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Lethal and sublethal effects of endosulfan, imidacloprid and indoxacarb on first instar larvae of *Chrysoperla carnea* (Neu.: Chrysopidae) under laboratory conditions

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Abstract

The common green lacewing is an important natural enemy used for pest control in greenhouses. It is also very common in many agricultural systems. Hence, studying lethal and sublethal effects of insecticides on this predator would be useful. Toxicity of endosulfan, imidacloprid and indoxacarb was assessed on 1st instar larvae of *Chrysoperla carnea* (Stephens) in laboratory. Residual bioassays were carried out in glass Petri dishes. The LC_{50} values, for endosulfan, imidacloprid and indoxacarb were estimated 251, 24.6 and 133 mg ai/l, respectively. Imidacloprid was the most toxic among insecticides tested. To assess the sublethal effects, the 1st instars were treated with LC_{25} of each insecticide. Thereafter, these effects were studied using fertility life table experiments. The analysis of variance revealed significant difference between treatments and control with respect to developmental time of the 1st instar larvae. However, no significant difference was observed among endosulfan, imidacloprid and indoxacarb treatments considering the larval developmental time. Differences between treatments and control were not significant for the developmental time of the 2nd and 3rd instars, pupae, sex ratio, adult longevity, and adult fertility. The results showed that only net reproduction rate (R_0) was significantly affected by treatments. The gross reproductive rate (GRR), intrinsic rate of increase (r_m), doubling time (DT), mean generation time (T) and finite rate of increase (λ) were not affected. The highest and the lowest amounts of r_m were 0.178 and 0.169 in control and indoxacarb, respectively. Imidacloprid was the most toxic of the insecticides tested on the 1st instar followed by indoxacarb and endosulfan. If results similar to laboratory findings are obtained in field conditions, these insecticides might be appropriate candidates for IPM programs.

Key words: *Chrysoperla carnea*, sublethal effects, intrinsic rate of increase, fertility life table, endosulfan, imidacloprid, indoxacarb

چکیده

بال‌توری سبز یکی از دشمنان طبیعی مهم است که در گلخانه برای کنترل آفات حشره‌ای استفاده شده و در اغلب سامانه‌های کشاورزی فعال است. بنابراین، بررسی اثرات کشندگی و زیرکشندگی حشره‌کش‌ها روی این شکارگر مفید خواهد بود. سمیت حشره‌کش‌های اندوسولفان، ایمیداکلوپرید و ایندوکساکارب روی لاروهای سن اول بال‌توری سبز *Chrysoperla carnea* (Stephens) در آزمایشگاه بررسی شد. از روش زیست‌سنجی تماس با باقی‌مانده در ظروف پتری شیشه‌ای استفاده گردید. مقادیر LC_{50} برآورد شده برای اندوسولفان، ایمیداکلوپرید و ایندوکساکارب به ترتیب ۲۵۱، ۲٤/٦ و ۱۳۳ میلی‌گرم ماده‌ی مؤثر در میلی‌لیتر بودند. در بین حشره‌کش‌ها، ایمیداکلوپرید سمی‌ترین ترکیب برای لاروهای سن اول بال‌توری بود. برای ارزیابی اثرات غیرکشندگی، لاروهای سن اول با LC_{25} هر حشره‌کش تیمار شدند. سپس این اثرات به روش جدول زیستی باروری برآورد گردید. بنابر نتایج تجزیه‌ی واریانس، در ارتباط با دوره‌ی نشو و نمای لارو سن اول بین شاهد و تیمارها تفاوت معنی‌داری بود، اما بین حشره‌کش‌های مختلف این اختلاف معنی‌دار نبود. دروه‌ی نشو و نمای سنین دو و سه لاروی، شفیرگی، طول عمر حشرات کامل نر و ماده، نسبت جنسی و باروری حشرات کامل تحت تأثیر قرار نگرفتند. فقط نرخ خالص تولید مثل (R_0) به طور معنی‌داری تحت تأثیر تیمارها قرار گرفت. تفاوت معنی‌داری بین تیمارها در سایر پارامترهای جدول زندگی شامل: نرخ ناخالص تولید مثل (GRR) ، نرخ ذاتی افزایش جمعیت (r_m)، میانگین زمان تولید نسل

(T)، زمان دوبرابر شدن نسل (DT) و نرخ متناهی افزایش جمعیت (λ) مشاهده نشد. بالاترین و پایین‌ترین نرخ ذاتی افزایش جمعیت ۰/۱۷۸ و ۰/۱۶۹ بود که به ترتیب در شاهد و تیمار ایندوکساکارب مشاهده گردید. بنابر نتایج به دست آمده، ایمیداکلوپرید سمیت‌ترین حشره‌کش مورد آزمایش روی لاروهای سن اول بود و ایندوکساکارب و اندوسولفان به ترتیب در مکان‌های بعدی قرار داشتند. اگر نتایج مشابهی در موقعیت مزرعه‌ای به دست آید، این حشره‌کش‌ها ممکن است کاندیدهای مناسبی برای برنامه‌های مدیریت تلفیقی آفات باشند.

واژگان کلیدی: *Chrysoperla carnea*، اثرات غیرکشندگی، نرخ ذاتی افزایش جمعیت، جدول زیستی باروری، اندوسولفان، ایمیداکلوپرید، ایندوکساکارب

Introduction

Green lacewing, *Chrysoperla carnea* (Stephens) is a polyphagous predator that is released (it's) for pest control in greenhouses and is also very common in many agricultural systems. This species is a powerful agent in biological control programs because of an expanded geographical distribution, high compatibility to different systems, high searching ability and an easy way to rear (Azema & Mirabzadae, 2004). Due to physiological similarities between pests and their natural enemies, insecticides usually cause the mortality in both groups (Croft, 1990). Insecticides compatible to biological control agents are useful tools in an integrated pest management program, so studying their effects on natural enemies is a necessity (Stark *et al*., 2004). De Bach & Bartlett (1951) were the first to study the effects of pesticides on beneficial arthropods.

Commonly, there are two types of toxicological studies on natural enemies: acute toxicity and sublethal effects (Croft, 1990; Desneux *et al*., 2007). Acute toxicity is assessed usually after a short exposure to a chemical (e.g. a few hours to a few days) and the endpoint is the death of the organism (Croft, 1990; Stark & Banks, 2003). Median lethal dose (LD_{50}) or lethal concentration (LC_{50}) is estimated in acute toxic studies (Croft, 1990; Stark & banks, 2003). Such data are used to compare efficacy of several chemicals on one species or sensitivity of various species to a chemical (Rumpf *et al*., 1997). Bringing in to contact with insecticide treated surfaces is one of the best methods for assessing the effect of insecticides residues on natural enemies (Tillman & Mulrooney, 2000). Regretfully, predictions about compatibility of an insecticide with a biological control agent are usually based on incomplete screening tests of which two factors are ignored: (1) screening experiments are usually performed on one developmental stage of beneficial insects (mostly adults) while other stages may be affected, (2) in screening tests only mortality caused by insecticides is studied, while sublethal effects may exist. For instance, reduced reproduction, shortened life span, weight loss, decreased longevity, and a change in sex ratio, host finding and searching ability may be observed (Staple *et al*., 2000). In IPM programs, sublethal effects of insecticides are important in relation to the decrease in the ability of entomophagus in regulating host or prey. This ability is affected by change in factors that affecting intrinsic rate of increase (r_m), and behavior (Croft, 1990).

To obtain a better understanding of the longer-term impacts of pesticides on ecosystems other approaches are required to estimate the effects of pesticides and other toxicants on both pest and beneficial species (Forbes & Calow, 1999; Stark & Banks, 2003; Stark *et al*., 2007). These approaches are called ecotoxicology. Demographic toxicology or life table response experiments have been suggested to evaluate the total effects of a toxicant, because it accounts for all effects a toxicant may have on a population (Stark *et al*., 2004). Forbes & Calow (1999) stated that demographic toxicological data are preferred to other types of toxicity data. Evaluations have indicated that ecotxicological analysis which is based on population growth rate would be a more accurate assessment of the impact of pesticides and other toxicants (Stark *et al*., 2004).

In Iran, *C. carnea* is quite active in many agricultural crops. In some areas, it is reared in laboratory and released in mass (Malkeshi *et al*. 2003). Since imidacloprid, indoxacarb and endosulfan insecticides are used in crops that green lacewing is abundant, the study of lethal and sublethal effects of these insecticides on *C. carnea* would be very useful.

Materials and methods

Insects

Colony of *C. carnea* was established using pupae obtained from the Division of Plant Protection of Agricultural and Natural Resource Research Center of Khorasan Province in November 2005. Adult insects were kept in plastic containers 16 cm in diameter and 24 cm in height, covered with a piece of cloth screen and fed on artificial diet consisted of 4 g brewers yeast, 7 g honey and 5 ml water. The mixture formed a paste that was smeared on transparent plastic tapes and put in rearing containers (Vogt *et al*., 2000). Extra water was provided using a wet sponge put on the screen on top of the container. The eggs deposited on the walls of rearing containers and the cloth screens were removed with a brush on daily basis. Newly hatched larvae were reared on the eggs of *Anagasta kuehniella* (Zell.). Rearing conditions were $25 \pm 2^{\circ}$C, $60 \pm 10\%$ relative humidity and a photoperiod of 16: 8 hours (L: D).

Insecticides

The insecticides tested were imidacloprid (35SC, Gyah Company, http://www.gyah.ir), indoxacarb (15SC, DuPont Company, http://www.dupont.com) and endosulfan (35EC, Partoenar Company, http://www.partonaragro.com).

Bioassays

The toxicity of insecticides was assessed on the 1st instars of *C. carnea*, using contact method. After determining the concentration range for each insecticide based on preliminary experiments, glass Petri dishes (90 mm diameters) were sprayed with 2 ml of each concentration at a pressure of 0.5 bar using Potter tower. The concentration ranges for endosulfan, imidacloprid and indoxacarb were 225-425, 10.5-63 and 75-225 mg ai/l, respectively. After the spray, the deposit was let to dry at ambient temperature, 15 1st instar larvae (6-18 h olds) were transferred to each Petri dish. Mortality was assessed 24 h, after treatment. The toxicity of the insecticides tested was compared based on 95% confidence limits of LC_{50} and LC_{90} ratios. If the 95% confidence interval included 1, then the difference between LC_{50}s was not considered significant (Robertson & Preisler, 1992). Each insecticide consisted of five concentration plus untreated control. Each treatment was repeated at least five times at different days.

Sublethal effects

In order to study the sublethal effects of each insecticide, a fertility life table was constructed using an insect cohort (60 eggs), and the fate of the cohort was pursued until the last female died. First instar larvae from the initial cohort were treated with LC_{25} of each of the insecticide (table 1). Twenty four hours after treatment, the surviving larvae were individually transferred to plastic Petri dishes 60 mm in diameters and were monitored daily until the adults appeared. The emerged adults from the surviving larvae were kept in pairs of male and female, and the eggs were collected and counted daily. To assess the effects of the insecticides on fecundity, the deposited eggs were kept until hatching. In order to avoid egg cannibalism by neonate larvae in eclosion interval, *A. kuehniella* eggs were also added to Petri dishes.

The population growth rate was calculated using the Lotka equation (Andrewartha & Birch, 1954): $\Sigma L_x m_x e^{-rmx} = 1$ (1)

where x is the age of cohort, L_x is the proportion of individuals survives to age x, m_x is the number of females produced per female of age x, and r_m is the intrinsic rate of increase for the population.

Table 1. Toxicity of the insecticides tested on the first instar *C. carnea*.

Insecticide	Category	n	Slope ± SE	LC_{25} (mg ai/l) (%95CL)	LC_{50} (mg ai/l) (95%CL)	LC_{90} (mg ai/l) (95%CL)	χ^2
endosulfan	organochlorinate	547	6.68 ± 0.76	181 (265-300)	251 (345.5-374)	391 (510-641)	0.9^{ns}
imidacloprid	neonicotinoide	487	2.12 ± 0.23	12 (9.2-14.1)	24.6 (21.7-28.1)	99.6 (80.4-148.4)	3.6^{ns}
indoxacarb	triazine	561	3.54 ± 0.35	86 (75.5-94.5)	133 (123-143)	306 (263-382)	0.2^{ns}

In addition to r_m, the other fertility life table parameters including: gross reproduction rate (GRR), net reproduction rate (R_0), generation time (T), doubling time (DT) and finite rate of increase (λ) were computed using the following equations (Cary, 1993; Maia, *et al*., 2000; Stark & Banks, 2003):

$$GRR = \sum m_x \qquad (2)$$

$$R_0 = \sum L_x m_x \qquad (3)$$

$$T = \frac{\ln R_0}{r} \qquad (4)$$

$$DT = Ln\ 2 / r_m \qquad (5)$$

$$\lambda = e^{rm} \qquad (6)$$

Statistical comparisons of life table parameters were carried out using jackknife technique (Meyer *et al*., 1986). There are several steps in jackknife method. First step is calculating each statistic using all n replications, represented here with P_{tot}. In the second step, each statistic is recalculated n times, omitting each time one of the replications and using remaining n-1 replications. These values were represented by Pi. In final step, jackknife pseudovalues, Pj are calculated as bellow:

$$Pj = n.\ P_{tot} - (n-1)\ Pi \qquad (7)$$

The average and variance of Pj's are used to estimate confidence intervals and compare statistics among treatments using traditional statistical methods of comparisons of means (Maia *et al*., 2000).

Data analyses

The probit method was used to estimate LC_{50}. The data were analyzed using SPSS 13.0 (SPSS, 2004). Jackknife pseudo-values that were computed for GRR, R_o, DT, T, r_m and λ for each treatment (using EXCEL) were estimated for the analysis of variance (ANOVA). Analysis of variance and mean comparison for the toxicity data and life table parameters were performed using SAS 9.0 (SAS, 2002). The means were compared by Duncan's Multiple Range Test.

Results and discussion

Results of the acute toxicity testing of insecticides for estimating LC_{50}, on the 1^{st} instar *C. carnea* are shown in table 1. The toxicity of the insecticides tested was as follows: imidacloprid > indoxacarb > endosulfan. The toxicity symptoms in larvae treated with indoxacarb were considerable. The symptoms included lack of coordination, cessation of feeding, paralysis and death. In some cases inability in casting the old head capsule was observed. Silver & Soderlund (2005) reported the same symptoms on larvae *Heliothis virescens* (Fabricius) treated with indoxacarb. More tolerance of *C. carnea* larvae to endosulfan was probably refers to its long history of field application. Elzen *et al*. (2000) found that endosulfan was less toxic to female *Catolaccus grandis* (Burks) than 10 other insecticides. Elzen (2001) also reported higher mortality in *Orius insidiosus* (Say) treated with imidacloprid than endosulfan. Nasreen *et al*. (2003) reported that concentration of 250 ppm treatment of indoxacarb caused 100% mortality in *C. carnea* larvae but in endosulfan treatment only 30% of the larvae were dead. At the present study, imidacloprid was more toxic to *C. carnea* than indoxacarb, which is nearly in agreement with the finding of above researchers. The results of the comparison of toxicities of the insecticides tested based on 95% confidence limits of LC_{50} ratios are presented in table 2. There was a significant difference between toxicity of the insecticides on the 1^{st} instar *C. carnea.*

Developmental time of the 1^{st} instar larvae was significantly affected by LC_{25} of all insecticides examined comparing to the control (df = 3, 172; F = 0.32; P = 0.0016); however, the difference among endosulfan, imidacloprid and indoxacarb was not significant. The extension of the developmental time of the treated individuals may be due to reduced food uptake as a consequence of insecticidal deterrence in the earlier instars (Galavan *et al*., 2005). Differences between treatments and control were not significant for the developmental time of 2^{nd} and 3^{rd} instars, pupae, sex ratio, adult longevity, and adult fertility. Sublethal effects of the insecticides on life table parameters are shown in table 3. Only net reproduction rate (R_0) was significantly affected by treatments (df = 3, 78; F = 2.82 and P = 0.04). There was a significant difference between indoxacarb treatment and control. Nevertheless, the difference among control, endosulfan and imidacloprid treatments was not significant. Rezaei *et al*. (2007) did not observe any difference in R_0 value between *C. carnea* treated in first instar by imidacloprid and control. Galavan *et al*. (2005) reported that indoxacarb caused a simultaneous reduction in adult fertility and also the survivorship of the 1st instar and adult of *Harmonia axyridis* (Pallas) in laboratory. Studebaker & Kring (2001) reported that imidacloprid and indoxacarb insecticides affected the survival of 3rd instars and adult *O. insidiosus*. Huerta *et al*. (2003) observed that imidacloprid was very toxic to the 3rd instar larvae of *C. carnea* and inhibited adult emergence. Our results indicated that the fertility of adults following larval treatment with indoxacarb was reduced, though the reduction was not significant. Grosch & Hoffman (1973) suggested that the reduction in fertility life table of parasite wasps treated with insecticides could be related to decreased in food uptake, change in physiology or cytotoxic destruction of eggs. De Cock *et al*. (1996) reported that when the females of pentatomid bug, *Podisus maculiventris* (Say) were exposed to imidacloprid, mortality was high, although oviposition and hatching were not affected by exposure of the females to sublethal doses. Kunkel *et al*. (1999) found that imidacloprid did not affect the population predatory arthropods in soil.

Table 2. Comparison LC_{50} and LC_{90} values of endosulfan, imidacloprid and indoxacarb on the first instar *C. carnea* using Robertson and Preisler method.

Insecticides	LC_{50}			LC90			Significance
	Ratio	Lower limit	Upper limit	Ratio	Lower limit	Upper limit	
endosulfan versus imidacloprid	9.97	8.65	11.49	4.54	3.16	6.52	significance
endosulfan versus indoxacarb	1.84	1.68	2.02	1.47	1.15	1.87	significance
imidacloprid versus indoxacarb	0.184	0.16	0.21	0.32	0.22	0.47	significance

The highest and the lowest amounts of intrinsic rate of increase (r_m) measured 0.178 and 0.169 in control and indoxacarb treatments, respectively; however, the difference between control and the treatments was not significant. Mean generation time (T) was the highest value in control, though it was not significantly different comparing to the insecticidal treatments. Doubling time (DT) and finite rate of increase (λ) were not significantly different among the treatments; although, they were lower than those of the control.

Table 3. Mean comparison of population growth parameters in 1st instars larvae *C. carnea* the treated using Duncan's Multiple Range Test.

Treatment parameter	Mean ± SE			
	Control	**Imidacloprid**	**Endosulfan**	**Indoxacarb**
GRR	505 ± 34.9[a]	460 ± 53.8[a]	375 ± 59.7[a]	433 ± 42.7[a]
R_0	405 ± 43.7[a*]	302 ± 49.05[ab]	245 ± 54.6[ab]	197 ± 39.9[b]
r_m	0.179 ± 0.005[a]	0.178 ± 0.007[a]	0.176 ± 0.008[a]	0.169 ± 0.007[a]
T	33.6 ± 0.99[a]	32.1 ± 0.77[a]	31.3 ± 0.82[a]	31.2 ± 1.16[a]
DT	3.87 ± 0.12[a]	3.87 ± 0.12[a]	3.92 ± 0.16[a]	4.0 7 ± 0.18[a]
λ	1.196 ± 0.006[a]	1.195 ± .009[a]	1.192 ± 0.008[a]	1.185 ± 0.009[a]

*Mean within each row followed by the similar letter in are not significantly different (P > 0.05).

In conclusion it can be stated that, in spite of the acute toxicity of imidacloprid, indoxacarb and endosulfan on 1st instars of *C. carnea,* these insecticides did not affect the life table parameters of the green lacewing. If the similar results are obtained in field conditions, these insecticides might be suitable candidates for use in integrated pest management programs.

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