LETHAL EFFECTS OF SPINOSAD ON *CHRYSOPERLA CARNEA* LARVAE (NEUROPTERA: CHRISOPIDAE) UNDER LABORATORY CONDITIONS

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Abstract: The use of selective insecticides could improve conservation of natural enemies and therefore contribute to the success of Integrated Pest Management (IPM) programs. In this study, the toxicity of one selective insecticide, Spinosad to common green lacewing *Chrysoperla carnea* Stephens was evaluated. Several stages of *C. carnea* larvae were exposed to Spinosad under laboratory conditions. The used quantities of Spinosad were less than the maximum recommended rate given on the product label. In contact bioassay tests, a direct relationship was detected between the concentration of Spinosad and mortality rate of first instar larvae. So that, the employing of 250 and 2 500 ppm of Spinosad caused 33 and 67 per cent mortality, respectively. Mortality rate was recorded 1–3 day post treatment. In implementation of 250 ppm of Spinosad on second and third instar larvae showed negligible mortality rate after 3 days whereas the first instars larvae suffered 33 per cent mortality. On the basis of collected data we could conclude that Spinosad is not to be considered to have an environmental safety profile on *C. carnea* similarly to well established biological insecticides.

Key words: common green lacewing, natural enemies, insecticide, bioassay, biocontrol agent

INTRODUCTION

The adverse impact of insecticides on natural enemies can be mitigated through choice of insecticide, dosage, or timing of insecticide application. Biological control and selective insecticides proved to be compatible tactics in Integrated Pest Management (IPM) programs (Galvan *et al*. 2005). Integrating biological control with selective insecticides also can minimize the likelihood of pest resurgence and possibly to reduce the number of insecticide applications (Hutchison *et al*. 2004).

The role of generalist predators as effective control agents is being supported by both biocontrol theory and practice (Symondson *et al*. 2002). The Chrysopidae family includes important predator species with adults feeding on plant nectar and pollen, whereas larvae show preference for certain soft-bodied prey such as "aphids", "whiteflies", "thrips", eggs, and larvae of lepidopterans and acari (Rimoldi *et al*. 2008). *Chrysoperla carnea* Stephens is a widespread polyphagous predator, used in biocontrol of aphids in greenhouses and very common in many agricultural systems. The use of lacewings in IPM programmes increased in recent years because, this insect may have an advantage over other introduced or resident natural enemies: a relatively broad tolerance to many insecticides, particularly during the larval and cocoon stages (Medina *et al*. 2001).

The impact of synthetic pesticides on beneficial arthropods and the human health risks posed by exposure to these chemicals are issues of growing concern (National Research Council 1996; Cisneros *et al*. 2002). This prompted the development of new compounds, such as imidacloprid, oxamyl, and cyfluthrin, with reduced environmental persistence and low mammalian and avian toxicity but a fairly broad spectrum of insecticidal activity (Harris 2000). An example is Spinosad (Dow AgroSciences), a mixture of spinosyns A and D that are tetracyclic macrolide compounds produced by an actinomycete, *Saccharopolyspora spinosa* Mertz and Yao, isolated from a Jamaican soil sample (Crouse *et al*. 2001). As these products are created by biosynthesis during fermentation of *S. spinosa*, Spinosad was classified as a bioinsecticide (Copping and Menn 2000).

Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera and Diptera (Xian-Hu *et al*. 2008). It is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and apparently the GABA receptors as well (Salgado 1998). Spinosad is classified by the U.S. Environmental Protection Agency as an environmentally and toxicologically reduced risk material (Cleveland *et al*. 2001). However, according to a recent review by Williams *et al*. (2003), among 25 parasitoid species tested, 78% of the laboratory studies and 86% of the field studies reported that Spinosad was moderately harmful or harmful to the parasitoids. Thus, the use of Spinosad-based products should be evaluated carefully with respect to the need for

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biological control by augmentative release and/or conservation of parasitoids. Galvan *et al*. (2005) reported that Spinosad decreased the survival of first instars, extended the time it took first instars to become adults, decreased gain weight and reduced the fertility of female *Harmonia axyridis.* Schneider *et al*. (2004) found that a sublethal dose of Spinosad also affected the life history parameters, such as a delay in development, a reduction in rate of pupae formation, pupal mortality, adult longevity and adult emergence in third-instar larvae of the endoparasitoid *Hyposoter didymator*.

As a result, the marketing strategy for Spinosad has focused heavily on its favorable environmental profile, reflected in the trade name "Naturalyte" used for this group of insect control products. Indeed, the safety profile of Spinosad was described as similar to that of benign biological pesticides (Thompson and Hutchins 1999). Formulation can have a marked impact on the biological activity of a pesticide toward both target and nontarget arthropod species (Croft 1990). Exposure results in cessation of feeding followed, some 24 h later, by paralysis and death. Conventional toxicity tests indicate that Spinosad has virtually no toxicity to birds and mammals. With a contact LC_{50} value of 200 ppm, Spinosad was also reported to be practically nontoxic to insect natural enemies such as *Orius* spp., *Chrysopa* spp., coccinelids, and the predaceous mite *Phytoseiulus persimilis* Athias-Henriot (Bret *et al*. 1997).

The objective of this study is to evaluate the toxicity of Spinosad on *C. carnea* larvae under laboratory conditions with the purpose of generating IPM guidelines for natural enemy conservation.

MATERIALS AND METHODS

Insect

A laboratory colony of *C. carnea* [grown at 25±2°C, 75±5% R.H., and a photoperiod of 16 : 8 (L : D)] was obtained from eggs received from Green Schema insectarium's mass rearing insects for biological control, Tehran (Iran) and maintained in culture for one to two generations at the University of Urmia, Urmia (Iran) before initiation of experiments. Larvae were reared on *S. cerealella* (Oliver) eggs and adults on an artificial diet as described by Vogt *et al*. (2000).

Insecticide

A sample of the commercial formulation of Spinosad (Tracer Naturalyte Insect Control) was obtained as a gift from Dow AgroSciences, Tehran. It contained 480 g of Spinosad active substance (a.s.) per liter.

Toxicity bioassays

Spinosad commercial formulation was applied at the doses of 250, 440, 800, 1 400 and 2 500 ppm for first instar and 500, 780, 1 220, 1 920 and 3 000 ppm for second and third instar larvae. Each dose was replicated 4 times, with 15 individuals per replication. Required solutions were prepared in distilled water and alkylarylpolyglycol ether was added (100% B.A.S.F, Germany) as surfactant to improve the adherence of the insecticide to the surface of

Petri dish. A Petri dish was lined with damp filter paper, and allowed to dry up for 1 h under laboratory conditions and 2- to 3-day-old larvae were used. In all experiments for a treatment, 15 larvae (24±6 h after emergence) were placed into plastic Petri dishes (150x15 mm). The larvae were fed on eggs of *S. cerealella* throughout the experiment. The Petri dishes were placed in incubators set at 27°C and 60±5% relative humidity. Mortality was recorded after 24, 48, and 72 h of exposure. Larvae were considered dead if they did not move when prodded with a soft paint brush.

Statistical analysis

Proportion mortality data were analyzed after 24, 48, and 72 h of exposure using ANOVA and treatment means were separated by Fisher's test LSD at p < 0.05 (SAS Institute 2000). To stabilize variance, proportion data were transformed [arcsin sqrt (x+0.001)] before analysis. The dose mortality response of the insecticide concentration was estimated using probit analysis in SPP (SPP 1999). Control mortality was corrected using Abbott's formula (Abbott 1925). LC_{50} and related statistics were estimated separately for the insecticide in each experiment.

RESULTS

First instars

The tested concentrations of Spinosad (250, 440, 800, 1 400 and 2 500 ppm) showed lethal effects to *C. carnea* larvae, with a dose response observed across rates of Spinosad. The mortality (mean ±SE) in all treatments (33.35±5 to 67.25±3%) was significantly more than in untreated control group (3.33±2.35%) (Fig. 1). Survival of *C. carnea* treated with Spinosad at 250 ppm was significantly higher than that of larvae which were treated with Spinosad at 2 500 ppm. There was a highly significant mortality effect of the concentration of Spinosad on first instars after 1 day postexposure ($x^2 = 1.042$, df = 4, p < 0.001) increasing through to 3 days postexposure ($x^2 = 1.138$, df = 4, p < 0.001).
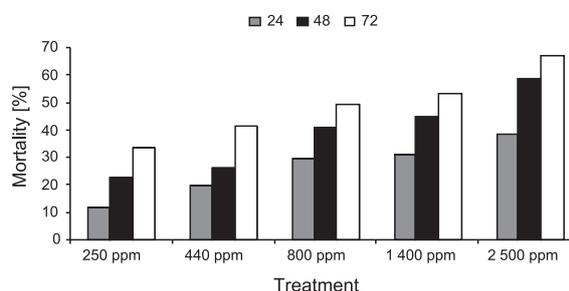

Fig. 1. Mean mortality percentages (% + SE) of first instars of *C. carnea* treated with 250, 440, 800, 1 400 and 2 500 ppm of Spinosad after 3 days

Second instars

In these experiments we used 500–3 000 ppm of Spinosad. A direct relationship was detected between mortality rate and Spinosad concentration. Mortality was increased significantly according to the concentration of Spinosad to which larvae were exposed from 1 day

($x^2 = .071$, df = 4, p < 0.001) to 3 days ($x^2 = 0.518$, df = 4, p < 0.001) after the initiation of the experiment. Mortality in control group did not exceed more than 6% after 3 days, whereas 3 d post treatment, mortality was moderately low in Spinosad treatments reaching 28.18±0.98% at 500 ppm and 45.83±2% at 3 000 ppm of Spinosad (Fig. 2).
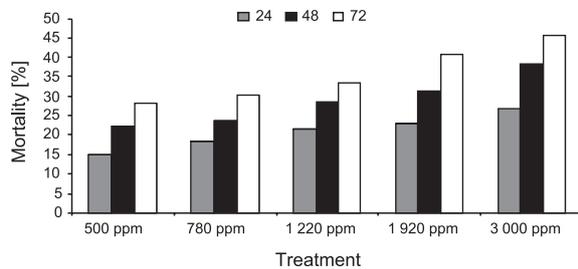

Fig. 2. Mean mortality percentages (% + SE) of second instars of *C. carnea* treated with 500, 780, 1 220, 1 920 and 3 000 ppm of Spinosad after 3 days

Third instars

Survival of third instars of *C. carnea* treated with Spinosad did not differ significantly from the untreated controls. After 3 days of exposure, significantly lower mortalities, ranging from only 26.82±1% to 41.99±1% at 500 ppm and 3 000 ppm, respectively, were recorded on third instars (Fig. 3).

The LC_{50} values and related statistics for different larval instars are given in table 1. On the basis of LC_{50} values, first instars larvae of *C. carnea* were more susceptible than the other two larval stages.
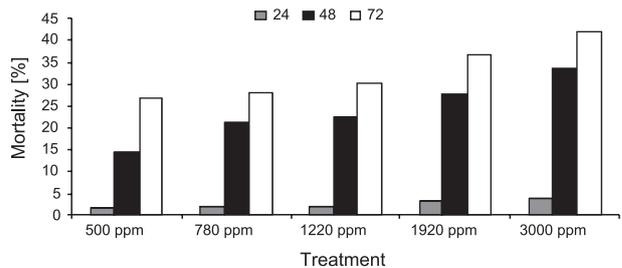

Fig. 3. Mean mortality percentages (% + SE) of third instars of *C. carnea* treated with 500, 780, 1 220, 1 920 and 3 000 ppm of Spinosad after 3 days

Table 1.   Probit analysis data for *C. carnea* larvae in treated with Spinosad after 3 days of exposure

	LC_{50} [ppm]	CL* (95%)	Intercept	Slop ±SE	p	X^2
First instars	581.18	417.22 – 754.83	1.19	1.37±0.68	0.076	1.13
Second instars	2883.34	7946.52– 1947.52	1.27	1.07±0.91	0.932	0.439
Third instars	4042.91	27011.51– 2366.68	1.78	0.890±0.86	0.864	0.739

*lower and upper confidence limit

DISCUSSION

Chrysoperla spp. are widespread and are major predators of larwae and adults of hemipteran pests. They are considered to be important natural enemies in a broad range of crops. Thus, the natural enemies chosen for study included a range of life histories and were from diverse taxonomic group (Neuroptera).

Knowledge of the impact of pesticides on beneficial arthropods is necessary for successful integration of biological control in agroecosystems (Croft 1990). Studies of pesticide impact on natural enemies usually address topical application or ingestion of a toxin, exposure of natural enemies to pesticide residues, or field studies assessing changes in natural enemy populations in response to pesticide application (Tillman and Mulrooney 2000; Martinson *et al*. 2001). Each approach provides different information about pesticide impacts to natural enemies. Studies on topical application and ingestion of toxins provide information on the effect of a compound when applied directly onto an insect or when it is ingested with food.

Chemical and biological control is both important for management of insect pests. For years, conventional insecticides were used in these systems, but they also may contribute to the reduction in natural enemy populations. In the past decade, new insecticides with unique mode of action showed high toxicity to insect pest populations, while being relatively nontoxic to natural enemies. New compounds, such as Spinosad may prove essential to the integration of chemical and biological control in IPM programs. The International Organization of Biological Control (IOBC) suggests a tiered approach to evaluate the potential effects of insecticides on natural enemies with laboratory studies followed by semi-field and field tests. According to the IOBC, once an insecticide is tested in the laboratory and shows no toxicity to natural enemies, no further semi-field or field studies are needed (Medina *et al*. 2001).

The fact that Spinosad is obtained from a naturally occurring soil organism does not automatically mean that it is safe and innocuous. In addition, Stark *et al*. (1995) pointed out the need for caution when making assumptions on pesticide impact on beneficial organisms based solely on laboratory-generated toxicity data. Toxicity of Spinosad to natural enemies is subject to controversy. Spinosad, when used according to good agricultural-horticultural practices, was found to be compatible with predatory mites (*Typhlodromus pyri* Scheuten, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius californicus* McGregor), predatory Heteroptera (*Orius insidiosus* Say, *Orius laevigatus* Fieber and *Macrolophus caliginosus* Wagner), Coccinellidae (*Hippodamia convergens* Gherin and *Coccinella septempunctata* L.) and Neuroptera (*C. carnea* and

Chrysoperla rufilabris Burmeister) evaluated at the stages commercially available for biological control (Miles and Dutton 2000), which is in accord with previous studies reported by Bret *et al*. (1997).

However, the results of this study showed that Spinosad at the high concentration resulted in 67% mortality of first instar larvae after 72 h of exposure. Selectivity of Spinosad on predators is under discussion because Spinosad is highly toxic by ingestion treatment to the earwing *Doru taeniatum* (Dohrn) and to a lesser extention to the staphylinid *Aleochara bilineata* Gyllenhal (Cisneros *et al*. 2002). Viñuela *et al*. (1998) reported a significant mortality of *Podisus maculiventris* (Say) nymphs when treated via ingestion and topical treatment with 15 and 50 mg a.i. litre onwards, respectively. Spinosad is considerably more toxic to parasitoid adults such as *Cotesia marginiventris* (Cresson), *C. plutella* (Kurdjumov), *Catolaccus grandis* (Burks) and *Trichogramma pretiosum* Riley (Elzen *et al*. 2001). Similarly contact bioassays of Spinosad at the recommended field rate caused 19–65% mortality in the parasitoid *C. grandis* (Burks) (Hymenoptera: Pteromalidae) compared to 56–73% mortality from methyl parathion, 38–83% from endosulfan, and 90–92% from malathion. However, both Spinosad and Malathion completely inhibited parasitoid reproduction when present at one-fourth of their respective recommended field rates (Elzen *et al*. 2000). Spinosad also showed high toxicity to second instars of predatory thrips *Scolothrips takahashii* Priesner and lady beetle *Stethorus japonicus* Kamiya (Mori and Gotoh 2001), and sublethal effects to adults of predatory mites and lacewings (Williams *et al*. 2003). In a particular case of *C. carnea* Medina *et al*. (2001) pointed out that Spinosad is practically non-toxic to larvae, it was show to be harmful to adults. Although some of these differences might be explained considering that results in the laboratory can be different from those obtained in the field, it is evident that the safety profile of Spinosad is not so clear. The effect of Spinosad on *C. carnea* strongly depends on the concentration applied and further studies are needed to take advantage of the potential that this new product offers in the pest control market.

More importantly, Spinosad is being evaluated for using against insect pests in delicate forest ecosystems (Wanner *et al*. 2000). Judging by the results of the present study, in which low to moderate concentrations of Spinosad caused a substantial mortality to insect natural enemies, deeply challenge the assertion, by representatives of Dow AgroScience (Thompson and Hutchins 1999), that Spinosad has a safety profile similar to benign biological pesticides.

CONCLUSIONS

This study provides data on a comprehensive examination of the acute toxicity of commonly used agricultural insecticide to various stages of *C. carnea*. Overall, results of the laboratory studies indicated that Spinosad was moderately toxic to *C. carnea* compared with conventional insecticides. The use of this insecticide would likely contribute to successful conservation of biological control in crops where common green lacewings are the most common natural enemies. Spinosad was selective with regard to acute toxicity, but further work is needed to evaluate the residual toxicity of this insecticide and its potential sub-lethal effects.

ACKNOWLEDGMENTS

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POLISH SUMMARY

ŚMIERCIONOŚNE DZIAŁANIE INSEKTYCYDU SPINOSAD NA LARWY *CHRYSOPERLA CARNEA* (NEUROPTERA, CHRISOPIDAE) W WARUNKACH LABOLATORYJNYCH

Używanie selektywnych insektycydów może zwiększyć ochronę wrogów naturalnych, a więc przyczynić się do odniesienia sukcesu w programach integrowanej ochrony roślin. W badaniach oceniano toksyczność selektywnego insektycydu Spinosad dla *Chrysoperla carnea* Stephens. w warunkach laboratoryjnych Larwy kilku stadiów *C. carnea* były eksponowane na Spinosad. Użyte ilości insektycydu były mniejsze niż maksymalna zalecana na etykiecie dawka. W kontaktowych biotestach wykryto bezpośredni związek pomiędzy stężeniem preparatu Spinosad i tempem zamierania larw pierwszego pokolenia. Użycie preparatu w dawkach – 250 i 2500 ppm, powodowało odpowiednio – 33 i 67% śmiertelności. Jej tempo oznaczano 1–3 dni po zabiegu. Zastosowanie 250 ppm insektycydu Spinosad, na drugie i trzecie stadium larwalne, wykazało znikome tempo śmiertelności po 3 dniach, podczas gdy larwy pierwszego pokolenia wykazały śmiertelność wynoszącą 33%. Na podstawie uzyskanych danych można wnioskować, że Spinosad nie jest bezpieczny dla środowiska, podobnie jak stosowane insektycydy biologiczne.