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Toxicity and side effects of three insecticides on adult *Chrysoperla carnea* (Neu.: Chrysopidae) under laboratory conditions

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Abstract

Green lacewing, *Chrysoperla carnea* (Stephens), is an important predator of arthropod pests such as aphids, psyllids, thrips and whiteflies. Toxicity of endosulfan, imidacloprid and indoxacarb was assessed on male and female *C. carnea* in the laboratory. Contact bioassays were carried out in glass Petri dishes. The LC_{50} values for indoxacarb, imidacloprid and endosulfan were 0.011, 0.053, and 0.343 g AI/L for males, and 0.019, 0.098 and 0.398 g AI/L for females, respectively. Males were more sensitive than females to all three insecticides. To assess the sublethal effects, using IOBC (International Organization for Biological Control) method, adults were treated with LC_{25} of each insecticide. Analysis of variance did not show significant differences among treatments regarding the developmental time of the first, second and third instars, pupae and sex ratio. Differences between treatments and control were significant regarding pre-oviposition, oviposition and post-oviposition periods, fecundity, fertility, longevity of male and female. Mean longevity for control, imidacloprid, endosulfan and indoxacarb were 30 ± 2.3, 24.3 ± 3.3, 21.3 ± 2.4 and 19.7 ± 1.4 days for males, and 36.9 ± 2.5, 31.8 ± 2.9, 27.7 ± 1.7 and 26.7 ± 2.6 days for females, respectively. The highest and the lowest rates of fecundity were 540 ± 49 and 206 ± 42 in control and indoxacarb, respectively. Based on the IOBC classification method, imidacloprid, endosulfan and indoxacarb were slightly harmful (%30 < Total Effect Index < %79) against adults. The adult stage was very sensitive to indoxacarb, imidacloprid and endosulfan. Hence, these insecticides should not be applied when the density of adults is high in the field.

Key words: *Chrysoperla carnea*, sublethal effects, insecticides, endosulfan, imidacloprid, indoxacarb

چکیده

سمیت و اثرات جانبی سه حشره‌کش روی حشرات بالغ *Chrysoperla carnea* (Neu.: Chrysopidae) در شرایط آزمایشگاهی

غلامرضا گل‌محمدی و میرجلیل حجازی

بالتوری سبز، *Chrysoperla carnea* (Stephens)، یکی از شکارگرهای مهم شته‌ها، پسیل‌ها، سفیدبالک‌ها و تریپس‌ها می‌باشد. در این تحقیق حساسیت حشرات نر و ماده بالتوری سبز به حشره‌کش‌های ایمیداکلوپرید، ایندوکساکارب و اندوسولفان در شرایط آزمایشگاهی ارزیابی شد. زیست‌سنجی به‌روش تماس باقی‌مانده در پتری‌دیش‌ها انجام شد. بنابر نتایج، میزان LC_{50} برای حشره‌کش‌های ایندوکساکارب، ایمیداکلوپرید و اندوسولفان برای حشرات نر به‌ترتیب ۰/۰۱۱، ۰/۰۵۳ و ۰/۳٤۳ و برای حشرات ماده به‌ترتیب ۰/۰۱۹، ۰/۰۹۸ و ۰/۳۹۸ گرم ماده مؤثر بر لیتر برآورد گردید. حشرات نر در مقایسه با حشرات ماده به هر سه حشره‌کش حساسیت بیش‌تری نشان دادند. در بررسی اثرات زیرکشندگی به‌روش سازمان بین‌المللی کنترل بیولوژیک (IOBC)، حشرات بالغ با مقادیر LC_{25} تیمار شدند. بنابر نتایج تجزیه واریانس، اختلاف معنی‌داری بین زمان نشو و نمای سنین اول، دوم و سوم لاروی، شفیرگی و نسبت جنسی در سه حشره‌کش مورد مطالعه وجود نداشت. بین تیمارها از نظر دوره‌های پیش از تخم‌ریزی، تخم‌ریزی و پس از تخم‌ریزی، باروری، زادآوری و طول عمر حشرات کامل نر و ماده اختلاف معنی‌دار وجود داشت. میانگین طول عمر حشرات کامل برای شاهد، ایمیداکلوپرید، اندوسولفان و ایندوکساکارب برای حشرات نر به‌ترتیب ۲/۳ ± ۳۰، ۳/۳ ± ۲٤/۳، ۲/٤ ± ۲۱/۳ و ۱/٤ ± ۱۹/۷ روز و برای حشرات ماده به‌ترتیب ۲/۵ ± ۳٦/۹، ۲/۹ ± ۳۱/۸، ۱/۷ ± ۲۷/۷ و ۲/٦ ± ۲٦/۷ روز بود. بیش‌ترین و کم‌ترین نرخ باروری به‌ترتیب با ٤۹ ± ۵٤۰ و ٤۲ ± ۲۰٦ عدد تخم به‌ازای هر ماده در تیمارهای شاهد و ایندکساکارب مشاهده گردید. بنابر طبقه‌بندی به‌روش IOBC، هر سه حشره‌کش در گروه کم‌خطر (۷۹٪ > شاخص کلی تأثیر > ۳۰٪) برای حشرات کامل قرار گرفتند. به‌طورکلی، حشرات کامل به ایندوکساکارب، ایمیداکلوپرید و اندوسولفان خیلی حساس بودند. بنابراین، در موقعی که تراکم حشرات کامل در مزارع بالا است، این ترکیبات نباید به‌کار برده شوند.

واژگان کلیدی: *Chrysoperla carnea*، اثرات زیرکشنده، حشره‌کش‌ها، اندوسولفان، ایمیداکلوپرید، ایندوکساکارب

Introduction

Green lacewing, *Chrysoperla carnea* (Stephens), is an important predator of arthropod pests of agricultural crops. The larvae voraciously feed on many soft-bodied arthropods including eggs and early instars of mites, lepidopterans, coleopterans and homopterans (Carnard & Principi, 1984). This predator can be found in orchards and farms. In some areas, it is mass reared and released as a biological control agent (Azma & Mirabzadae, 2004). At present, application of pesticides is one of the most effective tactics of pest control. Considering chemical control side effects such as development of resistance in pests, environment pollution, and destruction of natural enemies and non-target organisms, their application can be reduced using the integrated pest management programs (Croft, 1990). Therefore, selection of pesticides shall be made carefully to maximize the effects on target pests and minimize deleterious effects on beneficial organisms. Due to the physiological similarities among pest arthropods and their natural enemies, insecticides usually cause severe mortalities in both groups. Moreover, insecticides disrupt the feeding interactions in the ecosystems and in some cases result in increase of secondary pests' population (Metcalf, 1986; Croft, 1990). Determining the effects of pesticides on natural enemies can be useful in appropriate selection of these compounds for integrated pest management programs. In general, two types of toxicological assessments are studied on natural enemies: acute toxicity and sublethal effects.

Acute toxicity is determined after a short exposure of the organism to a single dose of a chemical. The stop point in these studies is the death of the organism. In these studies, LD_{50} or LC_{50} are estimated. These studies are usually used to compare the toxicities of different chemicals on a species or the sensitivity of several species to a chemical (Rumpf *et al.*, 1997; Desneux *et al.*, 2007). The acute toxicity studies do not reveal the disruptive effects of sublethal doses on behaviour and physiology of an organism (Croft, 1990).

As *C. carnea* is an important predator in many cropping systems, where imidacloprid, indoxacarb and endosulfan are applied, our objective was to understand the lethal and sublethal effects of these insecticides against *C. carnea* adults under laboratory conditions.

Materials and methods

Insects

The pupae of green lacewing were obtained from the Plant Protection Research Department of Khorasan Agricultural and Natural Resources Research Center (Mashhad, Iran). Pupae were kept in the greenhouse of the Plant Protection Department of the University of Tabriz (Tabriz, Iran) (26 ± 2 °C, 50 ± 10 % RH and a photoperiod of 16: 8 (L: D) hours) until the adults emerged. The adults were kept in clear plastic cylinder containers (16 cm in diameter × 24 cm high), which were covered with a mesh cloth. A thin layer of food source (mixture of yeast, water and honey at a ratio of 7: 4: 4) (Vogt *et al*., 2000) was smeared to a plastic tape which was inserted into the plastic container. Water was provided for the adults through a wet sponge placed on the mesh cloth. To harvest the eggs, the adults were transferred to fresh containers every day. The eggs were laid on a black paper which was attached to the interior wall of the cage. The eggs hatched after 3 days and the larvae were fed with eggs of Mediterranean flour moth, *Ephestia kuehniella* Zell. (Lep.: Pyralidae).

Bioassays

The tested insecticides were imidacloprid (350 SC, Gyah Company), indoxacarb (150 SC, DuPont Company) and endosulfan (35 EC, Partoenar Company). A range of concentrations for each insecticide (causing 25-75% mortality on males and females, separately) was assessed by contact method. The ranges of concentrations for endosulfan, imidacloprid and indoxacarb were 0.315-0.525, 0.210-0.525 and 0.007-0.045 for females and 0.028-0.245, 0.017-0.175 and 0.014-0.037 g AI/L, for males, respectively. Glass Petri dishes (90 mm in diameter) were sprayed with 2 ml of each concentration (1.68 $\mu l/cm^2$) (table 1) at a pressure of 0.5 bar using Potter tower (Burkard Manufacturing Company Ltd, Rickmansworth, Herts, UK). An untreated control was included along each treatment. After the spray, the deposit was dried at ambient temperature. Then 15 newly-emerged adults (24 h old) were transferred to each Petri dish. Mortality was assessed 24 h after treatment. The toxicity of the insecticides was compared based on 95% confidence limits of LC_{50} ratios based on Robertson & Preisler (1992) method. If the 95% confidence intervals was one or less, then the difference between LC_{50}s was not significant. Each treatment was replicated five times.

Sublethal effects

To study the sublethal effects of each insecticide, adults were treated with LC_{25} of each insecticide (table 2) using contact method. Twenty four hours after treatment, the survived adults were kept in pairs of male and female, and eggs were collected and counted daily (26 ± 2 °C, 50 ± 10% RH and a photoperiod of 16: 8 (L: D) hours. Thirty pairs of adults were used in each treatment. Pre-oviposition, oviposition and post-oviposition periods, fecundity, fertility, and longevity of female insects were estimated. To assess the effect on fertility, the deposited eggs were maintained up to hatching. To avoid egg cannibalism by neonate larvae in eclosion interval, *E. kuehniella* eggs were added to Petri dishes. To assess effects of each treatment on sex ratio and developmental time, three 50-egg samples were derived on 7, 14 and 25 days after initial oviposition, and were reared until adult emergence.

Insecticides were classified based on IOBC (International Organization for Biological Control) method. In this method, acute toxicity and sublethal effects on reproductivity of female is considered (Vogt *et al*., 2000). Total Effect Index (TEI) was calculated using the following formula: TEI = 100 - (100 - M) × R, where, M is mean corrected mortality, and R is the proportion of mean fecundity in treatment to mean fecundity in control (Talebi *et al.*, 2008). Therefore, insecticides were classified as: 'harmless' if TEI < 30, 'slightly harmful' if 30 < TEI < 79, 'moderately harmful' if 79 < TEI < 98, and 'harmful' if 98 < TEI.

Data analysis

The Probit method was used to estimate LC_{50}. The data were analyzed using SPSS 13.0 (SPSS, 2004). Analysis of variance and mean comparison for biological parameters were performed using SAS 9.0 (Proc GLM) (SAS Institute, 2002).

Table 1. Concentrations of insecticides applied against adult *Chrysoperla carnea* in the laboratory for bioassay tests.

Insecticides	Concentrations against male (gr ai/L)	Concentrations against female (gr ai/L)
Endosulfan	0.455, 0.403, 0.357, 0.315 and 0.280	0.525, 0.462, 0.406, 0.357 and 0.315
Imidacloprid	0.175, 099, 0.056, 0.031 and 0.018	0.210, 0.149, 0.135, 0.074 and 0.053
Indoxacarb	0.038, 0.022, 0.013, 0.008 and 0.004	0.045, 0.030, 0.019, 0.012 and 0.007

Table 2. Acute toxicity of three insecticides tested against male (M) and female (F) *Chrysoperla carnea* in the laboratory.

Insecticide	Sex	n	slope ± SE	LC_{50} (g ai/L) (95% CL)	LC_{90} (g ai/L) (95% CL)	χ^2
Endosulfan	F	310	6.89 ± 0.89	0.398 (0.378-0.419) a	0.611 (0.552-0.725) a	0.72
	M	303	6.17 ± 1.03	0.343 (0.323-0.363) a	0.553 (0.491-0.693) a	0.95
Imidacloprid	F	308	2.02 ± 0.35	0.098 (0.082-0.116) b	0.422 (0.289-0.883) b	1
	M	372	1.78 ± 0.2	0.053 (0.044-0.063) b	0.277 (0.196-0.466) b	0.96
Indoxacarb	F	321	2.01 ± 0 27	0.019 (0.016-0.023) c	0.083 (0.059-0.148) c	0.35
	M	311	1.64 ± 0.24	0.011 (0.009-0.014) c	0.069 (0.045-0.014) c	0.75

LC_{50} and LC_{90} values of each sex, followed by different letters in each column were significantly different based on Robertson-Preisler method.

Results and discussion

The results of acute toxicity assessment on male and female lacewings are shown in table 2. Based on LC_{50} values against males, indoxacarb was 30 and 4.6 times more toxic than endosulfan and imidacloprid, respectively. Indoxacarb was 21 and 5 times more toxic to females compared to endosulfan and imidacloprid, respectively. Based on LC_{50} values and their 95% confidence limits, it was concluded that susceptibility within each sex to the three insecticides, were significantly different. This difference in sensitivity was more prominent in imidacloprid and indoxacarb compared to endosulfan. Males were more sensitive than females. The ratio of sensitivity (male to female) for endosulfan, imidacloprid and indoxacarb were 1.16, 1.84 and 1.16, respectively. One of the reasons for this difference could be the smaller body size of the males compared to females, as it is seen in most of the insects (Croft, 1990). Another probable reason may be more fat reserves in females than males. Takeda *et al.* (1965) proposed higher sensitivity of male *Coccinella septempunctata* (Linnaeus) to malathion compared to females due to smaller amount of fat body in males. Elzen *et al*. (2000) reported that endosulfan was less toxic against female *Catolaccus grandis* (Burks) than male ones.

In the present study, it is observed that indoxacarb and imidacloprid are more toxic to *C. carnea* than endosulfan, which is similar to other reports. In another study, imidacloprid had the highest toxicity against first instar followed by indoxacarb and endosulfan (Golmohammadi *et al.*, 2009). Elzen (2001) reported higher mortality of *Orius insidiosus* (Say) treated with endosulfan. Cordero *et al*. (2007) reported 100% mortality (at 1/10 recommended field rate) on two parasitoid wasps, *Oomyzus sokolowskii* (Kurdjumov) and *Diadegma insulare* (Cresson), treated with imidacloprid. In another study, recommended field rate of imdacloprid caused 90% mortality on *D. insulare* (Hill & Foster, 2000).

Sublethal effects of the insecticides on biological parameters are shown in table 3. Biological parameters were significantly affected by LC_{25}. Fecundity of females was significantly different among treatments. Fecundity rates treated with imidacloprid, endosulfan and indoxacarb were reduced by 28.5, 45.7 and 61.8%, respectively. There was a significant difference between indoxacarb treatment and control, but the difference between control and imidacloprid treatment was not significant. Galavan *et al*. (2005) reported that indoxacarb caused a reduction in adult fertility and reduced survivorship of the first instar and adult *Harmonia axyridis* (Pallas) in the laboratory. Studebaker & Kring (2001) reported that imidacloprid and indoxacarb insecticides affected the survival of the third instars and adult *O. insidiosus*. The net reproductive rate (R_0) value of *C. carnea* when the first instar was treated with imidacloprid was not affected (Rezaei *et al.*, 2007). Grosch & Hoffman (1973) suggested that the reduction in fertility of parasite wasps, treated with insecticides, could be related to food uptake reduction, changes in physiology or cytotoxic destruction of eggs. De Cock *et al*. (1996) reported that when females of pentatomid bug, *Podisus maculiventris* (Say), were exposed to imidacloprid, mortality was high, but oviposition and egg hatch rate were not affected by sublethal exposure of females. Kunkel *et al*. (1999) found that imidacloprid did not affect predatory arthropods in soil.

Duration of pre-oviposition period was significantly different among treatments (table 3). The highest and the lowest durations were in indoxacarb and control treatments, respectively; however, the differences between control and endosulfan and imidacloprid treatments were not significant. Reduction in the time of reproduction (pre-oviposition period) caused intrinsic rate of increase of population to be lower than when the survivorship and fecundity is reduced. The ability of a pesticide to delay the oviposition of natural enemies is considered an important factor in the risk assessment of pesticides (Walthall & Stark, 1997).

The longevities of males and females were the highest in the control, while they were the lowest in indoxacarb treatment (table 3). Longevity of *Microplitis croceipes* (Cresson) was affected after feeding for 10 days on flower nectar of plants treated with systemic insecticides (acephat, aldicarb and imidacloprid) (Staple *et al*., 2000).

According to IOBC method, all three insecticides were slightly harmful (group II). The value of TEI for endosulfan, imidacloprid and indoxacarb were 39.3, 46.4 and 71.4, respectively. In another study, imidacloprid was slightly harmful (Group II) against *C.*

carnea in the laboratory (Talebi *et al.*, 2008). Imidacloprid, indoxacarb, acehate and oxamyl were in the harmful group, when their toxicity was assessed against the parasitoid wasp *Anaphes iole* (Girault). Release of natural enemies in cotton fields treated with insecticides has been regulated carefully to reduce the side effects of insecticides on natural enemies (Williams *et al.*, 2003).

In conclusion, in addition to acute toxicity of imidacloprid, indoxacarb and endosulfan against adult *C. carnea*, these insecticides affected the biological parameters of the green lacewing. Assuming that effects of these insecticides in the field, resemble those observed in the laboratory; these insecticides should preferably be applied when the density of adult insects is not high in the field.

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Table 3. Mean (± SE) fecundity, pre-oviposition, oviposition, post-oviposition, male and female longevity parameters when adult *Chrysoperla carnea* were treatment with LC_{25} of three insecticides.

Parameter	Mean ± SE			
	Indoxacarb	Endosulfan	Imidacloprid	Control
Fecundity (egg/female)	206 ± 42 c	293 ± 41 bc	386 ± 60 ab	540 ± 49 a
Pre-oviposition (day)	7.7 ± 1.3 b	4.4 ± 0.18 a	4.3 ± 0.3 a	3.4 ± 0.28 a
Oviposition (day)	14.2 ± 2.3 c	17.8 ± 1.6 bc	22 ± 3.2 b	30.4 ± 2.4 a
Post-oviposition (day)	5.7 ± 1.2 a	4.5 ± 0.8 a	5.5 ± 1.5 a	3.01 ± 0.7 a
Male longevity (day)	21.3 ± 2.4 b	19.7 ± 1.4 b	24.3 ± 3.3 ab	30 ± 2.3 a
Female longevity (day)	27.7 ± 2.6 b	26.7 ± 1.7 b	31 ± 2.9 ab	37 ± 2.5 a

Means within each row followed by different letters were significantly different based on Duncan's Multiple Range Test (P > 0.05).

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