

## Effects of Mineral Oils, Palizin and Buprofezin on Functional and Numerical Responses of Predatory Ladybeetle *Cryptolaemus montrouzieri* (Mulsant, 1850)

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### ABSTRACT

Predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant is an important biological agent in citrus orchards. The effect of six treatments included: two types of mineral oils, EC and mayonnaise at the concentration of 15 ml/L, buprofezin 0.75 ml/L, buprofezin+EC oil 0.5+5 ml/L, insecticidal soap palizin 2.5 ml/L, and water as the control were investigated on the functional and numerical responses of *C. montrouzieri* Mulsant under laboratory conditions. The experiment investigated predation rate of female adult coccinellids to varying densities (2, 4, 8, 16, 32 and 64) of mealybug pray, *Pseudococcus citri* Risso. The relationship between the rate of consumption and pray density was explored with a logistic regression. The results of logistic regressions revealed that the rate of prey consumption by the predator had risen as prey density increased, but eventually leveled off in both the control and all insecticide treatments (following Holling's type II functional response). Comparison of attack rates showed that the parameter significantly decreased when the predator was exposed to all insecticides but palizin. Predator handling times for oil mayonnaise, buprofezin and buprofezin+ EC oil treatments were significantly longer than that of the control. The longest handling time (1.6065 h) and the lowest attack rate ( $0.0351 \text{ h}^{-1}$ ) were estimated for buprofezin+oil and oil EC treatments, respectively. As prey density increased, increase in oviposition and decrease in the food exploitation efficiency were observed. The results confirmed that the functional response of *C. montrouzieri* was affected by tested compounds reducing the predatory potential. The exception was palizin that had a low effect on functional response events of treated *C. montrouzieri*.

**Key words:** predatory coccinellid, mealybug, insecticides, functional response, numerical response.

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## INTRODUCTION

*Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is an important biological control agent of citrus scales *Planococcus citri* Risso and *Pulvinaria aurantii* Cockerell in citrus orchards around the world, including Iran (Heidari & Copland 1992; Aghajanzadeh, Halagisani, & Gholamian, 2016). Both larvae and adults of this ladybird feed on the mentioned pests affecting their population growth (Saljoqi et al, 2015).

*C. montrouzieri* is very sensitive to many chemical compounds used in citrus orchards for pest control. The coccinellid can be affected by insecticide sprays which decrease its predation efficiency (Aghabaglou, Alvandy, Goldasteh, & Rafiei, 2013). Many insecticides such as chlorpyrifos, diazinon, buprofezin, malathion, gozathion and etrimfos (Nourbakhsh, 2020), mineral oils (Rajabpour, Seraj, Damavandian, & Shishebor, 2008) and insecticidal soaps (Halagisani, Naseri, Rafiee Dastjerdi, Aghajanzadeh, & Ghadamyari, 2019) are recommended against scale bugs in citrus orchards.

The functional response of a predator is one of the crucial factors in population dynamics of a prey-predator system (Saljoqi et al, 2015). Functional response tests show the potential of a predator's ability to suppress different prey densities. In fact, the functional response relates to the change in predation rate with changing prey density and describes the way natural enemies responds to the changing densities of its prey (Holling, 1959). Predator's attack rate (the rate of successful attack of prey by a predator; also known as searching efficiency or the rate at which a predator searches for finding its prey) and handling time (as a time it takes for a predator to encounter and consume a single prey) are two important parameters of functional response to evaluate the effectiveness of predators (Omkar & Kumar, 2013). In addition to the functional response, numerical response is another component of the predator-prey interactions and is defined as the change in predator numbers with the variation in prey density (Solomon, 1949). Progeny production by predator females can change in relation to prey density (Rahmani Piyani, Shishebor, Kocheili, & Eric, 2021). The functional response is known to be the key factor and defines the numerical response (Keith, Todd, Brand, Adamcik, & Rusch, 1977). The intake rate of a predator is described as a function of food density. Depending on the assumptions Holling (1959) separated functional response into three differently modelled types (type I, II and III). Most natural enemies show type II and III of functional response (Holling, 1959). Type I corresponds to the consumption of prey by the predator resulting in a linear increase. Type II occurs when the rate of predation decelerated reaching an asymptote. Type III involves a sigmoid relation also called S ascension (Santos et al, 2021).

The analysis of both the functional and numerical responses is helpful and provides information regarding the prey-predator relationship, which is required for the successful usage of a biological control agent (Rahmani Piyani, Shishebor, Kocheili, & Eric, 2021).

Several studies showed that insecticides adversely affect the functional and numerical responses of natural enemies. He, Zhao, Zheng, Desneux, & Wu (2012) reported that lady beetle, *Serangium japonicum* Chapin exposed to imidacloprid showed prolonged handling time and decreased attack rate while feeding on eggs of

*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). Two insecticides, imidacloprid and chlorpyrifos decreased attack rate and increased handling time of the predator *Macrolophus pygmaeus* (Hem.: Miridae) that had been feeding on *Tuta absoluta* (Lep.: Gelechiidae) (Sharifian, Sabahi, & Bandani, 2017). The numerical response of *Amblyseius largoensis* Muma (Acari: Phytoseiidae) was affected by exposure to abamectin, where just 60% of the females oviposited (Barros et al, 2022). Many studies had been done on the predatory behavior of *C. montrouzieri* (Jamila, 2011; Abdolahi Ahi et al, 2012; Saljoqi et al, 2015; Qin, Wu, Qiu, Ali, & Cuthbertson, 2019), but there are only a few information on the effect of pesticides on the type and parameters of the functional response (Pakyari, Kasirloo, & Arbab, 2016). Torres & Marcano (2015) tested the numerical response of *C. montrouzieri* to seven densities of third instar nymphs of *Maconellicoccus hirsutus* Green. However, no research has been done on the effect of pesticides on the numerical response of *C. montrouzieri*. In this study, the effects of two types of mineral oils, mayonnaise and emulsifiable concentrates (EC), buprofezin, buprofezin + oil combination, and the insecticidal soap palizin (the widely used insecticides in citrus orchards) examined on the functional and numerical response of *C. montrouzieri* to *P. citri*. The purpose of the study was to determine the type of functional response and estimate the parameters and the reproductive response of females of *C. montrouzieri* when affected by the pesticides. It is important in evaluating the performance of *C. montrouzieri* to a changing prey density and forecasting the suitability of the predator as a potential biocontrol agent. The information obtained would be useful in integration of the pesticides with the predatory coccinellid in integrated pest management of *P. citri*.

## MATERIALS AND METHODS

### Insect rearing

The colonies of the mealybug, *P. citri* and the coccinellid, *C. montrouzieri* were obtained from the insectarium in Amol, Mazandaran province (North Iran) in May 2018. The mealybug was reared on pumpkin, *Cucurbita moschata* Duchesne ex Poir. (Cucurbitaceae) at the temperature of  $26\pm 1$  °C, with  $60\pm 10\%$  R.H and 16:8 L: D photoperiod in a growth chamber. Every two weeks, new fresh pumpkins were added to the mealybug colony for the continuous supply of *P. citri*. The larvae of *C. montrouzieri* were kept in plastic containers ( $3\times 7$  cm<sup>2</sup>) in a rearing chamber and were provided with a daily diet of *P. citri*. After emergence, adults of *C. montrouzieri* were transferred to Petri dishes (60 mm in diameters). Egg sacs of mealybug were used as an oviposition substrate. After the copulation and egg laying, the eggs of *C. montrouzieri* were collected with a hair brush and cultured until the emergence of new adults. The same age adults were used for the functional response experiment.

### Chemicals

The compounds used in this study are mineral oils - EC (Ghazal Shimi, Iran, degree of sulphonation: 92% (90% chlorinated paraffin mineral oil + 10% emulsifier)

and mayonnaise (Cayan, Iran, degree of sulphonation: 80% (80% chlorinated paraffin mineral oil + 18% water+ 2% emulsifier), buprofezin insecticide (Nihon Nohyaku, Japan, 40% SC), and insecticidal soap, palizin (Kimia sabzavar, Iran, Coconut soap 65%).

### **Bioassay**

The insecticides were used at recommended field concentrations (Nourbakhsh, 2020). Mineral oils: EC and mayonnaise at the concentration of 15 ml/L, buprofezin 0.75 ml/L, buprofezin+oil (EC) 0.5+5 ml/L, and insecticidal soap palizin 2.5 ml/L. For bioassay, two hundred (1 day old) female and male adults of *C. montrouzieri* were used in each treatment. They were separated in different Petri dishes (60 mm in diameters) and placed into groups of 10 individuals per dish. Before the treatment, Petri dishes were placed in a refrigerator for 20 min to reduce the mobility of ladybeetles. Then each Petri dish containing adults of *C. montrouzieri* was sprayed with 1 ml of insecticide solution according to Karamaouna et al, (2013). Control Petri dishes were sprayed with water. After spraying, the excess solution was removed and Petri dishes were kept under laboratory conditions at  $25\pm 1^{\circ}\text{C}$ , 65% RH and a 16:8 light: dark photoperiod.

### **Functional response experiment**

Twenty-four hours after the treatment, only the surviving females of *C. montrouzieri* were collected for use in the functional response experiment. The surviving males were transferred to other Petri dishes with mealybugs as food and kept until numerical response experiment. With a mortality  $\leq 40$  percent for insecticide treatments in the bioassay, three hundred sixty females were used in the functional response experiment (sixty female adults in each treatment). Adults were starved for another 12 hours and then individually transferred to Petri dishes (60 mm in diameters). Mealybugs of the same age were used as prey for ladybeetles at different densities (2, 4, 8, 16, 32 and 64). After 24 h, the predators were removed and the number of consumed prey (Na) was recorded. Each density was replicated 10 times (10 female adults in each prey density) for insecticide treatments and the control. All experiments were carried out under laboratory conditions as mentioned above.

### **Numerical response**

For the numerical response experiment, three hundred sixty females from the functional response part were used (sixty female adults in each treatment). Females from each prey density were mated with the surviving treated males of the same age. Within 24 hours, after observing mating activity, females were transferred to separate Petri dishes provided with different densities of prey (2, 4, 8, 16, 32 and 64). Petri dishes were monitored daily and the number of eggs laid by females was counted. After removing the eggs for daily counting and prevention of cannibalism, each female was transferred to a new Petri dish containing the same prey density as before. With an average of 7.58 days pre- oviposition period, the number of eggs laid by each female was recorded daily for 15 days (Therefore, the whole experimental procedure including the functional response, time for the mating and the numerical response, lasted 17 days). The experiment was replicated ten times (10 female adults in each

prey density). Also, food exploitation efficiency in females was calculated using the following equation (Omkar & Kumar, 2013):

Food exploitation efficiency (%) = number of prey consumed/number of prey offered × 100

### Data analysis

The type of the functional response was determined by logistic regression analysis (SAS/STAT, CATMOD procedure version 9.0) of the proportion of prey killed ( $N_a$ ) in relation to the initial prey density ( $N_0$ ) (Trexler & Travis 1993). The data were fitted to the logistic regression which describes the relationship between  $N_a/N_0$  and  $N_0$  (Juliano, 2001):

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

Where  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the intercepts of linear, quadratic and cubic coefficients, respectively, and estimated using the method of maximum likelihood. If the linear parameter  $P_1$  is negative, a type II functional response is evident, whereas a positive linear parameter indicates density-dependent predation and thus a type III functional response (Juliano, 2001). After the determination of the shape of the curve, the handling times and attack coefficients of a type II response were estimated using Holling's disk equation (Williams & Juliano, 1985):

$$N_a = N_0(1 - \exp(-a(T_h N_a - T)))$$

Where  $N_a$  is the number of preys attacked,  $a$  is the attack rate,  $N_0$  is initial prey density,  $T$  is the total available time and  $T_h$  is the handling time.

Pairwise comparisons of functional responses parameters were performed using the indicator variable method (Juliano, 2001):

$$N_a = N_0 \left\{ 1 - \exp \left[ \frac{-a + Da(j)(T)}{(1 + (a + Da(j))(Th + DTh(j)))(N_0)} \right] \right\}$$

Where  $j$  is an indicator variable that takes on a value of 0 for the first data set and one for the second data set. The parameters,  $D_a$  is a difference in the attack rate and  $D_{Th}$  is a difference in the handling time between two treatments. In other words, the attack rate for one treatment is  $a$ , and that for another treatment is  $D_a$ . Testing for a significant difference in attack rates between two treatments is accomplished by testing the null hypothesis that  $D_a = 0$  (Juliano, 2001, Allahyari, Fard, & Nozari, 2004). To find the difference between two handling times (for control and treatment), it must be proved that  $D_{Th}$  is not equal to zero. If  $D_{Th}$  isn't significantly different from zero, the difference between  $T_h$  and  $T_h + D_{Th}$  is not significant and the two handling times are not statistically different (Juliano, 2001).

## RESULTS

### Consumption-prey density

The relationship between the number of prey density and the number of prey consumed for all treatments is showed in Fig. 1. In this figure, with the increase in the prey density, functional response decreases exponentially. The number of killed mealybugs was significantly higher in the control treatment than in insecticide treatments at all densities. The mean number of mealybugs that are consumed by *C. montrouzieri* at the highest prey density (64 mealybugs) was 21.5, 15, 16.2, 13.2, 11.2, and 20 mealybugs/predator in the control, EC oil, mayonnaise oil, buprofezin, buprofezin+ oil, and palizin treatments, respectively. There were significant differences in the number of mealybugs killed by treated *C. montrouzieri* at different prey densities (4 mealybugs:  $F = 6.76$ ,  $df = 5,54$ ,  $p < 0.0001$ ; 8 mealybugs:  $F = 24.27$ ,  $df = 5,54$ ,  $p < 0.0001$ ; 16 mealybugs:  $F = 16.43$ ,  $df = 5,54$ ,  $p < 0.0001$ ; 32 mealybugs:  $F = 28.99$ ,  $df = 5,54$ ,  $p < 0.0001$ , and 64 mealybugs:  $F = 26.54$ ,  $df = 5,54$ ,  $p < 0.0001$ ).

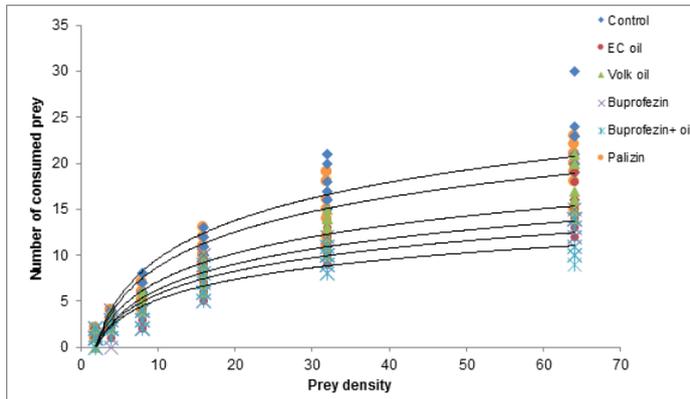


Fig. 1. Functional response of predatory coccinellid, *Cryptolaemus montrouzieri* exposed to the insecticides to different densities of mealybug, *Pseudococcus citri*.

### Attack rate/handling time-treatment

Estimated parameters of the logistic regression for the functional response for all treatments are presented in Table 1. Since the linear parameter ( $P_1$ ) was negative, the functional response for all treatments was type II. The values of attack rate ( $a$ ) and handling time ( $T_h$ ), estimated by Rogers' random attack equation are presented in Table 2. Insecticides caused adverse effects on functional response parameters of treated *C. montrouzieri* compared to control ladybeetles. The greatest attack rate value ( $0.0919 \text{ h}^{-1}$ ) and the shortest handling time value ( $0.9006 \text{ h}$ ) were obtained in the control treatment (Table 2). The longest handling time value ( $1.6065 \text{ h}$ ) and the lowest attack rate value ( $0.0351 \text{ h}^{-1}$ ) were estimated for buprofezin+oil and oil EC treatments, respectively (Table 2). Differences of functional response parameters of treated *C. montrouzieri* are presented in Table 3. Comparison of attack rates showed that the parameter significantly decreased when predator exposed to all insecticides

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but palizin (Table 3). Handling times for oil mayonnaise, buprofezin and buprofezin+oil treatments were significantly longer than that for the control. However, values for the attack rate and the handling time of *C. montrouzieri* treated by palizin were lower and longer than those for the control, but without significant statistical differences. Results of comparing functional response parameters of the two types of oils showed a significant difference for attack rates but no statistically *significant difference* for handling times. However, adding oil to buprofezin decreased attack rate and increased handling time to buprofezin treatment, but without a significant difference.

Table 1. Estimated parameters of the logistic regression for functional response of treated coccinellid, *Cryptolaemus montrouzieri* to different densities of mealybug, *Planococcus citri*.

Treatment	Parameters	Estimate	SE	$\chi^2$	p
Control	Intercept	2.0183	0.2754	53.73	0<.0001
	Linear	-0.0736	0.0166	19.71	0<.0001
	Quadratic	0.000492	0.000202	5.92	0.0150
	Cubic	-0.00004	0.000026	1.80	0.1796
Oil (mayonnaise) 15 ml/L	Intercept	0.8182	0.2236	13.39	0.0003
	Linear	-0.0487	0.0145	11.25	0.0008
	Quadratic	0.000298	0.000184	2.63	0.1047
	Cubic	-0.00002	0.000022	0.72	0.3971
Oil Ec 15 ml/L	Intercept	0.3620	0.2181	2.75	0.0970
	Linear	-0.0402	0.0145	7.72	0.0055
	Quadratic	0.000251	0.000185	1.86	0.1731
	Cubic	-0.00002	0.000022	1.06	0.3029
Buprofezin 0.75 ml/L	Intercept	0.2188	0.2181	1.01	0.3159
	Linear	-0.0362	0.0145	6.21	0.0127
	Quadratic	0.000183	0.000186	0.97	0.3254
	Cubic	7.683E-6	0.000021	0.13	0.7196
Buprofezin+ Oil Ec 0.5+5 ml/L	Intercept	-0.00758	0.2194	0.00	0.9724
	Linear	-0.0286	0.0147	3.77	0.0521
	Quadratic	0.000070	0.000189	0.14	0.7131
	Cubic	-3.89E-7	0.000022	0.00	0.9857
Palizin 2.5 ml/L	Intercept	1.5513	0.2481	39.10	0<.0001
	Linear	-0.0657	0.0154	18.10	0<.0001
	Quadratic	0.000455	0.000191	5.66	0.0174
	Cubic	-3E-7	0.000023	0.00	0.9897

### Food exploitation-treatment-prey density

Food exploitation efficiency was decreased with an increase in prey density in each treatment (Table 4). There were no significant differences in food exploitation efficiency among treatments at density of 2 mealybugs whereas at the densities of 4, 8, 16, 32 and 64 mealybugs significant differences were observed (Table 4). At the highest prey density (64 mealybugs), the lowest food exploitation efficiency was obtained in buprofezin+oil treatment and without a significant statistical difference in buprofezin treatment.

Table 2. Coefficient of attack rate (a) and handling time (Th) estimated by Rogers random attack equation in treated ladybeetle, *Cryptolaemus montrouzieri* to different densities of mealybug, *Planococcus citri*.

Treatments	Parameters	Estimate	SE	95% CI	
				Lower	Upper
Control	a' (h <sup>-1</sup> )	0.0919	0.0116	0.0687	0.1151
	T <sub>h</sub> (h)	0.9006	0.0468	0.8068	0.9943
Oil (mayonnaise) 15 ml/L	a' (h <sup>-1</sup> )	0.0501	0.00494	0.0402	0.0599
	T <sub>h</sub> (h)	1.1137	0.0621	0.9894	1.2380
Oil EC 15 ml/L	a' (h <sup>-1</sup> )	0.0351	0.00308	0.0289	0.0413
	T <sub>h</sub> (h)	1.0887	0.0709	0.9468	1.2306
Buprofezin 0.75 ml/L	a' (h <sup>-1</sup> )	0.0365	0.00387	0.0288	0.0443
	T <sub>h</sub> (h)	1.3357	0.0885	1.1585	1.5128
Buprofezin+oil 0.5+5 ml/L	a' (h <sup>-1</sup> )	0.0355	0.00384	0.0278	0.0432
	T <sub>h</sub> (h)	1.6065	0.0987	1.4090	1.8041
Palizin 2.5 ml/L	a' (h <sup>-1</sup> )	0.0715	0.00817	0.0552	0.0879
	T <sub>h</sub> (h)	0.9441	0.0523	0.8394	1.0488

Table 3. Differences of functional response parameters estimated by an equation with an indicator variable in treated ladybeetle, *Cryptolaemus montrouzieri* to different densities of mealybug, *Planococcus citri*.

Treatments	Parameters	Estimate	SE	95% CI	
				Lower	Upper
Control- Oil mayonnaise	D <sub>a</sub>	-0.0962	0.0431	-0.1814	-0.0109
	D <sub>Th</sub>	0.2085	0.0856	0.0390	0.3779
Control- Oil EC	D <sub>a</sub>	-0.1174	0.0405	-0.1977	-0.0372
	D <sub>Th</sub>	0.1903	0.0989	-0.00570	0.3862
Control -Buprofezin	D <sub>a</sub>	-0.1153	0.0417	-0.1979	-0.0328
	D <sub>Th</sub>	0.4261	0.1162	0.1960	0.6562
Control -Buprofezin+oil EC	D <sub>a</sub>	-0.1164	0.0406	-0.1968	-0.0359
	D <sub>Th</sub>	0.6910	0.1366	0.4205	0.9616
Control -Palizin	D <sub>a</sub>	-0.0577	0.0502	-0.1571	0.0417
	D <sub>Th</sub>	0.0399	0.0733	-0.1053	0.1850
Oil EC- Oil mayonnaise	D <sub>a</sub>	0.0213	0.00906	0.00332	0.0392
	D <sub>Th</sub>	0.0182	0.0941	-0.1682	0.2047
Buprofezin-Buprofezin+oil	D <sub>a</sub>	-0.00102	0.00774	-0.0163	0.0143
	D <sub>Th</sub>	0.2649	0.1338	-0.00011	0.5300

Da is a difference in the attack rate and DTh is a difference in the handling time between two treatments.

### Number of eggs-treatment-prey density

The number of eggs laid by females from each treatment was positively correlated with prey density (Fig. 2). With increasing prey density from 2 to 64, the number of eggs produced by a female was: in the control (F<sub>5,54</sub> = 45.7; P < 0.0001), mayonnaise oil (F<sub>5,54</sub> = 40.3; P < 0.0001), EC oil (F<sub>5,54</sub> = 50.94; P < 0.0001), Buprofezin (F<sub>5,54</sub> = 40.65; P < 0.0001), Buprofezin+oil (F<sub>5,54</sub> = 47.67; P < 0.0001), and palizin (F<sub>5,54</sub> = 46.83; P < 0.0001).

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Table 4. Food exploitation efficiency (%) of treated *Cryptolaemus montrouzieri* females (n=10) at different densities of mealybug, *Planococcus citri*.

Densities	Treatments						
	Control	Oil (mayonnaise) 15ml /l	Oil EC 15 ml/L	Buprofezin 0.75 ml/L	Buprofezin+oil 0.5+5 ml/L	Palizin 2.5 ml/L	
2	85.0±7.6 a	75±11.1 a	75.0±8.3a	60.0±10.0a	55.0±13.8a	85.0±7.6a	P=0.1849 F5,54=1.57
4	92.5±3.8 a	67.5±5.3 abc	55.0±5.0 bc	57.5±9.8 bc	50.0±5.2 c	77.5±5.8 ab	P<0.0001 F5,54=6.76
8	81.2±3.8 a	58.7±3.2 bc	46.2±4.1 cd	37.5±3.7 d	37.5±3.2 d	72.5±4.0 ab	P<0.0001 F5,54=24.2
16	68.7±2.0 a	51.2±2.9 b	43.7±2.4 b	46.8±3.8 b	41.2±2.6 b	65.6±2.8 a	P<0.0001 F5,54=16.4
32	55.3±2.1 a	40.0±1.5b	34.6±1.1 bc	31.2±1.1 c	29.6±1.0 c	47.8±3.1 a	P<0.0001 F5,54=28.9
64	33.5±1.8 a	25.3±1.2 b	23.4±1.0 b	20.6±0.6 bc	17.5±0.8 c	31.2±1.1 a	P<0.0001 F5,54=26.5

Means in the row followed by the same letters are not statistically different (Tukey,  $p \leq 0.05$ )

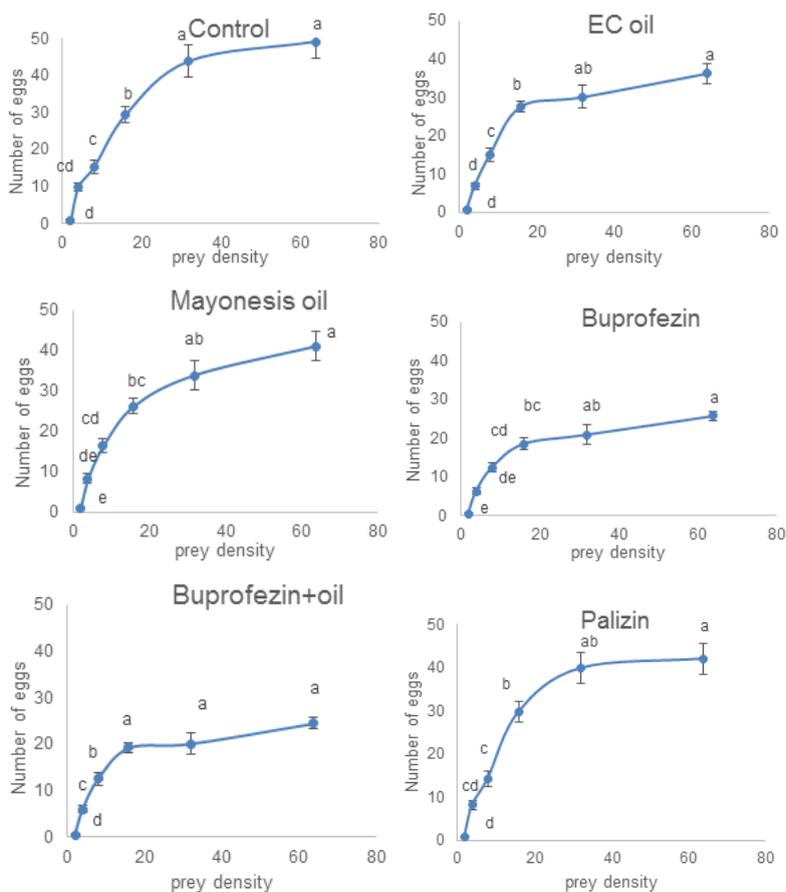


Fig. 2. The relationship between the number of eggs laid by females of *Cryptolaemus montrouzieri* and mealybug, *Planococcus citri* densities as prey

Points are mean  $\pm$  SE. Means with different letters indicate significant difference (Tukey,  $p \leq 0.05$ )

Table 5. Mean number ( $\pm$ SE) of eggs laid by treated *Cryptolaemus montrouzieri* females (n=10) at different densities of mealybug, *Planococcus citri*.

Densities	Treatments						
	Control	Oil (mayonnaise) 15 mL/L	Buprofezin 0.75 mL/L	Oil EC 15 mL/L	Palizin 2.5 mL/L	Buprofezin+oil 0.5+5 mL/L	
2	0.9 $\pm$ 0.3a	1.0 $\pm$ 0.3 a	0.8 $\pm$ 0.3 a	0.5 $\pm$ 0.3 a	0.4 $\pm$ 0.2a	0.8 $\pm$ 0.2a	P=0.73 F5,54=0.56
4	9.9 $\pm$ 1.2a	8.3 $\pm$ 1.3 a	6.8 $\pm$ 1.0 a	6.3 $\pm$ 0.8 a	6.0 $\pm$ 0.7a	8.1 $\pm$ 1.1a	P=0.12 F5,54=1.84
8	15.3 $\pm$ 1.8a	16.4 $\pm$ 1.8 a	15.1 $\pm$ 1.7 a	12.4 $\pm$ 1.3 a	12.5 $\pm$ 1.4a	14.2 $\pm$ 1.8a	P=0.49 F5,54=0.89
16	29.4 $\pm$ 2.2a	26.2 $\pm$ 1.9 ab	27.6 $\pm$ 1.5 a	18.5 $\pm$ 1.5 b	19.2 $\pm$ 1.2b	29.7 $\pm$ 2.4a	P<0.0001 F5,54=7.32
32	43.8 $\pm$ 4.4a	33.9 $\pm$ 3.6 ab	30.1 $\pm$ 3.0 ab	20.9 $\pm$ 2.5 b	20.1 $\pm$ 2.2b	39.8 $\pm$ 3.6a	P<0.0001 F5,54=8.48
64	49.0 $\pm$ 4.2a	41.1 $\pm$ 3.6 a	36.2 $\pm$ 2.6 ab	25.7 $\pm$ 1.3 b	24.5 $\pm$ 1.2b	42.0 $\pm$ 3.7a	P<0.0001 F5,54=9.92

Means in the row followed by the same letters are not statistically different (Tukey,  $p \leq 0.05$ )

Among treatments, there were no significant differences in the number of eggs laid at the densities of 2, 4 and 8 mealybugs (Table 5). Whereas at the densities of 16, 32 and 64 mealybugs, differences were statistically significant. At the density of 64 mealybugs, the lowest mean number of eggs laid by *C. montrouzieri* was related to buprofezin (25.7) and buprofezin+oil (24.5) treatments, and without a significant difference in the EC oil (36.2) treatment. The highest mean number of eggs was in the control treatment (49.0), followed by palizin treatment (42.0), while the differences in treatments with oils were not statistically significant.

## CONCLUSIONS AND DISCUSSION

Prey consumption can be decreased when predators are exposed to different insecticides (Sharifian, Sabahi, & Bandani, 2017). Pesticides may interfere with the feeding behavior of exposed insects and cause the behavioral alterations in motility include lack of motor coordination, tremors, downfalls, abdomen tucking and rotational movement for abdomen cleaning (Suchail, Guez, & Belzunces, 2001). Leg and proboscis tremor of the predatory bug, *Andrallus spinidens* Fabricius (Hem.: Pentatomidae) after pesticides application was observed. This abnormal behavior increased the time that takes for a predator to encounter and consume a single prey and reduced prey consumption (GholamzadehChitgar, Hajizadeh, Ghadamyari, Karimi-Malati, & Hoda, 2014). In this study, treated *C. montrouzieri* with the insecticides captured fewer prey compared to the control. The more adverse effects were observed in the treatments that used buprofezin. Buprofezin might either inhibit the activity of some enzymes related to the activity of coccinellid or it might influence the neuroendocrine system, which might adversely affect predation (Gu, Bei, & Gao, 1993; Deng, Xu, Cao, & Dai, 2008). Prey consumption was less affected in palizin treated *C. montrouzieri* than in the oils treated the coccinellid. It may be because different chemical structures and modes of action of the two compounds which will be discussed in more detail in the following.

According to the negative values obtained for the linear parameters  $P_1$ , *C. montrouzieri* exhibited type II functional response on *P. citri* in all treatments. The proportion of prey consumed decreased as the density of prey increased may be due to satiation of the predator (Toft, 2005). Similar results for *C. montrouzieri* were reported by Ghorbanian (2010) and Abdollahi Ahi et al (2012). According to Pakyari et al (2016) functional response of *C. montrouzieri* adults on different densities of *P. citri* was type II at 27°C and 65±5% RH. Fourth instar larvae, female and male adults of *C. montrouzieri* showed type II when fed on different densities of cotton mealybug, *Phenacoccus solenopsis* Tinsley at 28±1°C and 65±5% RH (Saljoqi et al, 2015). Functional responses of adult males and females of *C. Montrouzieri*, and its 3rd instar larvae on *P. citri* were studied at seven constant temperatures ranging from 15 to 40°C, at % 60 ± 10 RH. Results showed that coccinellid adults and its 3rd instar larvae were type II, except for the 3rd instar larvae at 40°C, which were type III (Mohasesian, Ranjbar Aghdam, & Pakyari, 2015). Type II of functional response has also been demonstrated for coccinellids, *Oenopia conglobata* Linnaeus feeding on different densities of *Schizaphis graminum* Rondani (PahlavanYali & Bozorg-Amirkalae, 2018) and *Harmonia axyridis* Pallas preying on crapemyrtle aphid, *Tinocallis kahawaluokalani* Kirkaldy (GholamzadehChitgar, 2019). Qin, Wu, Qiu, Ali, & Cuthbertson (2019) reported the functional response of *C. montrouzieri* predating on mealybug, *Dysmicoccus neobrevipes* Cockerell as type II. According to the results, insecticides exposure did not change the type of the functional response of *C. montrouzieri*. But the asymptote of the curve had a tendency towards lower values in insecticide treatments compared to the control. When treated with buprofezin+oil, asymptote of the functional response curve was lower than in treatments with other insecticides. A lower asymptote of treated coccinellids represents either a decrease in attack rate or an increase in handling time (Butt, Talib, & Khan, 2019). The predatory spider, *Oxyopes javanus* Thorell when exposed to sublethal concentrations of lambda cyhalothrin (95.5 a.i. µg/L), emamectin benzoate (125.14 a.i. µg/L) and imidacloprid (2475.1 a.i. µg/L) showed a lower asymptote in the functional response curves compared to the control, without a change in the type of a functional response in all treated and untreated groups (Butt, Talib, & Khan, 2019). Other studies also reported a significant decrease in prey consumption when natural enemies exposed to different insecticides without change in the type of functional response (GholamzadehChitgar, Hajizadeh, Ghadamyari, Karimi-Malati, & Hoda, 2014; Amini Jam, 2017; Sharifian, Sabahi, & Bandani, 2017). In contrast, exposure to imidacloprid affected the type of the functional response of the predator *Podisus nigrispinus* Dallas. *P. nigrispinus* presented a type III functional response in the absence of the insecticide, but the treatment with imidacloprid caused a type II response (Malaquias, Ramalho, Omoto, Godoy, & Silveria, 2014). *Coccinella septempunctata* L. larvae showed type II functional response after feeding on the *brassica aphids* (*Brevicoryne brassicae* L.) treated with thiamethoxam, lambda-cyhalothrin and cypermethrin, while imidacloprid, profenophos and chlorpyrifos treated aphids altered the functional response of larval coccinellids from type II to III. The change in response was linked to unconsciousness and disorientation induced by the insecticides targeting insect nervous system (Afza, Riaz, Afzal, & Majeed, 2021).

Feeding behavioral perturbations induced by insecticides with decreased capture, prolonged handling time and reduced attack rate negatively affect the efficiency of natural enemies (Ambrose, Rajan, & Raja, 2010). In this research, buprofezin+oil and oil EC treatments had the greatest effect on the functional response of *C. montrouzieri* compared to other insecticide treatments causing the longest handling time and the lowest attack rate, respectively. Oil in combination with buprofezin can have a synergistic effect and increase the diffusion and transport of insecticide molecules into the insect body (Mulrooney, Womac, & Greever, 1993). It can explain the more negative effect of buprofezin+ oil in comparison to buprofezin treatment on the predatory response of *C. montrouzieri*. Oils used in this study was petroleum products: stock emulsion type (mayonnaise) a polyphase product and miscible type (EC) a monophasic product (Tajbakhsh, & Heidari, 2016). After application of oils on insect's body, they penetrate the cuticle, diffuse and accumulate within lipid-containing tissues, primarily the fat bodies. Accumulation in the nerve ganglia cause the direct effect of suppressing synaptic transmission in the insect's ganglia (Najar-Rodríguez, Lavidis, Mensah, Choyd, & Waltera, 2008). Behavior abnormalities such as receptor coating after oil spray may occur in treated insects and cause host location failures (Simons, 1982). The negative behavioral effects may result time-consuming activities that prolong handling time and reduce prey consumption. It can explain significantly longer handling time in oil mayonnaise treated *C. montrouzieri* compared to the control. But for oil EC treated *C. montrouzieri*, there was no statistically significant difference in handling times with the control probably depending on the type of oil. Many researchers have reported that insecticides exposure can affect the attack rate and the handling time of coccinellids. Pakyari et al (2016) showed that *C. montrouzieri* exposed to LC<sub>10</sub> of fenpropathrin (7.63 µg a.i./ml) and LC<sub>30</sub> of abamectin (2.10 µg a.i./ml) resulted in the highest handling time and the lowest attack rate, respectively. Imidacloprid residual (LC<sub>50</sub>) increased handling time and decreased the attack rate of *S. japonicum* (He, Zhao, Zheng, Desneux, & Wu, 2012). On the other hand, palizin had a low effect on the functional response events of treated *C. montrouzieri* compared to the other insecticide treatments. Palizin as insecticidal soap (Potassium salts of fatty acids) dissolves the waxy layer on insect's body and damages the cell membrane. Saponification of the lipids in the cell tissues results in the cell membrane rupture and loss of vital fluids (Baniameri, 2008). Soft bodied insects such as aphids, whiteflies, and mealy bugs are more susceptible to desiccation. while, soaps have a minimal activity on some hard-bodied beneficial insects, such as adult lady beetles due to the insect's thickened cuticle (Weinzierl, 2000). A similar result associated with low harmful effects of palizin was reported by Amini Jam (2017) when the insecticidal soap effect was evaluated on the functional response of the parasitoid wasp, *Lysiphlebus fabarum* Marshall. The insecticide was effective against scale insects, *P. aurantii* and *P. citri* in citrus orchards (Ahmadi, Amiri-Besheli, & Hosieni, 2012; Halagisani et al, 2019) and relatively harmless to natural enemies (Kabiri & Amiri-Besheli, 2012; Gholamzadeh Chitgar, 2017).

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In this study, with increased prey density, food exploitation efficiency decreased. This finding coincides with the research findings of Omkar & Kumar (2013) on food exploitation efficiency of females ladybeetle, *Anegleis cardoni* Weise in response to changing prey densities of *Aphis gossypii* Glover. Zarghami, Mossadegh, Kocheili, Allahyari, & Rasekh (2015) reported the reduction in food exploitation efficiency of *Nephus arcuatus* Kapur, with increasing density of its prey *Nipaecoccus viridis* Newstead. It was caused due to satiation of the predator after consuming a sufficient amount of the prey (Omkar & Kumar, 2013). Probably, a greater reduction on food exploitation efficiency of *C. montrouzieri* in the treatments that buprofezin was used, is due to its more adverse effect on altering predator behavior.

With increasing prey density, more eggs by per female in each treatment were produced. An increase in food quantity facilitates the development of more numbers of ovarioles affecting the egg production by the predator (Evans, 2000). Dixon & Guo, (1993) reported that the egg production in ladybirds was determined by the availability of a prey. The number of eggs laid by females of *A. cardoni* was positively correlated with, *A. gossypii* density (Omkar & Kumar, 2013). The prey density-dependent productivity was also observed in *Scymnus coccivora* Ayyar preying on cotton mealybug, *P. solenopsis* (Kumari, Suroshe, Kumar, Budhlakoti, & Yana, 2021). Egg production in treated *C. montrouzieri* females with oils and palizin was less affected than buprofezin, probably due to differences in the mode of action. Buprofezin can negatively affect egg formation in adult insects by preventing the production of the hormone prostaglandin (Ishaaya, Mendelson & Melamed-Madjar, 1988; Uchida, Isawa & Sugimoto, 1987). The adverse effect of buprofezin on the egg production has been reported by several researchers (Grafton-Cardwell & Gu, 2003; James, 2004). Buprofezin (0.1 and 0.2 a.i./liter) had a destructive effect on the egg formation of the predatory coccinellid, *Delphastus catalinae* Horn (Liu & Stansly, 2004). Penetration of an insecticide into the reproductive system of female and male adults, may cause malformations and affect eggs production (Retnakaran, Granett, & Ennis 1985; Mathew, Vijayalaxmi, & Rahiman, 1992; Carpenter & Chandler, 1994).

According to the results, the natural control effects of *C. montrouzieri* would be weakened by the application of buprofezin. But palizin had less adverse effect on functional and numerical responses of the ladybeetle, *C. montrouzieri*. It can be used in conjunction with *C. montrouzieri* in the integrated pest management of citrus scales. The oils were found to be effective against *P. citri* and according to the results, they had less adverse effects than buprofezin on *C. montrouzieri* female adults. So, they can be used as an alternative to the insecticide. But use of oils for control of scale bugs should be at the proper dose and time to avoid hazardous effects on the predator. Moreover, greenhouse and field based studies are needed to determine the compatibility of the insecticides with *C. montrouzieri* and effectiveness of exposed *C. montrouzieri* against scale bugs under more realistic conditions.

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