5904)	0.91	44.83
Pyridalil	II	0.00354±0.000375 (0.5049–0.0208)	0.5046±0.0208 (0.4626–0.5465)	0.94	47.56

T_h – handing time; T/T_h – maximum attack rates (24 h)/handing time; SE – standard error parasitoid-host interaction, as the functional response, attack rates, and handling time was of major importance.

The handling times and coefficient of attack rates are shown in table 2. The negative values for the linear parameters obtained in the present study confirm the type II response for all treatments.

This study showed that control and cypermethrin had the lowest (0.4143±0.0155 h) and maximum (0.624±0.0349 h) handling time, respectively. The highest and lowest value of attack rate was observed in pyridalil (0.0035±0.0004 per hour) and cypermethrin (0.0029±0.00024 per hour), respectively.

DISCUSSION

Studies of the effects of pesticides on functional response can contribute to the success of IPM programs and to the release of the natural enemies. The studies are also a contribution since the LC_{30} value is crucial to assess possible sublethal effects in natural enemies surviving in such conditions of exposure in IPM (Croft 1990). Therefore in this study the sublethal effects of cypermethrin, two formulations of azadirachtin, methoxyfenozide and pyridalil insecticides were evaluated on the functional response of ectoparasitoid *H. hebetor.* The result of this study showed the attack rate of the parasitoid in the insecticide treatments was higher than the control, likely due to altering their behavior. The results indicated that there was a type II functional response of *H. hebetor* in response to host density in insecticide treatments as well as the control. Mahdavi-ParchinSofla (2011) conciliated the sublethal effects of abamectin, carbaryl, chlorpayrifos and spinosad on the functional response of ectoparasitoid *H. hebetor,* and reported a type III response for all treatments and the control. The results of this study are consistent with the results of Rafiee-Dastjerdi *et al.* (2009), who reported that the functional response of *H. hebetor* to host density in insecticide treatments and the control was type II. Claver *et al.* (2003) studied the effect of cypermetrin on the functional response of *Acanthaspis pedestris* (Stal) (Het., Reduviidae) and reported a functional response of type II which is consistent with our results. The results of the effects of the insecticides on handling time showed that cypermethrin had the highest adverse effect on the host-finding of *H. hebetor*. It is recognized that the functional response derived from laboratory studies may bear little resemblance to those that may be measured in the field (Munyaneza and Obrycki 1997). Houck and Strauss (1985) pointed out, however, that laboratory functional response studies can be used to infer basic mechanisms underlying natural enemy-prey-host interactions. Such studies provide valuable information for biological control programs. For example, comparisons of the attributes of different parasitoid species can be made, and baseline information can be established for quality control standards in mass-rearing projects (Montoya *et al*. 2000). In classical biological control, a density-dependent response of natural enemies to prey density is necessary to regulate prey populations (Murdoch and Oaten 1975). In conclusion, our results indicated that the insecticides can affect the functional response of parasitoid and also that functional response can play an important role in the quality control of a mass reared biocontrol agent. In addition to laboratory studies, more attention should be devoted to semi-field and field conditions to obtain more applicable results in the field.

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