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

Hajar Pakyari, Fazel Kasirloo, Abas Arbab

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±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 LC30 of abamectin. The maximum theoretical predation was increased when exposed to increasing sublethal concentrations from LC10 to LC30 of Abamectin but the highest theoretical maximum predation observed in LC20 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 implementions [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 described 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,
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 [10].

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 °C, 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 °C, 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 fenpropathrin (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 (LC10), 10.65 µg a.i./ml (LC20) and 16.70 µg a.i./ml (LC30), for fenpropathrin and 2.10 µg a.i./ml (LC10), 4.60 µg a.i./ml (LC20) and 5.70 µg a.i./ml (LC30), 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:

\[
N_a = \frac{N_0 e^{-\alpha T}}{1 + e^{-\alpha T}}
\]

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]:

\[
N_a = \frac{aTN_0}{1 + aT_hN_0}
\]

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
The functional response of Cryptolaemus 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 LC10, LC20 and LC30 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 Cryptolaemus Montrouzieri in LC30 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 LC10 to LC30 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 LC30 of fenpropathrin and the lowest attack rate (0.024±0.0008) observed in LC30 of abamectin. The theoretical maximum number of prey attacked by the Cryptolaemus 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 LC10 to LC30 of Abamectin. The highest maximum theoretical predation observed in LC20 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( P \pm SE )</th>
<th>( X^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-0.268 ±0.03</td>
<td>18.69</td>
<td>0.0001</td>
</tr>
<tr>
<td>LC10 Abamectin</td>
<td>-0.188 ±0.74</td>
<td>37.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>LC20 Abamectin</td>
<td>-0.069 ±0.03</td>
<td>4.51</td>
<td>0.034</td>
</tr>
<tr>
<td>LC30 Abamectin</td>
<td>-0.201 ±0.03</td>
<td>3.17</td>
<td>0.075</td>
</tr>
<tr>
<td>LC10 Fenpropathrin</td>
<td>-0.109 ±0.05</td>
<td>7.88</td>
<td>0.016</td>
</tr>
<tr>
<td>LC20 Fenpropathrin</td>
<td>-0.073 ±0.03</td>
<td>6.79</td>
<td>0.009</td>
</tr>
<tr>
<td>LC30 Fenpropathrin</td>
<td>-0.179 ±0.03</td>
<td>38.78</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Values in parentheses are 95% confidence intervals.

Table 2: Estimates (±SE, 95% confidence interval) of search rates \( (a) \), handling times \( (T_h) \) 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( a )</th>
<th>( T_h )</th>
<th>( T/T_h )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.051±0.005 (0.044-0.058)</td>
<td>0.0956±0.029 (0.087-0.102)</td>
<td>10.46</td>
<td>0.985</td>
</tr>
<tr>
<td>LC10 Abamectin</td>
<td>0.043±0.003 (0.036-0.050)</td>
<td>0.086±0.011 (0.064-0.107)</td>
<td>11.69</td>
<td>0.975</td>
</tr>
<tr>
<td>LC20 Abamectin</td>
<td>0.040±0.003 (0.034-0.045)</td>
<td>0.078±0.011 (0.057-0.099)</td>
<td>12.78</td>
<td>0.976</td>
</tr>
<tr>
<td>LC30 Abamectin</td>
<td>0.024±0.0008 (0.022-0.025)</td>
<td>0.019±0.006 (0.005-0.033)</td>
<td>52.35</td>
<td>0.995</td>
</tr>
<tr>
<td>LC10 Fenpropathrin</td>
<td>0.046±0.003 (0.040-0.052)</td>
<td>0.910±0.009 (0.074-0.108)</td>
<td>1.09</td>
<td>0.982</td>
</tr>
<tr>
<td>LC20 Fenpropathrin</td>
<td>0.031±0.004 (0.024-0.039)</td>
<td>0.057±0.019 (0.019-0.044)</td>
<td>17.63</td>
<td>0.947</td>
</tr>
<tr>
<td>LC30 Fenpropathrin</td>
<td>0.029±0.007(0.014-0.043)</td>
<td>0.188±0.089 (0.008-0.369)</td>
<td>5.31</td>
<td>0.830</td>
</tr>
</tbody>
</table>

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 Cryptolaemus 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 Cryptolaemus Montrouzieri exposed to insecticides had the highest \( T_h \) occurred in the LC10 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 LC30 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 acarphagous thrips, Scolotherips takahashi Priesner (Thysanoptera: Thripidae) females exposed to mancozeb showed prolonged handling time. Similarly, fenprofprthrin and abamectin caused significantly lower attack rates and prolonged handling time in both males and females of Scolotherips longicornis Priesner [24]. Disruption 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. Ambro 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 cypermethrin 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.

5. Acknowledgments
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6. References
8. Holling CS. Some characteristics of simple types of predation and parasitism. Canadian Entomology 1959; 7:385-399, XCI.


