Türk. entomol. derg., 2013, 37 (4): 399-407
ISSN 1010-6960

Orijinal araştırma (Original article)

Lethal effects of four insecticides on immature stages of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) in laboratory conditions

Laboratuvar şartlarında *Chrysoperla carnea* (Neuroptera: Chrysopidae) (Stephens)'nın ergin öncesi dönemleri uzerine dört insektisitin öldürücü etkileri

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Summary

Chrysoperla carnea (Stephens) is a general biocontrol agent of several insect pests in greenhouses. The lethal effects of four compounds, imidacloprid, lufenuron, thiametoxam and thiodicarb, on the eggs and 1st instar larvae of *C. carnea* were studied in laboratory conditions. Dipping bioassay tests were used for eggs and the residual contact method for larvae. Positive relationships were detected between the concentrations of insecticides and mortality rates of various stages. However, there were considerable variations in toxicity of insecticides. Thiodicarb had no effect on eggs, whereas thiametoxam with an LC_{50} value of 1.90 µg ai. L^{-1} showed the highest ovicidal activity. On larvae, thiametoxam was the most toxic (LC_{50}= 0.55 µg ai. L^{-1}) and lufenuron proved to be the least toxic (LC_{50} = 44.02 µg ai. L^{-1}). The use of thiametoxam should be carefully evaluated if employed in combination with *C. carnea* in IPM programs.

Key words: *Chrysoperla carnea,* imidacloprid, lufenuron, thiametoxam, thiodicarb.

Özet

Chrysoperla carnea (Stephens) seralarda birkaç zararlıyı kontrol altinda tutan genel bir biyolojik savaş ajanıdır. Imidacloprid, lufenuron, thiametoxam ve thiodicarb olmak uzere dört bilesigin oldurucu etkileri *C. carnea*'nın yumurta ve birinci dönem larvaları üzerinde, laboratuvar koşullarında denenmiştir. Bioassay testlerde yumurta için daldırma yöntemi, larvalar için rezidüel kontakt metodu kullanılmıştır. İnsektisitlerin konsantrasyonları ile degisik donemlerin ölüm oranları arasinda pozitif bir ilişki belirlenmiştir. Ancak, insektisitlerin toksisitesinde önemli farklılıklar görülmüştür. Thiodicarb yumurta üzerinde etkili olmamis, thiametoxam ise 1.90 µg ai. L^{-1} LC_{50} değeriyle en yüksek ovisidal etkiyi göstermiştir. Larvalar üzerinde, thiametoxam en zehirli (LC_{50} = 0.55 ug ai. L-1) ve lufenuronun en az zehirli olduğu kanıtlanmıştır (LC_{50} = 44.02 mg ai. L-1). Thiametoxamin IPM programlarında *C. carnea* ile kombinasyon halinde kullanılma durumu dikkatli bir şekilde değerlendirilmelidir.

Anahtar sözcükler: *Chrysoperla carnea,* imidacloprid, lufenuron, thiametoxam, thiodicarb.

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Alınış (Received): 22.04.2013 Kabul ediliş (Accepted): 09.09.2013

Introduction

Chrysoperla carnea (Stephens) is a widespread predator in biocontrol of soft body insect pests in agro-ecosystems and greenhouses (Hassan 1974; Corrales & Campos 2004). Adults of Chrysopidae feed on plant nectar and pollen, whereas their larvae are voracious and are efficient biological control agents against various phytophagous arthropods, such as mites, butterfly eggs, sharpshooters, whiteflies and thrips (New 1975; Bueno & Fereitas 2004; Rimoldi *et al*. 2008). The use of lacewings in IPM programs has increased dramatically in recent years because of their broad distribution worldwide and rearing (Medina *et al.* 2001).

In recent years, there has been considerable interest in development of plant protection programs that assure a more compatible use of chemical and biological methods of pest control. In these so-called 'integrated control programs' certain chemical control practices can destroy the pests without disrupting their effective natural enemies, thereby restricting subsequent pest increase. Among the most useful programs are those in which success depends upon the selection of pesticides that are less toxic to the most important natural enemies. Unlike the more complicated practices suggested for special timing or placement of toxicants to avoid pesticide contact with the natural enemies, this type of program requires less supervision and less knowledge of the differences in pest and natural enemy behavior (Hassan *et al.* 1985).

The use of selective insecticides allowing the survival of *C. carnea* populations is essential for IPM (Medina *et al.* 2003). Many laboratory and semi-field studies have demonstrated various responses of *C. carnea* to insecticides (Roger *et al*. 2007). Medina *et al.* (2003) showed that the egg and pupal stages of *C. carnea* were not affected by the chitin synthesis inhibitors, diflubenzuron and tebufenozide, whereas diflubenzuron was very toxic to larvae. The above-mentioned compounds had no effect on adults and their reproductive parameters. In another study, Bueno *et al.* (2004) indicated that abamectin was harmless and lufenuron was toxic to *Chrysoperla externa* Hagen (Neuroptera: Chrysopidae) eggs and larvae. According to the results of Rezaei *et al.* (2006), imidacloprid was harmless, although propargite and pymetrozine were slightly harmful on two-day-old larvae of *C. carnea*.

Imidacloprid and thiametoxam are systemic, chloronicotinyl insecticides, entering the pest through ingestion or direct contact. They act by disrupting nicotinic acetylcholine receptors in the insect nervous system (Mullins 1993). They are used worldwide on ornamentals, field crops and vegetables with application to seeds, soil and foliage for controlling many pests including aphids, scales insects, white flies, some coleopterans and lepidopterans. Imidacloprid and thiametoxam are absorbed by roots and transported via the xylem to the aerial parts of the plant (Horowitz *et al*. 1998; Ishaaya and Horowitz 1998; Buchholz and Nauen 2002).

Lufenuron, a benzoyl-urea compound which is thought to be a chitin synthesis inhibitor belongs to a class of insect growth regulators (IGRs). The IGRs have been proposed as alternative insect control agents (Graf 1993). They appear to act as inhibitors of a yet undefined step(s) in the chitin synthesis pathway, disrupting molting at critical developmental stages of insects (Hajjar 1979; Fournet *et al*. 1995). Lufenuron has been recommended for the control of *Cydia pomonella* Linnaeus (Lepidoptera: Totricidae) in Iran.

Thiodicarb is an oxime carbamate compound affecting the Diptera, and Lepidoptera via ingestion and direct contact. This compound showed high ovicidal activity against *Heliothis* spp. (Lepidoptera: Noctuidae) (Milne 2004).

Although long term effects of insecticides on natural enemies have attracted the attention of many researchers (Medina *et al.* 2002; 2003), short term effects have been rarely investigated. Various developmental stages of natural enemies might be exposed directly to insecticides, being unable to escape insecticide sprays.

The present study aimed to evaluate the toxicity of several insecticides in different groups including the neonicotinoids, imidacloprid and thiametoxam, the carbamate thiodicarb, and the chitin synthesis inhibitor lufenuron, on immature stages of *C. carnea*. All of these products are registered in Iran (Sheikhi Gorjan *et al*. 2009).

Material and Methods

Insect culture

A colony of *C. carnea* was reared in Plant Protection Section of the Agricultural and Natural Resource Research Center of Khorasan Razavi Province in January 2010. Molecular characterization of the geographical population had been already investigated (Ayubi *et al*. 2010). Adult insects were kept in plastic containers (16 cm in diameter and 24 cm in height) covered with a cloth screen, and fed on an artificial diet consisting of 4g brewer's yeast, 7g honey and 5ml water. The mixture formed a paste which was smeared on transparent plastic tapes and put in rearing containers (Vogt *et al*. 2000). Extra water was provided with a wet sponge attached to the top of the container. The eggs deposited on the walls and screens were removed daily using a brush. Newly hatched larvae were feed on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Rearing conditions were 25±2 ºC, 60±5% r. h. and a photoperiod of 16:8 (L: D).

Chemicals

The insecticides tested were imidacloprid (Confidor®, 35% SC, Aria chemistry Company), lufenuron (Match®, 50% EC, Syngenta Company), thiametoxam (Actara®, 35% WG, Syngenta Company) and thiodicarb (Larvin®, 80% DF, Syngenta Company).

Bioassay methods

A batch of 30, three-day old eggs was dipped into an insecticide solution in distilled water for 10 seconds, allowed to dry for 2h at room temperature, and then transferred to ventilated plastic boxes at the rearing room, based on the methodology described by Medina *et al*. (2001). The concentration ranges for imidacloprid, lufenuron and thiametoxam were: 0.58-52.5, 250-50000, 0.313-30 µg ai. L^{-1}, respectively. Control samples were treated with distilled water. The experiment was replicated 10 times. Newly emerged larvae were provided with 12 mg of processed eggs of *E. kuehniella* in each test box. Hatched eggs were evaluated 5-6 days after commencement of the experiment.

The toxicity of insecticides on the 1^{st} instars larvae was assessed using the residual contact method. An amount of 0.5 ml of each insecticide solution was added to each glass Petri dish (90 mm diameter) and allowed to dry at ambient temperature. The concentration ranges for imidacloprid, lufenuron, thiametoxam and thiodicarb were 3.5-52.5, 25-75, 0.125-2 and 2-416 µg ai. L^{-1}, respectively. Ten first instar, one day old larvae were transferred to each petri dish. The larvae of each petri dish were provided with 100 eggs of *E. kuehniella* to prevent cannibalism. Mortality was recorded after 24h. Each experiment was replicated 10 times.

Data analysis

Mortality data for each developmental stage were analyzed with the probit model (Finney 1971), using the Maximum Likelihood Program (POLO-PC, LeOra Software, Berkeley, California). The program POLO-PC calculates a theoretical 'natural response' for each experiment, based on the pattern of mortality at all concentration levels, including controls (Abbott 1925). The results include estimation of the LC_{50} (and other LCs, if required) and the 95% confidence limits, slope and intercept of probit mortality regression, and for the relevant statistical tests (such as "t" ratio, 'g' factor and heterogeneity). For comparison of the probit mortality lines, the program also provides the likelihood ratio tests of equality and

parallelism (Russel *et al.* 1977). Estimated median lethal concentration to kill 50% of insects was expressed as the LC_{50} (µg ai. L^{-1}). The resistance ratio and 95% confidence limits of this ratio were determined between data from different insecticide treatments, and comparisons were based on the procedure described by Robertson & Preisler (1992). The estimates of values of parameters needed for computing confidence limits of the resistance ratio were provided by individual probit analysis from the POLO-PC output.

Results

Eggs

The results showed that all insecticides had a lethal effect on eggs, except thiodicarb, which was ineffective even at concentrations higher than the maximum field recommended rate (Table 1). Order of the ovicidal activity of other insecticides was as follows: thiametoxam> imidacloprid> lufenuron. Lufenuron had the highest LC_{50} value against eggs (2185.88 µg ai. L^{-1}) (Table 1), being about 1152 and 564 times higher than those of thiametoxam and imidacloprid, respectively (Table 2). The eggs were more susceptible to thiametoxam (LC_{50} = 1.90 µg ai. L^{-1}) than to imidacloprid (LC_{50} = 3.87 µg ai. L^{-1}). A more appropriate comparison among toxicities of the three insecticides could be obtained using probit analyses of data. The dose-mortality responses of *C. carnea* eggs were compared in terms of differences in slopes and/or intercepts of probit regressions, and LC_{50} values. The slope values of probit regressions were in the range of 1.08-1.18. The heterogeneity factors were less than 1.0 for all of the insecticides, except thiametoxam. A heterogeneity factor greater than 1.0 indicated that the result was not within the 95% confidence limits, so a correction factor (g) was required. For all insecticides, the values for regression tests ("t" ratio) were greater than 1.96 and values for the potency estimation tests ("g" factor) were less than 0.5 at all probability levels.

Table 1. Probit analysis of toxicity[¥] of insecticides to the eggs and larvae of *C. carnea*

Pesticide	Stage	N[€]	Intercept (± SE)	Slope (± SE)	"t" ratio	Hetero-geneity	g(0.95) factor	Lethal concentrations (µg ai. L^{-1}) (95% CL)[£]	
								LC_{50}	LC_{90}
Lufenuron	Egg	1500	-3.61±0.33	1.08±0.86	12.62	0.12	0.024	2185.88 (1516.19-2976.29)	33458 (23850-50727)
	Larvae	500	-9.34±0.79	5.68±0.48	11.84	0.20	0.027	44.02 (41.47-46.74)	73.97 (67.43-83.57)
Imidacloprid	Egg	1500	-0.72±0.12	1.23±0.10	12.31	0.61	0.025	3.87 (2.81-5.07)	42.46 (31.48-61.62)
	Larvae	500	-2.57±0.23	2.23±0.19	11.64	0.64	0.028	14.08 (12.12-16.38)	52.73 (41.59-72.29)
Thiodicarb [δ]	Larvae	500	-4.03±0.35	3.35±0.28	11.74	0.28	0.027	15.88 (14.36-17.56)	38.28 (32.75-47.07)
Thiametoxam	Egg	1500	-0.32±0.92	1.18±0.91	12.98	1.02	0.011	1.90 (1.14-2.84)	23.08 (14.69-42.76)
	Larvae	500	0.64±0.94	2.46±0.20	12.27	0.84	0.025	0.55 (0.47-0.63)	1.81 (1.47-2.39)

[¥] Dipping bioassay used for eggs and residual contact bioassay for larvae
[€] N= total number of insects tested (including control)
[£] CL= confidence limits
[δ] No mortality recorded for eggs even at concentrations above field recommendation (10^6 µg ai. L^{-1}).

The slopes of the three probit mortality regressions for the insecticides were not significantly different, as revealed by the acceptance of the likelihood ratio test of parallelism (X^2=1.73, *df*=2, *P*=0.42), so the common slope of 1.161 ± 0.048 was calculated. The intercepts of probit mortality regressions for the three insecticides were significantly different, as revealed by rejection of the likelihood ratio test of equality (X^2=676.43, *df*=4, *P*<0.001).

Table 2. LC_{50}s ratios and their respective 95% confidence limits calculated to compare toxicity[¥] of insecticides to the eggs of *C. carnea*

Insecticidal comparison	Ratio	95% CL[€] of ratio
Lufenuron/ Thiametoxam	1152	732.56-1810.74*
Lufenuron/Imidacloprid	564	360.07-884.78*
Imidacloprid/Thiametoxam	2	1.34-3.11*

[¥]Toxicity tested by dipping method
[€]Lower and upper 95% CL calculated as described by Robertson & Preisler (1992)
*Significant difference at P<0.05.

Larvae

On larvae, lufenuron caused 90% mortality at the concentration of 75 µg ai. L^{-1}. Similar mortality was obtained by thiametoxam at 2 µg ai. L^{-1}. Based on probit analysis, the highest LC_{50} value (44.02 µg ai. L^{-1}) was achieved by lufenuron (Table 1).The results of residual contact of insecticides on 1st instar larvae indicated that there was positive relationship between mortality rate and insecticide concentration. Thiametoxam was the most potent toxicant on 1st instar larvae with an LC_{50} of 0.55 µg ai. L^{-1}; the LC_{50} values of lufenuron, thiodicarb and imidacloprid were, respectively, 80, 29 and 25 times higher than that of thiametoxam. The LC_{50}s ratios of the insecticides showed that their toxicities to larvae differed significantly, except for imidacloprid and thiodicarb (Table 3).

Table 3. LC_{50}s ratios and their respective 95% confidence limits calculated for comparing toxicity[¥] of imidacloprid, lufenuron and thiametoxam to the larvae of *C. carnea*

Insecticidal comparison	Ratio	95% CL[€] of ratio
Lufenuron/ Thiametoxam	80.43	68.86-93.94*
Thiodicarb/ Thiametoxam	29.02	24.35-34.58*
Imidacloprid/ Thiametoxam	25.73	20.89-31.70*
Lufenuron/Imidacloprid	3.12	2.65-3.68*
Lufenuron/Thiodicarb	2.77	2.46-3.11*
Thiodicarb/Imidacloprid	1.13	0.94-1.35 NS

[¥] Toxicity tested by residual contact method
[€] Lower and upper 95% CL calculated as described by Robertson & Preisler (1992)
* Significant difference at P<0.05
NS Not significant.

The slope values of probit regressions were in the range of 2.23-5.68. The heterogeneity factor of less than 1.0 for all of the insecticides indicated that the results were within the 95% confidence limits, so no correction factor (g) was required. For all insecticides, the values for regression tests ("t" ratio) were greater than 1.96 and the potency estimation tests ("g" factor) were less than 0.5 at all probability levels. The slopes of probit mortality regressions differed significantly among insecticides, as revealed by rejecting the likelihood ratio test of parallelism (X^2=56.47, *df*=3, *P*<0.001), as did the intercepts, as revealed by the likelihood ratio test of equality (X^2=614.55, *df*=6, *P*<0.001).

Discussion

Chrysoperla species are widespread and major predators of larvae and adults of hemipteran pests. They are considered to be important natural enemies in a broad range of crops. Knowledge of the impact of pesticides on beneficial arthropods is necessary for successful integration of biological control in agro-ecosystems (Croft 1990). Both chemical and biological control are important for management of insect pests. For years, conventional insecticides were used in these systems, but they may also contribute to the reduction in natural enemy populations.

The present results indicated that thiodicarb had no ovicidal activity, meanwhile thiametoxam showed high toxicity to eggs (LC_{50} = 1.90 µg ai. L^{-1}). The egg phase is the most tolerant stage to the action of pesticides. The tolerance of eggs to some insecticides has already been observed for some chrysopids (Velloso *et al*. 1997; Carvalho *et al.* 1998; Bueno *&* Freitas*,* 2004) and particularly for *C. carnea* (Bartlett 1964).

The high estimated LC_{50} values for lufenuron on immature stages (Table 1) suggested that lufenuron seems to be a suitable candidate for use in IPM programs. This compound showed no toxicity at doses higher than field recommendation in Iran (1 lit.ha^{-1}). Another chitin synthesis inhibitor compound, tebufenozide, had no effect on survival, fecundity or fertility of *C. carnea* adults treated by residual assay (Vinuela *et al.* 1998). Similar conclusions regarding the harmlessness of these compounds in residual treatments of predators have been obtained with *Amblyseius longispinus* Evans, *A. andersoni* Chant and the coccinelid *Stethorus punctum* Weise (Biddinger & Hull 1995). Sechser *et al. (*1994) indicated that the insect growth-regulatory (IGR) insecticide, diofenolan (CGA 59 205) had no effect on *C. carnea* adults but disrupted molting of the third instar larvae. Medina *et al*. (2002) stated that the chitin synthesis inhibitor compounds are relatively harmless to *C. carnea* larvae in the worst-case scenario (laboratory). However, Medina *et al*. (2003) suggested that the effect on *C. carnea* strongly depends on the concentration used and further studies are needed to take advantage of the potential use of IGRs in the pest control market.

Thiametoxam and imidacloprid are categorized in the neonicotinoid compounds group, being neurotoxins with a novel mode of action, with the latter was much more noxious to immature stages of *C. carnea* (Table 1). The estimated LC_{50} values for thiametoxam and imidacloprid seem to be much lower than field application doses, as recommended in Iran (0.3 kg. ha^{-1} and 1 lit.ha^{-1}, respectively). Thiametoxam was classified as harmful for beneficial insects, reported as a highly effective, broad spectrum insecticide with potential value for the control of a wide range of crop and veterinary pests (Nasreen *et al.* 2005). However, it may severely disrupt natural enemy populations, so it's suitability for inclusion in IPM programs should be carefully evaluated. Lawson *et al*. (1999) stated that thiametoxam is often applied to the soil, protecting systematically the plant. Therefore, flexibility in the application of thiametoxam with limited leaf surface residues results in excellent pest control without disrupting natural enemies.

The present study showed that imidacloprid showed high toxicity on larvae (Table 1). Huerta *et al.* (2003) demonstrated that imidacloprid was a very toxic insecticide against *C. carnea,* causing a high mortality on larvae and adults. Elbert *et al*. (1998) reported 40% *C. carnea* larval mortality in a field trial with 120-250 g ai. ha^{-1} of imidacloprid. Rezaei *et al*. (2006) stated that imidacloprid is considered as category 1 by IOBC, because of its high toxicity on all developmental stages of *C. carnea.* Similar results were reported by Van De Veire *et al.* (2002) for the predatory bug *Orius laevigatus* (Hemiptera: Anthocoridae) for the neonicotinoid compounds, thiametoxam and imidacloprid.

The present study may contribute to successful conservation of biological control in crops where common green lacewings are the most common natural enemies. Having focused on short term effects, the authors are aware of the importance of long term effects of insecticides on natural enemies in the IPM programs, particularly those of chitin synthesis inhibitors (Medina *et al*. 2003). IOBC classification of these insecticides requires evaluation of other developmental stages. Moreover, semi-field and field research will be needed to evaluate the residual toxicity of these insecticides and their potential sub-lethal effects.

Acknowledgments

The authors are thankful for the assistance of Dr G. Golmohammadi of the Plant Protection Research Institute of Iran. This work is a part of the MSc thesis of the first author. Thanks are due to Faculty of Agriculture, Ferdowsi University of Mashhad, Iran for its financial support.

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