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Original Contribution

SIDE EFFECTS OF IMIDACLOPRID AND ABAMECTIN ON THE MEALYBUG DESTROYER, *CRYPTOLAEMUS MONTROUZIERI*

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ABSTRACT
The effect of sublethal concentration (LC_{50}) of the imidacloprid and abamectin on *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), an efficient predator of the citrus mealybug, were tested under laboratory conditions. The toxicities of the insecticides were assayed using the topical application method and the LC_{50} value was determined based on a dose–response analysis. The LC_{50} values of female, for imidacloprid and abamectin were estimated to be 23.9 and 66.7 µg a.i./ml, respectively. Laboratory exposure of new emerged females to an LC_{50} level of imidacloprid and abamectin, caused significant decreases in adult female longevity, egg production and oviposition periods of surviving beetles. Population increase parameters were also adversely affected by insecticides application. Life table assays revealed that imidacloprid and abamectin caused significant reduction in the intrinsic rate of natural increase (r_m value). The results showed little compatibility between *C. montrouzieri* and the use of imidacloprid and abamectin.

Key words: Coccinellidae, *Cryptolaemus montrouzieri*, Imidacloprid, Abamectin, LC_{50}, Life table

INTRODUCTION

Worldwide, insecticides play a major role in integrated pest management (IPM). However, pesticide compatibility with biological control agents is a major concern to IPM practitioners and combining these two methods is still a difficult task even though more selective pesticides are now available (1). Arthropod predators and parasitoids directly contact insecticide residues applied to plants, resulting in both increased mortality and sublethal (developmental time, fecundity, locomotory behavior) effects (2, 3).

Coccinellids predators are well known for their ability to control pests in a wide range of cropping systems throughout the world (4). However, in agroecosystems their effectiveness is limited by the extensive use of insecticides (5). The mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) a key predator of citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), is native to Australia and has been used in many control programs against a number of mealybug species around the world (4, 6). This biocontrol agent was introduced to northern of Iran for control of *P. citri.*

Imidacloprid and abamectin be used in Iran to control *P. citri* and *Phyllocoptruta oleivora* Ashmead (Hemiptera: Pseudococcidae) in citrus trees and tea shrubs. Although imidacloprid is one of the most widely used insecticides in the world (7), many studies have shown its negative effects on coccinellid species, as found for adult stage of *Harmonia convergens* (8), *Coleomegilla maculata* (9), *Cycloneda sanguinea* (10), *Stethorus punctum* (11), *Rhyzobius lophanthae* (12), *Cryptolaemus* sp. (13), *Adalia bipunctata* (14) S. *tsugae* (15), and *H. axyridis* larvae (11, 16). Also susceptibility to abamectin has been shown for several ladybeetles species, as found

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for *H. axyridis* larvae (17), *Stethorus punctum* larvae and adults (18, 19).

Imidacloprid and abamectin has been abundantly tested against coccinellid predators and a broad range of its sublethal effects have been evaluated (15, 16, 20).

Therefore, the objective of this study was to evaluate the lethal and sublethal effects of imidacloprid and abamectin on population growth rates of *C. montrouzieri*.

MATERIALS AND METHODS

1. Insect rearing

Predators were reared on *Planococcus citri* infested squash and potato in 25×25×30cm cages in the laboratory at 25±1°C, 60%±10 RH, and a photoperiod of 16: 8h L: D. adult *C. montrouzieri* were sexed by examination of the first pairs of legs; in males they are reddish brown to yellow while in females they may range from grey to black (21).

2. Insecticides and chemicals

Imidacloprid and abamectin (technical grade with 95% purity) were obtained from the Gyah Corporation, Iran.

3. Dose–response bioassay

The toxicities of imidacloprid and abamectin on the new emerged male and female adults of *C. montrouzieri* were assayed using the topical application method. The concentrations of the insecticides were chosen based on dose-response tests. Concentrations used in the final bioassay ranged from 20 to 100 µg a.i./ml for abamectin and 10 to 40 µg a.i./ml for imidacloprid. Insecticides were dissolved in acetone and 1µl of each concentration was applied on the ventral abdomen with a microapplicator (Burkard Ltd., England) (19). Control was tested with acetone using the same method. After treatment, the insects were maintained with the mealybugs in plastic petri dishes at 25±1°C, 60±10% RH, and a photoperiod of 16: 8h L: D. Mortality was determined 24h after treatment. Mortality curves were estimated by probit analysis using SPSS 16.0.

4. Effect of LC_{50} concentration on biological parameters of the treated unmated females

The fifteen new emerged female individuals survived 24 hours after treatment with LC_{50} concentration of insecticides, were used for this experiment. Each female was exposed to an untreated male from stock colony, individually. The dead males were replaced with new ones. In all experiments, the various stages of citrus mealybug, *Planococcus citri* were provided as a food source. Mortality and oviposition were recorded daily until the death of the last female in both treatments and controls. Population growth parameters calculated according to Bechmann (22). The Jackknife method was used to estimate the pseudo-values of population growth parameters and compare them statistically (23). Analysis of variance (ANOVA) was used to analyze the effect of the LC_{50} concentrations of the insecticides on preoviposition, oviposition, and post-oviposition periods, total and daily fecundity and longevity of *C. montrouzieri*. Means were grouped by Tukey's multiple range tests. Statistical analysis was carried out using SPSS 16.0.

RESULTS AND DISCUSSION

1. Dose–response bioassay

The toxicity of imidacloprid was approximately 3 times higher than that of abamectin for *C. montrouzieri* females based on LC_{50} values. The estimated values of LC_{50} for female and male *C. montrouzieri* were 23.91and 17.25 µg a.i./ml (nanogram of active ingredient per insect) for imidacloprid and 66.73 and 67.21 µg a.i./ml (ng a.i/insect) for abamectin, respectively. The toxicity of imidacloprid on male and female *C. montrouzieri* was significantly different, as inferred by the confidence intervals of LC_{50} **(Table 1)**. No mortality was recorded in the controls.

A topical application of imidacloprid to the ventral abdomen of individual beetles resulted in a 6 d LD_{50} value of 0.71 ng imidacloprid per beetle for *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) a predator of the hemlock woolly adelgid (Hemiptera: Adelgidae) (15) and a LC_{50} value of 364 µg a.i./ml (ng imidacloprid per beetle) for *Harmonia axyridis* (Coleoptera: Coccinellidae) (19). These results suggest that the toxicity of imidacloprid to *C. montrouzieri* was lower than *S. tsugae* but was much higher than *H. axyridis*.

The estimated LC_{50} values of abamectin for *C. montrouzieri* female was 66.73 µg a.i./ml while a topical application of abamectin to the ventral abdomen of individual *H. axyridis* was 4.88 µg a.i./ml (19) showing that toxicity of abamectin to *C. montrouzieri* was lower than for *H. axyridis*.

Table 1. *Probit analysis for the dose response of imidacloprid and abamectin on adult females and males of C. montrouzieri*

insecticide	Sex	No. *	95% CL	χ^2(df)	Probability	LC_{50}	Slope ± SE
imidacloprid	female	200	(19.9-30.0)	0.57(3)	0.90	23.91	2.73±0.63
	male	200	(14.6-19.6)	0.45(3)	0.93	17.25	4.04±0.69
abamectin	female	200	(56.7-85.9)	3.11(3)	0.38	66.73	3.06±0.65
	male	200	(57.9-83.6)	5.25(3)	0.15	67.21	3.45±0.67

* Ten individuals per replicate, four replicates per concentration, five concentrations per assay; LC: lethal concentration (μg a.i./ml or ng a.i./ insect); CL: confidence limits; a.i.: active ingredient

2. Effect of LC_{50} concentration on biological parameters and reproductive performance of the treated unmated females

Laboratory exposure to the LC_{50} concentration of imidacloprid and abamectin significantly affected pre-oviposition (F = 5.03, df = 2, 42, P= 0.011) and oviposition (F = 197.62, df = 2, 42, P< 0.0001) periods, fecundity (F = 2.54, df = 2, 42, P= 0.0001), fertility (F = 3.96, df = 2, 42, P= 0.0001) and female longevity (F = 172.52, df = 2, 42, P= 0.0001) of surviving adults. Although post-oviposition periods (F = 0.731, df = 2, 42, P= 0.49) were not significantly different between treated and untreated females, the oviposition period, fecundity, fertility and female longevity of treated females decreased significantly compared to control **(Table 2)**.

The course of the age-specific survival rate (lx) and the age-specific number of progeny per day (mx) of *C. montrouzieri* for treated and untreated females are presented in Figure 1. The developmental success of *C. montrouzieri* from egg to adult was 100% **(Figure 1)** and an adult sex ratio of 1♂: 1♀ was obtained. Untreated *C. montrouzieri* spent about 80% of their lives in the adult stage and did not reach their peak of reproduction (m_x = 10) until the adults were 60 days old, at the same time the probability of reaching this age was 100%. The treated females with LC_{50} concentrations of abamectin and imidacloprid were spent about 50 and 70% of their lives in the adult stage, respectively and both groups reached their peak of reproduction 7 days after reaching adulthood (m_x = 1.5). The probability of reaching this age was about 90% at both cases.

Table 2. *Mean (±SE) effects of LC_{50} concentration of imidacloprid and abamectin on biological parameters of C. montrouzieri*

Parameter	Treatment		
	Control	imidacloprid	abamectin
Pre-oviposition period (days ± SE)	3.07 ± 0.21a	3.67± 0.23a	2.80± 0.14b
Oviposition period (days ± SE)	96.27± 1.52a	26.60± 5.13b	13.53± 1.18c
Post-oviposition period (days ± SE)	1.80± 0.74 ns	1.67± 0.79ns	0.80± 0.20ns
Fecundity (eggs ± SE)	921.80± 12.26a	86.40± 10.70b	41.07± 5.10c
Fertility (eggs ± SE)	747.27± 10.34a	33.93± 4.35b	15.53± 2.46b
Egg hatch (% ± SE)	81.13± 0.89a	39.91± 1.46b	36.08± 2.70b
Female longevity (days ± SE)	101.13 ± 1.71a	31.93± 5.50b	17.13± 1.32c

Means (fifteen individuals) in a row followed by different letters are significantly different (P< 0.05) (Tukey test)

The life-table parameters of the treated females are shown in **Table 3**. The net reproductive rates (R_0) in LC_{50} concentration of imidacloprid and abamectin were significantly lower than the control (F = 78530, df = 2, 42, P<0.0001). Further the intrinsic rate of increase (r_m) (F = 1622, df = 2, 42, P<0.0001) and the finite rate of increase (λ) (F = 1698, df = 2, 42, P<0.0001) were significantly lower than those in the control. The mean generation time (T) was also found to be significantly lower in the LC_{50} groups of both imidacloprid and abamectin than the control (F = 812.47, df = 2, 42, P<0.0001).

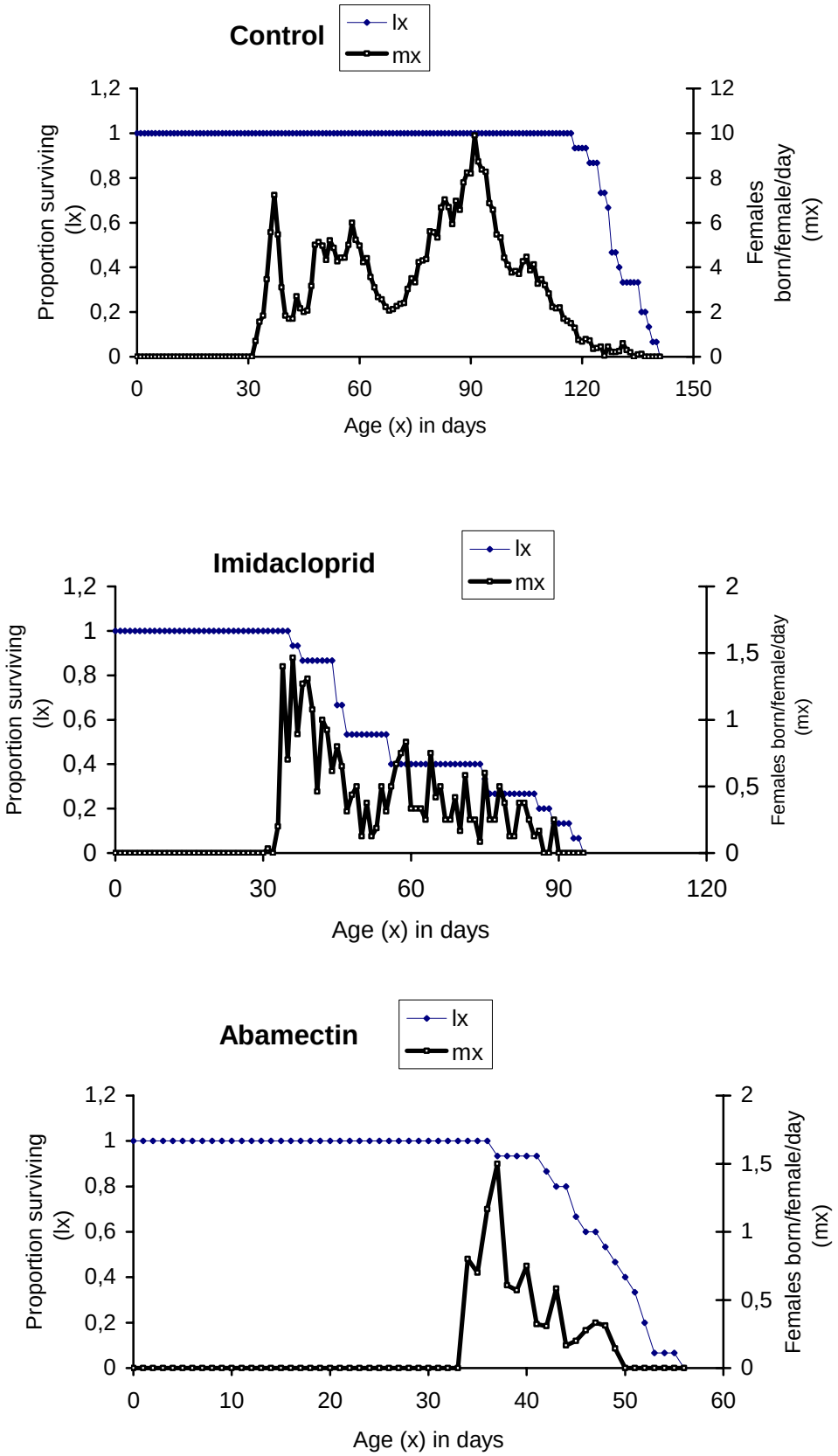

Figure 1. Effect of LC_{50} concentration of imidacloprid and abamectin on survivorship and age-specific fecundity curves of *C. montrouzieri* treated 24 h after appearance. The period of immature stages, from egg to adult, lasted about 30 days that be shown at first part of figure.

Table 3. *Mean (±SE) life-table parameters of C. montrouzieri females treated with LC_{50} concentration of imidacloprid and abamectin*

Parameter	Treatment			
	Control	imidacloprid	abamectin	F[1]
R_0 (females/female)	373.63 ± 0.37a	17.00± 0.15b	7.77± 0.09c	78530**
r_m (females/female/day)	0.108± 0.00a	0.065± 0.00b	0.054± 0.00c	1622**
λ (females/female/day)	1.11± 0.00a	1.07± 0.00b	1.05± 0.00c	1698**
T (days)	42.41± 0.01a	40.69± 0.13b	37.90± 0.024c	812.47**

Means in a row followed by different letters are significantly different by Tukey test at P< 0.05
1: At all cases df = 2, 42
**: P < 0.01

Few studies have been carried out on the reproductive effect of imidacloprid on beneficial coccinellid**.** Similar to our results, imidacloprid caused a significant reduction of adult longevity and fecundity of the predator *Hippodamia undecimnotata* (Coleoptera: Coccinellidae) under laboratory conditions (20). Foliar applications of imidacloprid reduced progeny production and survival of *Rodolia cardinalis* (Coleoptera: Coccinellidae) adults and inhibited development of larvae to adult (2).

Seal et al. (2006) reported that imidacloprid and abamectin were very harmful and harmful for *Cryptolaemus* sp., respectively. Also it was reported that abamectin was toxic to two ladybeetles, *Cycloneda sanguinea* Linnaeus and *H. axyridis* Pallas and concluded it was not compatible with IPM programs in citrus ecosystem (10).

CONCLUSIONS

In integrated pest management programs, *C. montrouzieri* will likely be exposed to insecticide residues on crops. In field situations, exposure of coccinellid predators to pesticides, including imidacloprid and abamectin, may occur as a result of ingestion of treated prey, contact with treated surfaces or by direct exposure to sprays (3). Our study focused on the latter mechanism, with particular reference to reproduction parameters.

It is estimated that the LC_{50} for imidacloprid corresponded to 0.2 times the recommended field concentration [ca 100 μg a.i./ml, (12, 14)]. Also the results indicated the reproductive biology of *C. montrouzieri* is strongly affected by exposure to insecticides. Exposure of females to insecticides resulted in adults that laid a lower number of eggs and had shorter life spans. Moreover, females laid significantly fewer eggs in both insecticide treatments than the control (81% and 95% reduction in average fecundity for imidacloprid and abamectin, respectively). The consequence of these effects was a substantial adverse impact on the population increase parameters of the predator. These results indicate that IPM programmes for *Planococcus citri* that use abamectin or imidacloprid, would be detrimental to *C. montrouzieri*.

Our results show little compatibility between *C. montrouzieri* and the use of imidacloprid and abamectin. However, laboratory data may be of limited value to predict compatibility of insecticides and natural enemies in situ (8, 18) and further field or semi-field studies are needed to confirm the present results under real-world conditions.

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