# Sublethal effects of some insecticides on the functional response of *Ae*nasius bambawalei Hayat, 2009 (Hymenoptera: Encyrtidae)

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Received June 17, 2021; accepted April 23, 2022. Delo je prispelo 17. junija 2021, sprejeto 23. aprila 2022

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Abstract: Aenasius bambawalei Hayat, 2009 is one of the most effective natural enemies of Phenacoccus solenopsis Tinsley, 1898. The sublethal effects of dimethoate, imidacloprid, and thiodicarb on the functional response of A. bambawalei to different densities of third instar nymphs of P. solenopsis were evaluated under laboratory conditions. Young females were exposed to the insecticides and then introduced to the host densities of 2, 4, 8, 16, 32, and 64 for 24 h. The results revealed a type III functional response in control and insecticide treatments. The handling time and maximum attack rates of A. bambawalei females were adversely affected by insecticides. The longest handling time and the lowest value of maximum attack rate were observed in thiodicarb treatment, 5.03 h and 4.76, respectively. Therefore, for the simultaneous application of biological and chemical control of P. solenopsis, the influence of insecticides on the functional response behavior of natural enemies must be evaluated.

Key words: parasitoid; biological control; chemical control; IPM

Subletalni učinki nekaterih insekticidov na funkcionalen odziv vrste *Aenasius bambawalei* Hayat, 2009 (Hymenoptera: Encyrtidae)

Izvleček: Vrsta Aenasius bambawalei Hayat, 2009 je najučinkovitejši naravni sovražnik škodljivca Phenacoccus solenopsis Tinsley, 1898. Subletalni učinki dimetoata, imidakloprida in tiodikarba na funkcionalni odziv vrste A. bambawalei na različne gostote nimf tretjega štadija škodljivca P. solenopsis so bili ovrednoteni v laboratorijskih razmerah. Mlade samice so bile izpostavljene insekticidom in nato prinešene gostitelju v gostotah 2, 4, 8, 16, 32, in 64 za 24 h. Rezultati so pokazali funkcionalni odziv tipa III pri kontroli in obravnavanjih z insekticidi. Na čas obravnavanja in največji napad samic vrste A. bambawalei so negativno vplivala obravnavanja z insekticidi. Najdaljši čas obravnavanja (5,03 h) in najmanjša vrednost maksimalnega napada (4,76) sta bila opažena pri obravnavanju s tiodikarbom. Zaradi tega moramo pri hkratnem biološkem in kemijskem uravnavanju škodljivca P. solenopsis predhodno ovrednostiti vpliv insekticidov na funkcionalni odziv naravnega sovražnika.

Ključne besede: parazitoid; biološki nadzor; kemijski nadzor; IPM

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## 1 INTRODUCTION

Extensive application of synthetic insecticides has caused unsuitable effects on ecosystems such as insect resistance, pest outbreaks, pesticide residues in soil and products, and undesirable effects on non-target organisms (Ambrose et al., 2010). On the other hand, in an integrated pest management (IPM) program, success mainly depends on the simultaneous use of chemical compounds and biological control agents and insecticides are essential elements for pest population suppression (Abedi et al., 2012). Thus, in such systems, the compatibility of insecticides with biocontrol agents is the main concern for IPM managers (Martinou & Stavrinides, 2015).

Parasitoid wasps can regulate the pest population and prevent the pest outbreak (Hentz et al., 1998). One of the major interactions between parasitoids and pests that can influence pest suppression is functional response (Holling, 1959). In parasitoids, functional response refers to the relationship between the number of hosts attack by a female parasitoid during a given time interval as a function of host density (Solomon, 1949). According to Holling (1959, 1966), there are three types of functional responses. In type I, the parasitoid has a constant search rate overall densities and the result is a linear response until parasitoid satiation (Hassell, 1978). In type II, the parasitoid response to the pest density is curvilinear and in higher densities changes to plateau. Type II response integrates parasitoid handling time, which defines as the time parasitoids spend for overcoming and parasitizing a host. Moreover, parasitoid cleaning and resting behavior before starting to search for a new host are included. Type III shows a sigmoid curve that rises to a plateau when the parasitoid feels satiation. The sigmoid form is due to a slow increase in the attack rate of the parasitoid in higher densities of the host (Holling, 1959, Hassell, 1978).

Cotton mealybug, *Phenacoccus soleopsis* Tinsley (Hemiptera: Pseudococcidae), has been reported as a serious pest on cotton in the United States for the first time (Fuchs et al., 1991) and then from India and Pakistan (Hodgson et al., 2008, Sahito et al., 2011). It was also described as a pest of *Hibiscus rosa-sinensis* L. in Nigeria and Iran (Akintola & Ande, 2008, Joodaki et al., 2018). *P. soleopsis* is highly polyphagous and more than 202 plant species are attacked across the world including ornamentals, fruits, weeds, and field crops (Kumar et al., 2009, Fand & Suroshe, 2015). Several chemical insecticides are used for managing mealybug infestation; however, due to the wax covers the whole body and the cryptic habit of the pest, the efficiency of the method is limited (Fand & Suroshe, 2015). The pest is attacked by 23 species of predators and 7 species of parasitoids in Iran and all of the reported parasitoids belong to the family Encyrtidae (Mossadegh et al., 2015). Among them, only the solitary parasitoid, *Aenasius bambawalei* Hayat is effective in suppressing the pest population and has a key role in its natural parasitism (Fand & Suroshe, 2015).

Pesticide exposure is one of the several factors which can influence the functional response of Natural enemies (Martinou et al., 2015). The effect of sublethal concentrations of insecticides on different parasitoids such as Diaeretiella rapae (McIntoch, 1855) (Hym.: Braconidae) (Rezaei et al., 2014), Habrobraon hebetor Say, 1836 (Hym.: Braconidae) (Abedi et al., 2012, Mahdavi et al., 2013, Rashidi et al., 2018), Dolichogenidea tasmanica (Cameron, 1912) (Hym.: Braconidae) (Paull et al., 2014), and Eretmocerus mundus Mercet, 1931 (Sohrabi et al., 2014) have been reported in previous studies. Nevertheless, there is no available information on the sublethal effects of insecticides on A. bambawalei. In the current study, the sublethal effects of dimethoate, imidacloprid, and thiodicarb on the functional response of the parasitoid wasp, A. bamabawalei, were investigated on P. solenopsis.

## 2 MATERIALS AND METHODS

## 2.1 INSECT REARING

Different life stages of *P. solenopsis* were collected from twigs of *Hibiscus rosa-sinensis* L. available at the campus of Agricultural Sciences and Natural Resources University of Khuzestan. Young potato, *Solanum tuberosum* L., sprouts (0.5-1.5 cm length) were used as a laboratory host of the mealybugs and the transfer was carried out using a fine brush. Then, the potato sprouts were kept in a container ( $24 \times 10 \times 16$  cm) covered with fine mesh. The newly established colony was used in the experiments.

To create the colony of *A. bambawalei*, the parasitized nymphs of *P. solenopsis* were collected from the same *H. rosa-sinensis* twigs. A separate container was used to keep the mummies until the adults' emergence. While the adult parasitoids appeared in the containers they were moved by an aspirator to new containers containing 3<sup>rd</sup> instar nymphs of *P. solenopsis*. Both colonies were maintained in the incubators at 27  $\pm$  2 °C, 65  $\pm$  5 % R. H., and 14 l: 10 D h, and all the experiments were carried out in the mentioned conditions.

#### 2.2 INSECTICIDES

Dimethoate, imidacloprid, and thiodicarb were tested in the experiments. Table1 shows more information about insecticides.

#### 2.3 FUNCTIONAL RESPONSE BIOASSAY

For these experiments, the sublethal concentrations of 1, 0.5, and 25 ppm of dimethoate, imidacloprid, and thiodicarb were used, respectively. The cylindrical plastic containers (15 cm high and 7 cm diameter) were considered as exposure cages and impregnated to the insecticidal solution for 30 seconds. Distilled water was used as control. Then, they were allowed to dry for 24 h. After this time, 50 newly emerged mated female wasps (less than one day old) were released in each exposure cage for 24 h. Then, randomly selected females were transferred to the new containers consist of different densities of 2, 4, 8, 16, 32, and 64 third instar nymphs of P. solenpsis. The containers were transferred to the incubators with the above-mentioned conditions for 24 h. Then the females were removed and the containers were kept in the incubator until the appearance of mummies. The number of parasitized nymphs was recorded. Ten replications were considered for this experiment.

#### 2.4 STATISTICAL ANALYSIS

Logistic regression analysis of the proportion of host-parasitoid  $(N_a/N_o)$  as a function of host density  $(N_o)$  was used to determine the functional response type of *A. bamabawalei* as recommended by Juliano (2001). This was done by fitting the below polynomial function:

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

Where  $N_a$  is the number of parasitized hosts,  $N_0$  is the initial number of hosts offered, and  $P_0$ ,  $P_1$ ,  $P_2$ , and

Table 1: Information about experimental insecticides

 $P_3$  are intercept, linear, quadratic, and cubic parameters, respectively. These parameters were calculated using the method of maximum likelihood (PROC CATMOD, SAS Institute, 2001). If the linear parameter ( $P_1$ ) is negative, the type of functional response is II, whereas a positive linear parameter reveals a type III functional response (Juliano, 2001).

In the second step, a nonlinear least-squares regression (PROC NLIN; SAS Institute Inc., 2001) was used to calculate the functional response parameters  $(T_h$  and either *a* for type II functional response or *b*, *c*, and *d* for type III functional response) using Roger's random parasitoid equation (Rogers, 1972; Juliano, 2001). The parameters were estimated according to the following equations:

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

where  $N_a$  is the number of parasitized hosts,  $N_o$ , the initial number of hosts, is the instantaneous searching efficiency (attack rate), *T* is the total amount of time available for searching (24h), and is the handling time.

For modeling the type III functional response, the searching efficiency (a) in equation (2) was substituted with equation (3) with a function of host density.

$$a = \frac{(d+bN_0)}{(1+CN_0)} \tag{3}$$

Where *a*, *b*, *c*, and *d* are constants and must be estimated. In cases where both *d* and *c* were not significantly different from 0; the case observed in this study, led to  $a = bN_0$  which was inserted into Equation (2). This yielded the following formula (Hassell, 1978):

$$N_a = N_0 [1 - \exp(-bTN_0/1 + bT_h N_0^2)]$$
(4)

Since the data from the above experiment fitted the type III functional response, the functional response parameters were obtained using (3) and (4).

The maximum attack rate  $(T/T_h)$  which indicates the maximum number of hosts that can be parasitized by an individual parasitoid during 24 h, was estimated using calculated  $T_h$  (Hassell, 2000).

Insecticide	Active ingredient	Company	Formulation	Dosage
Confidor®	Imidacloprid	Aria Chimi	35 % SC	0.5 ppm
Roxion®	Dimethoate	KimiyaGohareKhak	40 % EC	1 ppm
Larvin®	Thiodicarb	Moshkfam Fars	80 % DF	25 ppm

## 3 RESULTS

The logistic regression analysis of the proportion of *P. solenopsis* parasitized by *A. babmawalei* indicated Type III functional response for this parasitoid in control and all insecticidal treatments. The linear coefficient,  $P_1$ , was positive and the quadratic coefficient,  $P_2$ , was negative in all treatments (Table 2).

Therefore, the proportion of host parasitized was density-dependent, which shows a type III functional response (Figure 1).

Roger's random parasitoid equation was used for data analysis in control and other treatments. According to the results of nonlinear least square regression parameters, c and d were not significantly different from zero; therefore, they were removed from the model and a reduced model was used (Equation 4). Estimated b values for the control, dimethoate, imidacloprid, and thiodicarb were 0.00620, 0.00304, 0.00344, and 0.00267, and estimated handling times in these treatments were 2.87, 4.92, 3.70, and 5.03 h, respectively (Table 3). Comparing the asymptotic 95 % confidence of b values in Table 3 indicated that imidacloprid had no significant effect on the value of the b of A. bambawalei; however, it was significantly reduced in dimethoate and thiodicarb treatments. Nevertheless, handling time  $(T_{\mu})$  significantly increased in all insecticide treatments. The values of  $r^2$ , coefficient of determination, revealed that Rogers's random parasitoid equation properly explained the functional response of *A. bambawalei* in all treatments (Table 3).

The maximum number of  $3^{rd}$  instar nymphs that could be parasitized by *A. bambawalei*  $(T/T_h)$  decreased from 8.35 in control to 6.49, 4.88, and 4.76 in imidacloprid, dimethoate, and thidicarb, respectively (Table 3).

#### 4 DISCUSSION

Pesticides are prevalent elements in integrated pest management programs and the study of their behavioral effects such as functional response on natural enemies can be helpful for the success of IPM. According to the results of the current study, the functional response of A. babmawalei in control and insecticide treatments was of type III. Parasitoids with type III functional response are density-dependent and can increase their search rate on higher densities of the host. Although a previous overview on functional response indicated that type II is more common in parasitoids (Fernandez-arhex & Corley, 2003), several studies indicate type III in parasitoid wasps such as Eretmocerus mundus Mercet on Bemisia tabaci Gennadius, 1889 (Sohrabi et al., 2014), Aphidius colemani Viereck, 1912 on Aphis gossypii Glover, 1877 (Van Steenis & El-Whawass, 1995) and Praon volucre (Haliday, 1833) on Myzus persicae (Sulzer, 1776) (Tazerouni et al., 2016). According to an earlier study, type II functional response was

coccus solenopsis parasitized by Aenasius bambawalei as a function of initial host density				
Treatment	Parameters	Estimate	SE	P value
Control	$P_0$ (Constant)	-0.4730	0.3778	0.2105
	$P_{I}$ (Linear)	0.0528	0.0578	0.3614
	P (Quadratic)	-0.00344	0.00220	0.1189

Table 2: Maximum likelihood estimates from logisitic regression analysis of the proportion of third instar nymphs of *Pheno*coccus solenopsis parasitized by *Aenasius bambawalei* as a function of initial host density

	$P_{1}$ (Linear)	0.0528	0.0578	0.3614
	$P_2$ (Quadratic)	-0.00344	0.00220	0.1189
	$P_{3}$ (Cubic)	-0.000035	0.000022	0.1055
Dimetoat	$P_0$ (Constant)	-1.6313	0.4598	0.0004
	$P_{l}$ (Linear)	0.0853	0.0689	0.2160
	$P_2$ (Quadratic)	-0.00388	0.00261	0.1366
	$P_{3}$ (Cubic)	0.000036	0.000026	0.1619
Imidacloprid	$P_0$ (Constant)	-1.1003	0.4118	0.0075
	$P_{I}$ (Linear)	0.0659	0.0625	0.2919
	$P_2$ (Quadratic)	-0.00350	0.00238	0.1408
	$P_{3}$ (Cubic)	0.000034	0.000024	0.1458
Thiodicarb	$P_0$ (Constant)	-1.4500	0.4536	0.0014
	$P_{1}$ (Linear)	0.0488	0.0689	0.4787
	$P_2$ (Quadratic)	-0.00249	0.00262	0.3421
	$P_{3}$ (Cubic)	0.000022	0.000026	0.3894

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**Figure 1:** Functional response of *A. bambawalei* to different densities of 3<sup>rd</sup> instar nymphs of *P. solenopsis* in different insecticide treatments. From the top: dimethoate, thiodicarb, imidacloprid, and control treatments. Symbols are observed data and lines were predicted by the model (Equation 2)

Treatments	b	T <sub>h</sub>	T/T <sub>h</sub>	$r^2$
Control	$0.00620 \pm 0.00103$	$2.87\pm0.0980$	8.35	0.96
	(0.00484- 0.00825)	(2.6781-3.0704)		
Dimethoate	$0.00304 \pm 0.000881$	$4.92\pm0.3197$	4.88	0.96
	(0.00128-0.00480)	(4.2774-5.7773)		
Imidacloprid	$0.00344 \pm 0.00106$	$3.70\pm0.2602$	6.49	0.86
	(0.00131-0.00556)	(3.1747-4.2163)		
Thiodicarb	$0.00267 \pm 0.000740$	$5.03\pm0.3203$	4.76	0.88
	(0.00119-0.00415)	(4.3940-5.6765)		

**Table 3:** Parameters ( $\pm$  SE) estimated by random parasitoid equation indicating functional response of *A. bambawalei* to different treatments

The values in parentheses are 95 % confidence intervals; b: constant;  $T_h$ ; handling time;  $T/T_h$  maximum attack rate

recorded for A. bambawalei to different densities of P. solenopsis at selected temperatures which is varied from the results of our study (Joodaki et al., 2018). Several factors including difference in experimental conditions, parasitoid strain, host plant cultivar, host species, the age of parasitoid and host, physiological state of the host may be responsible for the change of functional response type in parasitoid wasp (Sagarra et al., 2001, Ambrose et al., 2010, Asadi et al., 2012, Pasandideh et al., 2015, Tazerouni et al., 2016, Joodaki et al., 2018). In this study, the type of functional response did not change depending on different insecticide treatments. There is no available information about other studies reporting the effects of insecticides on the functional response of A. bambawalei. On the other hand, Habrobracon hebetor Say, 1836 showed type III functional response to different densities of Anagasta kuheniella Zeller, 1879 in control and all insecticide treatments (Mahdavi et al., 2013). In another study, the type of functional response of Diaretiella rapae (McIntosh) did not change when it was exposed to primicarb and thiamethoxam (Rezaei et al., 2014). However, Sohrabi et al. (2014) reported an alteration in type III functional response of Eretmocerus mundus Mercet in control treatment as well as imidacloprid to type II in buprofezin treatment. Moreover, the sublethal concentrations of primicarb, cypermethrin, and dimethoate changed the functional response of D. rapae from type II to type III (De-Jiu et al., 1991).

Handling time (the time needed to subdue and consume the prey item) and attack rate are important parameters used to assess the parasitoid functional response (Juliano, 2001). In this study, all insecticides had a significant negative effect on the handling time of parasitoids; however, the influence of thiodicarb was even higher than the other insecticides and had the most adverse effect on the host-finding of the parasitoid. The difference in handling time in insecticide treatments may be due to their various mode of action, which influence the neural system of parasitoids. The longer handling time in parasitoids exposed to insecticides could be related to the commotion of their neural system (Rezaei et al., 2014). An increase in handling time under insecticide treatments has been reported in different parasitoids such as *H. hebetor* when exposed to spinosad (Dastgersi, 2009), and cypermethrin (Abedi et al., 2012).

Among all insecticide treatments, dimethoate and thiodicarb had the most, and imidacloprid had the least negative effect on the value of maximum attack rate of the wasp. The parasitism rate of *A. bambawalei* in imidacloprid treatment was 1.3 times more than dimethoate and thiodicarb; however, in control, it was distinctly higher than insecticide treatments. Insecticides can cause a repellent effect on parasitoids or decrease their host finding ability due to boosting disturbance and reducing the olfactometric abilities, which may result in a reduction in parasitism rate (Decourtye et al., 2004).

# 5 CONCLUSIONS

The results confirmed the negative impact of insecticides on the functional response of parasitoids and probably the success of biological control programs. Hence, for the simultaneous application of biological and chemical control in integrated pest management programs, the influence of insecticides on the different behavior of natural enemies such as functional response has to be evaluated. Such information is essential to estimate the appropriate time to release natural enemies in the case of using insecticides.

## 6 ACKNOWLEDEMENTS

The authors would like to thank Agricultural Sciences and Natural Resources University of Khuzestan, Iran, for the financial support of this research project.

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