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Study on Ovicidal and Side Effects of Diazinon and Imidaclopride on *Cryptolaemus Montrouzieri* Mulsant (Coleoptera: Coccinellidae)

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ABSTRACT

Lethal and sub lethal effects of two insecticides, diazinon and imidaclopride, were studied on *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) in the laboratory conditions. The newly laid eggs of *C. montrouzieri* were exposed to the insecticides by dipping method. The obtained results indicated that diazinon and imidaclopride were moderate toxic (33-66%) to egg of *C. montrouzieri* (according to IOBC standard method). The significant differences were observed for pupal period, post oviposition, longevity and gross fecundity rate (GFR), Mean eggs per day, net reproductive rate (R0) and doubling time (DT) of *C. montrouzieri* between two examined insecticides. Imidaclopride showed the highest inhibiting effect on stable population growth parameters of *C. montrouzieri.*

Keywords: *Cryptolaemus montrouzieri*, lethal effect, Development parameters, Reproductive parameters, population growth parameters, diazinon, imidaclopride.

1. Introduction

Biological control or the use of natural enemies such as parasitoids, predatory beetles, predatory bugs, as well as beneficial bacteria, fungi, and nematodes is a current strategy to manage greenhouse pests [46]. However, the only use of biological control and natural enemies may not always be sufficient to control pests in greenhouse [28]. The use of insecticides compatible to biological control agents [39] and protection of biological control agents are useful tools in integrated pest management program (IPM) [22]. However, conservation of them is limited for incompatibility between insecticides and natural enemies. Continued use of insecticides has harmful effects on non-target arthropod because beneficial species sensitive than their prey or host [36]. So, study on undesirable effects of insecticides on non-target insects and natural enemies are crucial [45], [39-41]. Selective application of insecticide can reduce adverse effects of insecticide on natural enemies [17]. *Cryptolaemus montrouzieri* Mulsant *(*Col.: Coccinellidae) is a worldwide Bio control agents [10]. This predator is a very efficient natural enemy of pest species, such as whitefly [18], aphids [16], [20], mealy bugs [18, 21], scales [11, 14], and mites [9]. *C. montrouzieri* is the most commonly used biological control for citrus mealy bugs in greenhouses [42] and both adults and larvae of *C. montrouzieri* are predator of mealy bugs [10]. Unfortunately, this predator as same as other natural enemies is so susceptible to chemical pesticides.

Imidaclopride is the first neonicotinoid insecticide [30]. This insecticide acts on post synaptic acetylcholine receptors in the insect nervous system [1]. Imidaclopride is effective against sucking insect, including aphids, scale insects, whiteflies, some Coleoptera, and some Lepidoptera [15].

Diazinon is a contact organophosphate pesticide and used for to control various insect pests [7]. There is little information about undesirable effects of these two insecticides on *C. montrouzieri.* This study was carried out to evaluate the lethal and sublethal effects of imidaclopride and diazinon on development and population growth parameters of *C. montrouzieri.*

2. Materials and Method

2.1 Insects

a. Prey: Colony of *Planococcus citri* (Risso) were supplied by Plant Protection Department of Behshahr, Mazandaran province, Iran. *P. citri* were reared on potato tubers as host plant in a climate-controlled growth chamber (25±2 °C, 65±5% RH).

b. Predator: *Cryptolaemus montrouzieri* were supplied by Baghdaran Sabz Dezfoul, Khuzestan province, Iran. *C. montrouzieri* were reared on *p. citri*) in a climate-controlled growth chamber (25±2 °C, 65±5% RH).

2.2 Insecticides

in this study Imidacloprid 35% SC, Golsam gorgan (500 PPM and 250 PPM) and Diazinon 60% EC, Golsam gorgan(1000 PPM and 500 PPM) were tested.

2.3 lethal Effect of Imidaclopride and Diazinon on the egg Cryptolaemus montrouzieri

At the beginning of the experiments, five concentrations of diazinon (1000 and 500 ppm) and imidaclopride (500 and 250 ppm) were prepared and then 25 newly laid eggs of *C. montrouzieri* were collected from the laboratory colony and then eggs on the paper were dipped for 10 seconds in each concentration. Thereafter, exposed eggs were kept in a climate-controlled growth chamber under and under 25±1 °C and 65±5% RH. This experiment was carried out in four replications for each concentration. The eggs mortality was recorded after 72 hours. The distilled water was used as control treatment. The percentage of corrected mortality was calculated using suggested formula by[2].

2.4 Sub lethal Effects of Imidaclopride and Diazinon on the egg Cryptolaemus montrouzieri

Based on the results of previous experiment, sub lethal effects of diazinon (500 PPM) and imidaclopride (250 PPM) were investigated on biology and population growth parameters of *C. montrouzieri* in a climate-controlled growth chamber (25±2 °C and 65±5% RH). In this experiment, 100 one-day-old eggs of *C. montrouzieri* were dipped for 10 seconds into above-mentioned concentrations of each insecticide and then separately maintained on potato tubers into climate-controlled growth chamber (25±2 °C and 65±5% RH). The growth and development of examined individuals were monitored and recorded every 24 h. After emergence of adult beetles, male beetles were offered to females for mating and the number of laid eggs was counted every day. This procedure continued until death of all female beetles.

2.5 Data analyses

From the reproduction and survival data of *C. montrouzieri* and using suggested formula by Carey JR [8], some reproductive and population growth parameters including intrinsic rate of natural increase (r_m), finite rate of increase (λ), net reproduction rate (R_0), mean generation time (T), doubling time (DT), gross fecundity rate and mean eggs per day were calculated.

The data were analyzed using SPSS 15 (2006), MINITAB 13 (2000). For the analysis of variance using (ANOVA). The demographic parameters and their corresponding standard errors were estimated by the Jackknife technique [24].

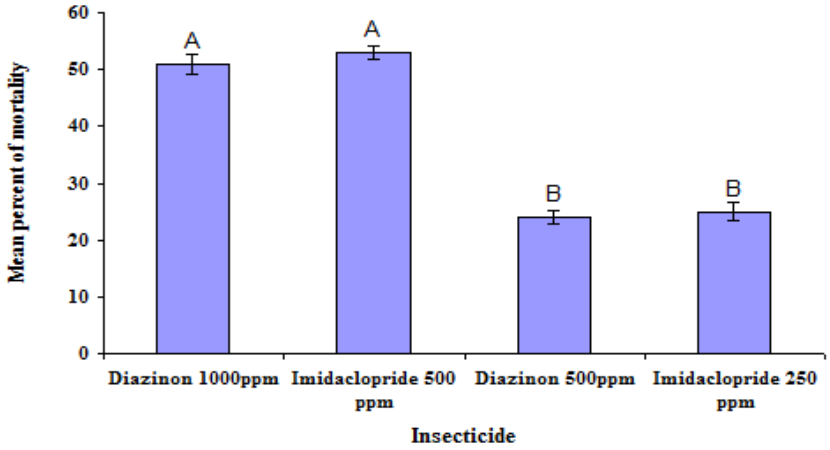

Fig 1: Mean percentage of egg mortality of *Cryptolaemus montrouzieri* in different concentration of diazinon and imidaclopride

3. Results & Discussion

3.1 Lethal effects of imidaclopride and diazinon on eggs of *Cryptolaemus montrouzieri*

The obtained results in this experiment showed that the highest and lowest percentage of mortality were occurred in 500 PPM concentration of imidaclopride (53%) and diazinon (23/5%) (Fig. 1) Significant differences were observed between eggs mortality in different concentration of both insecticides (Tukey test after one-way ANOVA). Based on the satudies [3], imidacloprid and diazinon [43] impact of quantitative on the egg predators. But In the present study, Diazinon and Imidaclopride were moderate toxic on eggs of *C. montrouzieri*. While Cock DA *et al.* [12] reported that when the females of pentatomid bug, *Podisus maculiventris* (Say) were exposed to imidacloprid, mortality was high.

3.2 Sub lethal effects of diazinon and imidaclopride on development of *C. montrouzieri*

According to the results only 61 and 65%. Eggs hatched when they treated with the Imidaclopride 250 ppm and Diazinon 500 ppm. Hatching began in sixth day and continued till eighth day. In both treatments 47% turned mature. Table 1 and 2 show Growth period lengths during different developmental stages in C. montrouzieri females and males according to these results, there were no significant differences among incubation and larval periods of both males and females between two examined insecticides.

While, pupal period and total preimaginal development of males and females had significantly influenced by diazinon and imidaclopride.

Table 1: Development times (Mean±SE) of various life stages of females of *Cryptolaemus montrouzieri* under sub lethal concentration of diazinon and imidaclopride

	Stage of development (days)			
	Incubation period	**Larva period**	**Pupal period**	**Preimaginal development**
Diazinon	5.71±0.86 [a]	15.75±0.06 [a]	7.37±0.48 [a]	29.53±0.9 [a] 30.58±0.12 [b]
Imidaclopride	5.72±0.08 [a]	15.43±0043 [a]	6.68±0.37 [b]	

Means with the same letters in the same columns are not significantly different (P<0.05, Tukey after One-way ANOVA)

Table 2: Development times (Mean±SE) of various life stages of male of *Cryptolaemus montrouzieri* under sub lethal concentration of diazinon and imidaclopride

		Stage of development		
	Incubation period	**Larvae period**	**Pupal period**	**Pre-imaginal development**
Diazinon	5.89±0.01 [a]	15.57±0.13 [a]	7.80±0.76 [a]	29.47±0.11 [a] 30.40±0.14 [b]
Imidaclopride	5.55±0.12 [a]	15.16±0009 [a]	8.20±0.70 [b]	

Means with the same letters in the same columns are not significantly different (P<0.05, Tukey after One-way ANOVA)

Table 3: Adult and total longevity (mean ± SE) of *Cryptolaemus montrouzieri* under sub lethal concentration of diazinon and imidaclopride

Insecticide	preoviposition	oviposition	Post oviposition	adult longevity	Total longevity
Diazinon	7.32±0.8 [a]	23.85±0.14 [a]	0.75±0.08 [a]	31.85±0.15 [a]	61.39±0.18 [a] 62.13±0.13 [b]
Imidaclopride	7.03±0.03[a]	23.44±0041 [a]	1.06±0.6 [b]	31.55±0.09 [a]	

Means with the same letters in the same columns are not significantly different (P<0.05, Tukey after One-way ANOVA)

Post-oviposition period and total longevity of female beetles were significantly different between diazinon and imidaclopride, while no considerable differences were observed on pre-oviposition, oviposition and adult longevity (Tukey after one-way ANOVA). Based on the studies [13] sublethal doses of toxicants may change life span, development rates. Lashkari MR *et al.* [24] observed that Egg development of *C. montrouzieri* was 4 to 5 days on *Maconellicoccus hirsutus* at 24-28 ºC. And with the results match looks of this study. The duration of total stage of C. *montrouzieri* larvae were 15 days. While Baskaran RKM [6] reported that larval period *C. Montrouzieri* was 12.42 days on planococcus citri. This difference may be due to the effect of insecticides. The pre-pupal and pupal period of *C. montrouzieri* reared on *P. citri* was in Imidaclopride 250 PPM (6.68±0.37 days) and in Diazinon 500 ppm (7.37±0.48 days). Babu TR and Azam KM [5] reported that at temperatures of 20, 27.5 and 30 ºC, the average pupal period was 14.3, 6.1 and 6.2 days, respectively when reared on *M. hirsutus* Green. The pre-oviposition period of *C. montrouzieri* was in Imidaclopride 250 ppm (7.03days) and in Diazinon 500 ppm (7.32 days). Mani M *et al.* [26] reported that the pre-oviposition period was 5 to 7 days on mealy bugs. While Mullins JW [30] observed that under lab temperature (25±1 ºC and 45±5% RH) the Pre-oviposition period of *C. montrouzieri* was 2 to 8 days with an average of 5.1±0.64 days when reared on *P. citri*. This difference may be due to the effect of insecticides. Oviposition and post oviposition period were in Imidaclopride 250 ppm (23.44, 1.06 days) and in Diazinon 500 ppm (23.85, 0.75 days). While, [12] reported that imidacloprid were not affected on oviposition and hatching of *Podisus maculiventris* (Say). According to studies up [30] oviposition and post-oviposition period of *C. montrouzieri* averaged 109.3±14.61 and 5.4±0.73 days respectively when reared on mealy bug, *P. citri,* under lab temperature (25±1 ºC and 45±5% RH). This difference may be due to the effect of insecticides.

In this study adult period of *C. montrouzieri* was in Imidaclopride 250 ppm (31. 55 days) and in Diazinon 500 ppm (31.85 days) and longevity was in Imidaclopride 250 ppm (62.13 days), in Diazinon 500 ppm (61.39 days). While [24] observed that adult period of *C. montrouzieri* was 121 to 138 days on *P. citri* also [26, 4] reported that adult period of *C. montrouzieri* was 52 to 80 days. [32] reported that under laboratory conditions (27±3 ºC and 58±3% RH) average longevity was 57 days. [31] reported that under laboratory conditions (25±1 ºC and 45±5% RH) average longevity of *C. montrouzieri* was 39 to 159 days with an average of 120.8±17.4 days

3.3 Reproductive parameters

Based on the obtained results, greater values of Gross fecundity rate and mean eggs per day were obtained for *C. montrouzieri* under sub lethal concentration of diazinon and these differences were significant (table 4).

Table4- reproductive parameters of *Cryptolaemus montrouzieri*

	Insecticide	
	Diazinon	**Imidaclopride**
Gross fecundity rate	254.72±1/88 [a]	207.57.±1 .58 [b]
Mean eggs per day	8.49±0.37 [a]	6.29±0.04 [b]

Means with the same letters in the same row are not significantly different (P<0.05, Tukey after One-way ANOVA)

Sublethal doses of insecticide may reduction of growth or fecundity or increase in mortality rates or development times [19]. In this study, the mean eggs per day was in Imidaclopride 250 ppm (6.29 eggs), Diazinon 500 ppm (8.49 eggs).While, [32] reported that the mean number of eggs per female and mean eggs per day was 805±92.07 and 7.0±0.58 respectively when reared on *P. citri*. Based on the satudies [39] sublethal doses of insecticide may change fecundity, egg viability. While [34] reported Imidacloprid had no significant effect on fecundity *Chrysoperla carnea* (Stephens).

3.4 Population growth parameters

The obtained results in this experiment showed that greater values of Net reproductive rate (R0) and finite rate of increase (λ) and lowest value of doubling time of (DT)were obtained for *C. montrouzieri* under sub lethal concentration of diazinon and these differences were significant (table 5). Intrinsic rate of natural increase (rm) is an important demographic parameter, for predicting the potential of population growth of an animal [35]. In this study rm *was* 0.08 while [32] reported the rm of 0.092±0.002 for *C. montrouzieri* on *P. citri* under laboratory conditions (27±3 ºC and 58±3% RH). Also, [34] reported imidacloprid and propargite had no significant effects on the intrinsic rate of natural increase (rm). While Lashkari MR *et al.* [24] evaluated the efficiency of imidacloprid and pymetrozine on population growth parameters of cabbage aphid, *Brevicoryne brassicae* L. (Homoptera: Aphididae) and rm were lower in imidacloprid and pymetrozine treatments than in testifier. The net reproductive rate (Ro) of *C. montrouzieri* was in Imidaclopride 250 ppm (27.98), Diazinon 500 ppm (34.36) females/female, generation time (T) in Imidaclopride 250 ppm (39.06), Diazinon 500 ppm (39.80) days, doubling time (*DT*) in

Imidaclopride 250 ppm (8.27), Diazinon 500 ppm (7.79) days and finite rate of increase (λ) in Imidaclopride 250 ppm (1.08), Diazinon 500 ppm (1.09) day-1. Persad A and Khan A [32] reported that the net reproductive rate (Ro), generation time (T), doubling time (DT) and finite rate of increase (λ) was 227.18 females/female, 40.13 and 5.13 days and 1.14 day-1 respectively when reared on *M. hirsutus*. Özgokce MS *et al.* [31] observed that Ro, T, DT and λ was 340.703 females /female, 59.350 and 7.2 days and 1.101 day-1 respectively when reared on *P. citri*. This difference may be due to the effect of insecticides Imidaclopride and Diazinon.

Table 5: population growth parameters of *Cryptolaemus montrouzieri*

	Insecticide		
	Diazinon	Imidaclopride	
(R0)	34.36±0.19 [a]	27.98 ±0.21[b]	Female/ generation/ Female
(rm)	0/08±0/00 [a]	0/08 ±0/00 [a]	Female/Female/ Day
(λ)	1/09±0/00 [a]	1/08±0/00 [b]	Day
(T)	39/80±0/09 [a]	39/06±0/18 [a]	Day
(DT)	7/79±00/0 [a]	8/27 ±01/0 [b]	Day

Means with the same letters in the same row are not significantly different (P<0.05, Tukey after One-way ANOVA)

In a conclusion, the study of Diazinon and imidacloprid indicated that the both insecticide were moderate toxic on eggs *C. montrouzieri* Also, they had no significant effect on fecundity *C. montrouzieri* but Imidaclopride showed the highest inhibiting effect on stable population growth parameter.

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