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## Study on Side Effects of Diazinon and Imidaclopride on *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) Under Laboratory Conditions in Indirect Method in First and Second Generation (Prey Treated with Insecticide)

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**ABSTRACT**

The study is based on sub lethal effect of Diazinon and Imidacloprid insecticide density evaluation on biological-reproductive parameters and also population growth of *Cryptolaemus montrouzieri* Mulsant ladybird under controlled conditions (25±2 °C and 65±5% RH) and only focused in the first 72 hours of their lives which were being fed on prey treated mealybug with LC-25 density. Adult longevity of female insect in first generation treated with Diazinon and Imidaclopride was (in order) 20.21 and 19.45, development times in second generation were 30.15 and 30.39 and adult longevity in second generation was 32.17 and 30.93 days. Gross fecundity rate (first generation) in Diazinon and Imidacloprid treat in order were 60.03 and 60.71, Gross fecundity rate (second generation) 270.71 and 247.14, Gross hatch rate 40.82 and 29.16, Net fecundity rate 162.98 and 98.56 and Net fertility rate 110.35 and 47.31, Mean eggs per day 4.61 & 3.03 and mean fertile eggs per day 3.14 & 1.45. Intrinsic rates of natural growth (rm) were 0.9 & 0.07 in Diazinon and Imidacloprid treat.

**Keywords:** *Cryptolaemus montrouzieri*, diazinon, imidaclopride, biology, reproductive, growth parameters.

**1. Introduction**

The Coccinellidae family has an important role in controlling the population of pests. However in the most pest management programs; they performed weakly to protect them<sup>[13]</sup>. Protection of natural enemy means: Correction and improvement of environment in favor of natural enemies<sup>[6]</sup>. Therefore with the recognition of the effect of insecticide and use of plant resistance variation against pests, we are able to minimize the harmful effect of insecticides on natural enemies, because using insecticides have the most effect on ladybird's population<sup>[13]</sup>. Insecticides could affect ladybirds population directly (direct contact with pesticide residue) and indirectly (prey treated with pesticide)<sup>[6]</sup>.

The vast study done regarding the effect of insecticides on predator ladybird's species<sup>[9]</sup>. Predator ladybirds have shown a high sensitivity to some of the insecticides which are used in order to control citrus pests<sup>[4]</sup>, fruit orchards<sup>[3]</sup>, Cotton<sup>[11]</sup>; Thus, below, we will mention the effect of some of insecticides on ladybirds. These studies showed low toxicity in acephat insecticide and high toxicity in Imidacloprid and Abamketin<sup>[3]</sup> on larvae and adult longevity of ladybirds.

Because of the effective role of ladybirds compare to other predators in biological control and integrated pest management, study about the effect of pesticides on these insects has special importance ;therefore here the effects of sub lethal Diazinon and Imidacloprid insecticides on *Cryptolaemus montrouzieri* was considered.

**2. Materials and methods**

In order to do these tests, a colony primary sources of citrus mealybug was provided as a prey from Plant pest and disease Center (located in Tonekabon-Mazandaran-Iran), And which was nurtured on potato tubers in temperature of 27±2 °C and relative humidity of 65±5%.

Also 15 pairs of *Cryptolaemus montrouzieri* (male and female) were provided as predators (from Green Gardener Insectariums, Dezfool-Khoozestan-Iran) which were released on mealybugs in order to feed and nurture in above mentioned temperature condition.

**2.1 Studied insecticides**

1. Diazinon: Emulsion 60% (Golsam formulate, GorganGolsam-Iran), recommended doze 1 in 1000
2. Imidacloprid: Suspension 35% (Golsam formulate, GorganGolsam-Iran), recommended doze 0.5 in 1000

**2.2 Sub lethal effects of imidaclopride and diazinon on *Cryptolaemus montrouzieri***

In this test, for 72 hours, one hundred one-day mating female insects was fed on treated mealy bug nymphs with Diazinon and Imidacloprid insecticides. After this period they were fed on their former diet. This test done on 25± 2 °C and 65±5% RH and their growth stages was studies daily. In this test, sub lethal effects of Imidacloprid and Diazinon on biological parameters were measured for first and second generation, reproduction for first generation, fecundity rate and population growth rate for second generation. The study was continued up to *Cryptolaemus Montrouzieri* death time.

**2.3 Data analyses**

In this study biological parameters, reproduction and population growth were evaluated. They used “SPSS 15”<sup>[16]</sup> and MINITAB13<sup>[12]</sup> in order to analyze these data, for Variance analyzes “ANOVA”, and for evaluating population growth parameters “Jackknife”<sup>[11]</sup>.

**3. Results& Discussion**

**3.1 Sublethal effects of diazinon and imidaclopride on development *cryptolaemus montrouzieri***

The result of sub lethal effect on Diazinon and Imidacloprid on adult longevity first generation in table 1, development times in

table 2, adult longevity second generation in table 3 are shown. According to the result ,there were significant differences among first generation adult longevity only “lifetime period” and development times of second generation “pupal stage ” and adult longevity second generation “all stages except preoviposition period”.

The result of adult longevity between two generations, compare with each other, is a significant difference among all stages in both generations. Also many researchers evaluated *Cryptolaemus montrouzieri* ladybird life time.

According to<sup>[17]</sup> studies, *Cryptolaemus montrouzieri* ladybird's life time separately on artificial food and mealy bug are 53±2.70 and 59.6±2.87.

In their studies, <sup>[10]</sup> reported that oviposition period of *Cryptolaemus montrouzieri* ladybird on mealybug is 45-68 days.

However in this test oviposition period for Diazinon treat 24.39±0.04 and for imidacloprid treat 22.34±0.10 was reported; the result difference might be because of pesticides effect.

**3.2 Reproductive parameters**

The result of this test showed that there was a significant difference among all reproductive parameters in first generation except gross fecundity rate. Also there was a significant difference among gross fecundity rate in second generation (table 4).

The result of studies done by<sup>[8]</sup> on the stages of *Cryptolaemus montrouzieri* ladybird ‘s oviposition showed ,while feeding on Tea mealybug in 26 °C, the ladybird lays 7.09±1.2 eggs-in average-daily.

However, in this test the average number of eggs per day was less. In this research, fecundity deduction and the less number of laid eggs confirmed the result of<sup>[7]</sup> on natural enemy which treated with Metadion and in conflict with the result of<sup>[2]</sup> over the effect of DDT on *Colemegilla maculate* Lengi. Also from comparing GFR in first and second generation we got the result that Diazinon and imidacloprid had strong effect on this parameter which is similar to the result of<sup>[14]</sup> research about Deltametrin & Fenitrotion on *T. grandis* and *T. semistriatus*.

**Table 1:** Adult and total longevity (mean ± SE) of *Cryptolaemus montrouzieri* under sub lethal concentration of diazinon and imidaclopride first generation

Insecticide	preoviposition	oviposition	Post oviposition	adult longevity
Diazinon	7.63±0.64 <sup>a</sup>	10.63±0.08 <sup>a</sup>	1.90±0.03 <sup>a</sup>	20.21±0.09 <sup>a</sup>
Imidaclopride	7.38±0.07 <sup>a</sup>	10.08 ±0.27 <sup>a</sup>	1.98±0.2 <sup>a</sup>	19.45±0.1 <sup>b</sup>

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 females of *Cryptolaemus montrouzieri* under sub lethal concentration of diazinon and imidaclopride in second generation

	(days)Stage of development			
	Incubation period	Larva period	Pupal period	Pre-imaginal development
Diazinon	7.24±0.05 <sup>a</sup>	16 ±0. 00 <sup>a</sup>	5.84 ±0.04 <sup>a</sup>	30.15±0.04 <sup>a</sup>
Imidaclopride	7.39±0.1 <sup>a</sup>	16.01±0. 00 <sup>a</sup>	6 ±0. 00 <sup>b</sup>	30.39±0.1 <sup>a</sup>

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

**3.3. Population growth parameters**

All population growth parameters had significant differences with each other except Finite rate of population growth .In between, the least amount “rm” and the most mount“ DT” were belonged to lady

birds which were fed on Imidacloprid prey treated .So we could get the result that Imidacloprid compare to Diazinon has had more effect on ladybird. (Tab. 5)

As a conclusion, we could say Diazinon and Imidacloprid

insecticides effected biological stages, reproductive parameters & population growth and caused the deduction oviposition period and the number of laid eggs. Also have deducted the fecundity rate and other parameters. Once more, the result of this research shows the

necessity of using Demographic toxicity method to estimate the general and definite effect of pesticides on pest and natural enemies.

**Table 3:** Adult and total longevity (mean  $\pm$  SE) of *Cryptolaemus montrouzieri* under sub lethal concentration of diazinon and imidaclopride in second generation

Insecticide	preoviposition	oviposition	Post oviposition	adult longevity
Diazinon	7.00 $\pm$ 0.00 <sup>a</sup>	24.39 $\pm$ 0.04 <sup>a</sup>	1.82 $\pm$ 0.05 <sup>a</sup>	32.17 $\pm$ 0.08 <sup>a</sup>
Imidaclopride	7.00 $\pm$ 0.00 <sup>a</sup>	22.34 $\pm$ 0.1 <sup>b</sup>	1.59 $\pm$ 0.09 <sup>b</sup>	30.93 $\pm$ 0.2 <sup>b</sup>

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

**Table 4:** reproductive parameters of *Cryptolaemus montrouzieri*

	Insecticide	
	Diazinon	Imidaclopride
Gross fecundity rate (first generation)	60.03 $\pm$ 0.79 <sup>a</sup>	60.71 $\pm$ 0.81 <sup>a</sup>
Gross fecundity rate (second generation)	270.71 $\pm$ 3.00 <sup>a</sup>	247.14 $\pm$ 3.30 <sup>b</sup>
Gross fertility rate	40.82 $\pm$ 0.53 <sup>a</sup>	29.16 $\pm$ 0.40 <sup>b</sup>
Gross hatch rate	0.68 $\pm$ 0.0 <sup>a</sup>	0.48 $\pm$ 0.0 <sup>b</sup>
Net fecundity rate	162.98 $\pm$ 1.93 <sup>a</sup>	98.56 $\pm$ 0.72 <sup>b</sup>
Net fertility rate	110.35 $\pm$ 0.75 <sup>a</sup>	47.31 $\pm$ 0.34 <sup>b</sup>
Mean eggs per da	4.61 $\pm$ 0.06 <sup>a</sup>	3.03 $\pm$ 0.04 <sup>b</sup>
Mean fertile eggs per day	3.14 $\pm$ 0.04 <sup>a</sup>	1.45 $\pm$ 0.02 <sup>b</sup>

Means with different letters in the same rows are significantly different (P<0.05, Tukey after One-way ANOVA)

**Table 5:** population growth parameters of *Cryptolaemus montrouzieri*

	Insecticide		
	Diazinon	Imidaclopride	
(R <sub>0</sub> )	50.91 $\pm$ 0.54 <sup>a</sup>	19.49 $\pm$ 0.23 <sup>b</sup>	Female/Female/Generation
(r <sub>m</sub> )	0.96 $\pm$ 0.00 <sup>a</sup>	0.07 $\pm$ 0.00 <sup>b</sup>	Female/Female/Day
(λ)	1.10 $\pm$ 0.00 <sup>a</sup>	1.07 $\pm$ 0.00 <sup>a</sup>	Day
(T)	40.8 $\pm$ 0.08 <sup>a</sup>	40.3 $\pm$ 0.08 <sup>b</sup>	Day
(DT)	7.19 $\pm$ 0.00 <sup>a</sup>	9.40 $\pm$ 0.00 <sup>b</sup>	Day

Means with different letters in the same rows are significantly different (P<0.05, Tukey after One-way ANOVA)

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