Journal of Entomology and Zoology Studies

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JEZS
Journal of Entomology and Zoology Studies

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Effect of sublethal doses of Abamectin and fenpropathrin on functional response of *Cryptolaemus Montrouzieri* (Coleoptera: Coccinellidae) predator of *Planococcus citri* (Hemiptera: Pseudococcidae)

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Abstract

The sublethal affects of abamectin and fenpropathrin on the functional response of predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant a potential biological control agent, were investigated on 4th-instar nymphs of *Planococcus citri* Risso as prey. The experiment was conducted in varying densities (2, 4, 8, 16, 32, 64 and 128) of *P. citri* at 28 ± 1 °C, $60\% \pm 10\%$ relative humidity (RH) and a photoperiod of 16:8 h (L: D). The results of logistic regressions showed a type II functional response in the control and all insecticide concentrations. Comparison of functional response curves revealed that tested insecticides markedly decreased the mean of preys consumed by *C. montrouzieri*. The search rates found for predators decreased when exposed to increasing sublethal concentrations from LC_{10} to LC_{30} of both insecticides. The handling times of females exposed to insecticides were in all cases lower than in the control treatment. The maximum handling time observed in LC_{10} of fenpropathrin and the minimum attack rate observed in LC_{30} of abamectin. The maximum theoretical predation was increased when exposed to increasing sublethal concentrations from LC_{10} to LC_{30} of Abamectin but the highest theoretical maximum predation observed in LC_{20} of fenpropathrin. The studies suggested that the adverse effect of these insecticides on *C. montrouzieri* must be considered in integrated pest management programs (IPM).

Keywords: *Cryptolemus montrouzieri*, *Planococcus citri*, sub-lethal concentrations, attack rate, handling time

1. Introduction

The citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae) is a key pest of citrus and other crops. It is a polyphagous pest known to be found on 175 plants [1]. While feeding, citrus mealybugs produce large quantities of honeydew, and secondary injury can be motive by the growth of sooty molds [1]. Control of *P. citri* is difficult due to its particular cryptic behavior and to its wax cover that protects these insects from insecticide implementations [2].

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae) is a very efficient natural enemy of the citrus mealybugs and has worldwide distribution [3]. Both adults and larvae of these beetles eat the citrus mealybugs completely [3]. Successful control of the citrus mealybug species by this predator has been reported in many control programs [4]. Extensive research has been guided on *C. montrouzieri* feeding on citrus mealybugs [5, 6]. It has received much attention from investigator as well as farmers as a potential biological control agent [6]. Interest in utilizing this beneficial predator as one of the most important components of integrated pest management (IPM) programs for field and horticultural crops has recently increased as growers have found alternatives to insecticides for managing pests. Since *C. montrouzieri* is a generalist predator, the effective and proper utilization of this predator is necessary for a positive effect in the IPM programs.

Functional response is the main element in prey-predator interactions which was considered by Solomon [7] for the first time. It is descripted as the change in number of preys attacked by a natural enemy per unit of time in relation to prey densities [7, 8]. Functional response has been organized into three basic categories suggested by Holling (1959) [8]. Most of the predators and parasitoids exhibit type II functional response but type III has been observed in some species too [9].

There are many factors such as insecticides that affect the functional response of a natural enemy [10]. Insecticides can change the behavior of predators through their neurotoxic activity,

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even in low doses. They may also reduce the predator's behaviors such as searching rate, response to prey kairomones and pheromonal communication. However, changes in foraging patterns and diminish of odor learning have been reported [11].

Side-effects of insecticides on survival of natural enemies, including *C. montrouzieri*, have been tested since the 1980's [12, 13] but only a few studies have examined sublethal effects of insecticides on the functional response of natural enemies [14]. There is no previous study on sublethal effects of insecticides on *C. montrouzieri*.

In this paper, we present the impact of abamectin and fenpropathrin on the functional response of *C. montrouzieri*. Abamectin and fenpropathrin was chosen as a representative of insecticides commonly used against many pests of fruit and vegetable [15]. Both insecticides have been shown to be toxic to many predatory insects [16, 17]. Such information can be utilized to predict the potential for integrating the use of these insecticides with one of the natural enemies of *P. citri*.

2. Materials and Methods
The research work was organized at Entomology laboratory of Islamic Azad University of Takestan, Iran during 2014.

2.1. Rearing of host insect, *P. citri*
The culture of *P. citri* was reared on different vegetables (Potato sprouts and Pumpkin) under controlled conditions at a constant temperature of 28±1 ℃, with 60±10% R.H and 16:8 L: D photoperiod in growth chamber and also in plastic jars of different sizes. The citrus mealybug colonies were renewed by replacing the older vegetables with new fresh vegetables to make sure continuous supply of *P. citri* to the predator throughout the experimental duration.

2.2. Rearing of predator, *C. montrouzieri*
C. montrouzieri adults were reared under controlled conditions of 28±1 ℃, with 60±10% R.H and 16:8 L: D photoperiod in growth chamber. Then adults were kept in plastic container and diets were provided on potato sprouts and pumpkin infested with *P. citri* crawlers every morning. The diets were renewed daily with new infested crawlers. The plastic containers were covered with fine nylon mesh to allow ventilation at the top. Sprouts of potatoes and pumpkin were provided as an oviposition substrate for the collection of eggs. *C. montrouzieri* adults usually gave eggs singly and the eggs were collected daily early in the morning during the time of observations. The eggs were kept in another plastic container and tissue paper was provided at the bottom to avoid dry. Upon hatching the larvae were fed citrus mealybug crawlers in the same container till adult emergence. The adults were shifted to stock colony to maintain the culture continuously. The rearing jars were checked every morning for the collection of eggs. Eggs were collected with hair brush and also on respective vegetable leaves.

Chemicals and application
Commercial formulations of fenpropathrine (Meothrin, 20% EC, Giah, Iran) and abamectin (Vermectin®EC 1.8%, Giah, Iran) were utilized in the experiments. To assess the effects of the insecticides on adult *C. montrouzieri*, a modified leaf-dip technique was used [25]. Fresh pumpkin pieces (5 cm in diameter) were treated with sublethal concentrations of

fenpropathrin and abamectin and allowed to dry for about 3 h. The insecticide concentrations were 7.63 μg a.i./ml (LC$_{10}$), 10.65 μg a.i./ml (LC$_{20}$) and 16.70 μg a.i./ml (LC$_{30}$), for fenpropathrin and 2.10 μg a.i./ml (LC$_{10}$), 4.60 μg a.i./ml (LC$_{20}$) and 5.70 μg a.i./ml (LC$_{30}$), for abamectin. Each treatment was replicated six times.

2.3. Experimental Procedures
The experiment was performed to calculate the functional response of adult female of *C. montrouzieri* at the same laboratory conditions used for rearing the predator. Individual predator were assigned to one of seven prey densities (2, 4, 8, 16, 32, 64, 128 4th-instar nymphs) for 24 h after which the predators were removed and the number of remaining intact nymphs counted. Six replications were conducted for each prey density.

2.4. Data analysis
The functional response data were calculated in two steps: (1) the type of the functional response was determined by logistic regression of the proportion of prey killed as a function of initial density and (2) the random predator equation was fitted to data after determining the functional response type [18]. In the logistic regression analysis, the polynomial function from Juliano (2001) [18] was utilized:

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

where N_a is the number of prey eaten, N_0 is the initial prey density and P_0, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively, estimated using the method of maximum likelihood [18]. Significant negative or positive linear coefficient (i.e. P_1) from the regression indicates type II or III, respectively [18].

For type II functional responses an attempt was made to fit the data to Rogers' random predator equation [19], which, however, in all cases failed to provide a good description of the data. The data were consequently fitted to Holling's disc equation (2) [20]:

$$N_a = \frac{aTN_0}{1 + aT_h N_0} \quad (2)$$

Where T is the time available for searching during the experiment (here 24 h), a the instantaneous attack rate, and T_h the amount of time that the predator handles each prey (handling time).

The logistic regression analysis was performed using a SAS program provided by Juliano (2001) [18]. The functional response parameters were estimated using SAS PROC NLIN [21]. Separation of statistically different parameter estimates was made using upper and lower limits of the 95% confidence interval.

3. Results
In all treatments, *C. montrouzieri* exhibited a type II functional response to 4th-instar nymphs, as the linear coefficient of the logistic regression was estimated as significantly negative (linear coefficient P_1 (±s.e.) (Table 1). Holling's disc equation (eq. 2 above) [32] could be used to describe the type II

functional response of *C. montrouzieri* providing estimates of both search rates (*a*) and handling times (*T_h*) significantly different from zero (Table 2). Similarly, the model for type II functional responses (eq. 2 above) adequately described the functional response of females exposed to LC$_{10}$, LC$_{20}$ and LC$_{30}$ of fenpropathrin and abamectin (Table 2).

The relationship between number of prey density and number of prey consumed for abamectin and fenpropathrin is showed in Fig.1 and 2. In these figure, the response curve rises in a negatively accelerating manner to a plateau that exhibited type II of functional response. The proportion of preys consumed by a predator with a type II functional response declines exponentially as the prey density increases. Type II is curvilinear and the saturation level is reached in a gradual manner. Comparison of functional response curves showed that functional response curve of *C. montrouzieri* in LC$_{30}$ of fenpropathrin and abamectin was significantly lower than the other treatments.

The search rates found for predators decreased when exposed to increasing sublethal concentrations from LC$_{10}$ to LC$_{30}$ of both insecticides. The handling times of females exposed to insecticides were in all cases lower than in the control treatment. The longest handling time (0.910±0.008) observed

in LC$_{10}$ of fenpropathrin and the lowest attack rate (0.024±0.0008) observed in LC$_{30}$ of abamectin

The theoretical maximum number of prey attacked by the *C. montrouzieri* estimated by the asymptote (T/T_h) of the functional response curve was 10.46 4th-instar nymphs per day for females (attained in the control). The maximum theoretical predation was increased when exposed to increasing sublethal concentrations from LC$_{10}$ to LC$_{30}$ of Abamectin. The highest maximum theoretical predation observed in LC$_{20}$ of fenpropathrin.

Table 1: Linear coefficients of the logistic regression analysis of the proportion of *Planococcus citri* 4th-instar nymphs eaten by female *Cryptolaemus Montrouzieri* exposed to sublethal doses of abamectin or fenpropathrin versus the initial prey density

Treatment	P_1±SE	X^2	P
Control	-0.268 ±0.03	18.69	0.0001
LC$_{10}$ Abamectin	-0.188 ±0.74	37.64	0.0001
LC$_{20}$ Abamectin	-0.069 ±0.03	4.51	0.034
LC$_{30}$ Abamectin	-0.201 ±0.03	3.17	0.075
LC$_{10}$ Fenpropathrin	-0.109 ±0.05	5.78	0.016
LC$_{20}$ Fenpropathrin	-0.073 ±0.03	6.79	0.009
LC$_{30}$ Fenpropathrin	-0.179 ±0.03	38.78	0.0001

Table 2: Estimates (±SE, 95% confidence interval) of search rates (*a'*), handling times (T_h (*h*)) and R^2 values for the models (eq. 2) describing the functional response of female *Cryptolaemus Montrouzieri* exposed to sublethal doses of abamectin or fenpropathrin versus the initial prey density

Treatment[1]	a	T_h	T/T_h	R^2
Control	0.051±0.005 (0.044-0.058)	0.0956±0.029 (0.087-0.102)	10.46	0.985
LC$_{10}$ Abamectin	0.043±0.003 (0.036-0.050)	0.086±0.011 (0.064-0.107)	11.69	0.975
LC$_{20}$ Abamectin	0.040±0.003 (0.034-0.045)	0.078±0.011 (0.057-0.099)	12.78	0.976
LC$_{30}$ Abamectin	0.024±0.0008 (0.022-0.025)	0.019±0.006 (0.005-0.033)	52.35	0.995
LC$_{10}$ Fenpropathrin	0.046±0.003 (0.040-0.052)	0.910±0.009(0.074-0.108)	1.09	0.982
LC$_{20}$ Fenpropathrin	0.031±0.004(0.024-0.039)	0.057±0.019 (0.019-0.044)	17.63	0.947
LC$_{30}$ Fenpropathrin	0.029±0.007(0.014-0.043)	0.188±0.089 (0.008-0.369)	5.31	0.830

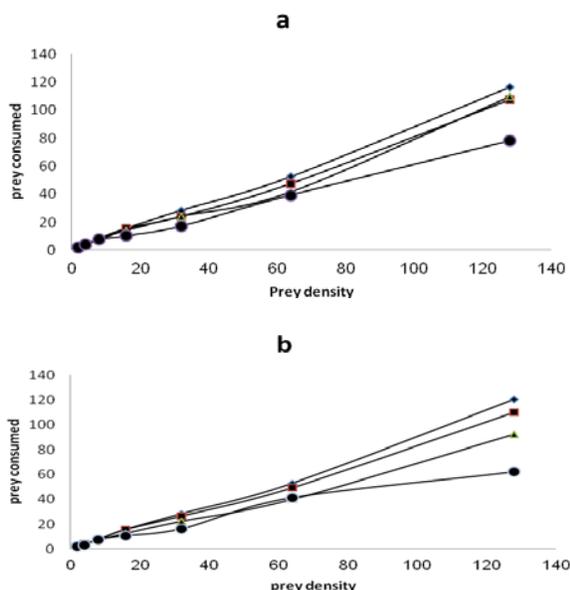Values in parentheses are 95% confidence intervals

Fig 1: Functional responses of *Cryptolaemus Montrouzieri* exposed to sublethal doses of abamectin (a) or fenpropathrin (b) while feeding on insecticide-treated *Planococcus citri* 4th-instar nymphs. ——♦—— control; ——■—— LC$_{30}$; ——▲—— LC$_{20}$; ——●—— LC$_{10}$.

4. Discussion

The negative values for the linear parameters ($P_1 < 0$) obtained in this research confirm type II functional response for all treatments, in which the proportion of preys consumed by a predator decline exponentially as the prey density increases, therefore the linear term is negative. The logistic regression model thus can be recommended as a tool for further analyzing functional response curves. The common functional responses among arthropod predators are type II and III [9]. The results of this research are consistent with the results of Mohasseian *et al.* [22], which showed that the functional response of *C. montrouzieri* on various densities of *P. citri* at different temperatures was type II. Similarly, this type of functional response has also been demonstrated for coccinellid efficiency and handling time compared to control. Our findings showed that *C. montrouzieri* exposed to insecticides had the highest T_h occurred in the LC$_{10}$ of fenpropathrin and abamectin. Moreover, effects of insecticides on *a* revealed that the value of this parameter decreased when exposed to increasing sublethal concentrations and the lowest value was observed in LC$_{30}$ of both insecticides. The searching rate (*a*) is the proportion of the total area searched by a predator/unit of searching time. It determines how rapidly the functional

response curve approaches the upper plateau. Moreover, it is a function of (1) maximum distance at which the predator can perceive the prey, (2) speed of movement of predator that can perceive the prey, (3) proportion of attacks that are successful [23]. Li *et al.* [15] reported that acarophagous thrips, *Scolothrips takahashii* Priesner (Thysanoptera: Thripidae) females exposed to mancozeb showed prolonged handling time. Similarly, fenpropathrin and abamectin caused significantly lower attack rates and prolonged handling time in both males and females of *Scolothrips longicornis* Priesner [24]. Disrubtion of predation by insecticides may drastically reduce the efficiency of natural enemies. The negative effects of insecticides on functional response have been reported in many natural enemies. Ambrose *et al.* [25] investigated the impact of Synergy- 505® (chlorpyrifos 50% and cypermethrin 5% E.C), on functional response of *Rhynocoris marginatus* (Fab.) and demonstrated that this chemical compound caused a less pronounced type II functional response with decreased numbers of prey killed, attack rate, searching rate, and prolonged handling time in 4th and 5th nymphal instars, adult males and females reflecting reduced predatory potential. Gholamzadeh Chitgar *et al.* [26] showed that diazinon, fenitrothion and chlorpyrifos had a negative effect on functional response of *Andrallus spinidens* Fabricius (Hem.: Pentatomidae). They demonstrated that the predatory bugs exposed to insecticides had higher handling time and among the insecticides, fenitrothion caused the most negative effect on searching rate. In addition, Abedi *et al.* [27] indicated that the application of cypermethrin on *Habrobracon hebetor* Say (Hym.: Braconidae) caused longest handling time and lowest attack rate. In addition, the effect of cypermetrin on the functional response of *Acanthaspis pedestris* (Stal) (Hem.: Reduviidae) showed that the insecticide negatively affected the functional response occurrences such as attack rate, handling time and also reduced the predatory efficiency [14].

However, most of the researches and present study also illustrated negative effects of insecticides on the functional response of natural enemies. However, a positive effect on predation rate has been demonstrated for some low-dose of insecticides. For example, a stronger functional response was seen in wolf spiders, *Pardosa pseudoannulata* Boes and Strand (Araneae: Lycosidae) [28] and increased consumption was seen in *Mallada signata* (Schneider) (Neur.: Chrysopidae) [29]. These results can be described by the general theory of hormoligosis (the stimulation of reproductive physiology by sublethal doses of insecticides) [30] or by the perturbation of predator avoidance ability of prey.

Furthermore, our results are valuable as a step in the further evaluation of compatibility between *C. montrouzieri* as a biological control agent and insecticides. More researches are needed to determine the effect of environmental factors on the functional response of coccinellid predators. Under field conditions, interactions between the utilize of chemicals and factors such as the effect of scale [31], the presence of predators like as ants and spiders, spatial complexity [32] and weather may adversely influence the effectiveness of natural enemies [33]. By understanding these interactions, we will be able to develop integrated management programs for *P. citri* involving the exploitation of *C. montrouzieri*.

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