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Research Article

Toxicity of spinosad against developmental stages of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae)

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Abstract: Colorado potato beetle, *Leptinotarsa decemlineata* is one of the major insect pests of potato. Toxicity of spinosad, as a bio-rational insecticide, was investigated against various developmental stages of this pest. Bioassays were conducted by using the eggs, neonates, first, second, third and fourth instar larvae and adults. The potato leaves were impregnated with different concentrations of spinosad and applied for the adults and different larvae bioassays. The eggs were tested through dipping its masses into the insecticide solutions. LC_{50} values of neonates, first, second, third and fourth instar larvae and unsexed adults after 24 hours were 2.06, 3.19, 4.75, 6.46, 20.24 and 11.97 ppm (of commercial formulation), respectively. Results show that spinosad did not possess any ovicidal effects and the fourth instar larvae and neonates were the most tolerant and the most susceptible stages, respectively. Susceptibility of the neonates (up to 24 hrs after hatching) was significantly higher than that of first instar larvae (24-48 hrs after hatching). Developmental stages of Colorado potato beetle responded differentially to this insecticide. Since the control of *L. decemlineata* mostly relies on early season measures against the most susceptible stage(s), by considering no ovicidal effect, our results propose a limited interval, for avoiding the highly tolerant larvae.

Keywords: Colorado potato beetle, Laboratory bioassay, Ovicidal effect, Spinosad

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Introduction

Use of synthetic insecticides is the most practical approach in Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) control programs. Exposure of its populations to insecticides has caused high pressure for resistance selection. Organochlorine insecticides were the first ones in this regard, where Harris and Svec (1976) reported DDT-resistant populations. Organophosphorous, carbamate and pyrethroid resistant populations have also been described (Heim *et al*., 1990; French *et al*., 1992; Pap *et al*., 1997). Development of resistance to newly introduced insecticides is a practical problem involved in CPB control (Cutler *et al*., 2005; Zhao *et al*., 2000).

Ardabil is the main potato production area of Iran and is the place where CPB was reported for the first time in Iran (Nouri Ganbalani, 1986). It was a quarantine pest before the report, but nowadays, CPB is the most important biological destructive agent of potato fields of Iran. We found some high degree of resistance to a

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cyclodiene insecticide, endosulfan, in several CPB populations including Ardabil population (Mohammadi Sharif *et al*., 2007). In addition, resistance to OP compounds has been reported from some potato production areas of Iran (Malek Mohamadi *et al.,* 2010). The CPB management specialists have to look for new effective insecticides.

Spinosad is fermentation byproduct of the soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao, and its active ingredients consist of spinosyns A (85%) and D (15%) (Zhang *et al*., 2008). This insecticide is a nerve poison where postsynaptic nicotinic acetylcholine and GABA receptors are involved in its toxic actions (Salgado, 1998; Watson, 2001). Spinosad is effective against some insect pests belonging to various orders (Bond *et al*., 2004; McLeod *et al*., 2002; Mendez *et al*., 2002; Morishita, 2001). Despite a few undesirable effects on some useful insects (Babul Hossain and Poehling, 2006), spinosad is an environmentally friendly pesticide (Bond *et al.,* 2004, Cisneros *et al.,* 2002 and Roe *et al.,* 2010). This pesticide can be effectively combined with microbial agents (Mendez *et al.,* 2002), is rapidly decomposed in soil, does not cause cross-resistance and is a safe material in worker exposure issue (Davey *et al.,* 2001). These make it a useful agent in crop production systems.

In this study, we aimed to determine the efficacy of spinosad against eggs, neonates, 1st, 2nd, 3rd, 4th instar larvae as well as adults of Colorado potato beetle.

Materials and Methods

Insect rearing

Adults of Colorado potato beetle were collected from Ardabil potato fields and reared in plastic pots (with the diameter and height of 16 and 24 cm, respectively) at 26 ± 2 °C, 50 ± 5% RH and 16: 8 h (L: D) photoperiod. Bottom of the pots were filled by sand as high as 8 cm. The sand surface was covered with foam lamina where three potato lateral stems with 2-3 branches were planted in sand through holes that were created in the foam. Paper towels coated inner surface of the pots. Then, 15 mated-adults were released on potato foliages and the pots covered with net cloth. The eggs were daily collected by monitoring the potato leaves and inner surfaces of the pots and were maintained in the plates covered with wet filter paper. The larvae were reared in transparent plastic containers (19 × 13 × 6 cm) under the same conditions and were fed with unsprayed potato foliages. Prepupae were transferred to the pots filled with sterilized sands where they bored into sands and completed the pupal stage. New emerged adults (250-300 in number) were released into a cage (100 × 65 × 70 cm) prior to transfer into the pots. The cages were supplied with fresh potato stems for simulation of natural condition and increasing mating occurrence. The insects were reared for three generations during the experiments.

Bioassays

The female adults laid their eggs on the surface of potato leaves or paper towel. The eggs were treated by dipping the pieces of towels containing 1-day egg masses into the concentrations (1000 to 5000 ppm) of Spinosad (Spintor® 2 SC, 240 g a.i./L) (Dow Agroscience, 2012). The batches were numerically uniformed by killing some eggs with a thin needle. Tween 80 was used as surfactant at 0.8% (vol: vol). The treated egg masses were maintained in the Petri plates covered with wet filter paper and at the same environmental conditions of the rearing. Spinosad-treated hatched eggs were counted until 2 days after finishing the hatching of control treatment.

The 1-day old larvae and adults were assayed through feeding by spinosad treated potato leaves. Potato leaves were dipped in the tested solution with gentle shaking. Larvae were removed gently by fine brush and placed on treated leaves inside of the transparent plastic containers (7 × 4 × 14 cm). The containers were ventilated through a small hole on the lid covered with 23 mesh net cloth. The concentrations used for

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treating the neonates and 1st instar larvae were 1, 1.8, 3.2, 5.6 and 10 ppm, for 2nd instar larvae were 2, 3.5, 6.3, 11.3 and 20 ppm, for 3rd instar larvae 2, 3.8, 7.1, 13.3 and 25 ppm, for 4th larvae were 10, 17, 28, 48 and 80 ppm and for the adults were 6, 9, 13.5, 20 and 30 ppm, based on preliminary tests. Commercial formulation of the insecticide was used for bioassays. Control treatments were conducted in the same manner but by using distilled water. The mortalities were recorded 24 hours after treatment and the larvae and adults which did not respond to stimulation were considered as dead. All bioassays were performed at 26 ± 1 °C and 16: 8 h (L: D) photoperiod. Each treatment was replicated 4 times.

Dose-mortality data was analyzed by probit procedure (Finey, 1971) of SPSS (SPSS, 1999) and graphs designed by Excel (version 2003).

Results

Spinosad did not have any lethal effects against CPB eggs. However, neonates died a few hours after hatching, possibly as a consequence of contact with or chewing the spinosad-impregnated eggshells. In a primary experiment, the concentrations of 4000 and 5000 ppm had no ovicidal effect. Susceptibility of the first instar larva was investigated against the neonates with a little feeding (up to 24 hrs after hatching) and 1st instar larvae with normal feeding (24 to 48 hrs after hatching). Based on 95% confidence limit (CI) of LC_{50}, neonates were significantly more susceptible than the 1st instar larvae (Table 1). Their susceptibility was not significantly different according to CIs of LC_{90} values. Considering the stomach toxicity of spinosad, more susceptibility would be expected for 1st instar larvae in comparison with neonates; but, their slopes were the same (Fig. 1), the minute and fragile neonates were more sensitive.

Susceptibility of other larval stages decreased with age and the fourth instar larvae were the least sensitive. The adults were more susceptible than 4th instar larvae and significantly more tolerant than 2nd and 3rd instar larvae (Table 2). Susceptibility of the last two stages was not significantly different in either LC_{50} or LC_{90} levels; but significant difference was observed in 3rd and 4th instar larval responses. Slope of log concentration-probit line in adults' bioassay was greater than the last three larval stages (Fig. 2).

Toxicity ratio was assessed by dividing highest field recommended dose in ppm by that of laboratory measured LC_{50} (McLeod *et al.,* 2002). The highest field recommended dose of spinosad for CPB control practices is 6 oz/acre or 438 ml/hectar, based on commercial formulation (Dow Agrosciences, 2012). The field recommended dose (129 ppm) has been calculated by considering the amount of water needed for optimum coverage of vegetable crops (McLeod *et al.,* 2002). The highest and lowest spinosad toxicity ratios were 62.6 and 6.3 for neonates and fourth instar larvae, respectively (Table 3).

Table 1 Toxicity of spinosad against neonates and 1st instar larvae of Colorado potato beetle.

Developmental Stages	n	Slope ± SE	LC_{50} (95% CI)[a]	LC_{90} (95% CI)[1]	χ^2 (df)[2]	p-value
Neonate	384	2.17 ± 0.24	2.06 (1.70-2.44)	8.02 (6.25-11.54)	1.782 (3)*	0.619
1st instar larva	358	2.10 ± 0.24	3.19 (2.68-3.80)	12.94 (9.57-20.38)	0.227 (3)*	0.973

1. Concentration of insecticide (based on commercial formulation) quoted in parts per million.
2. χ^2 (df) values marked by * indicate good fit of the data to the probit model ($P < 0.05$).

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Table 2 Toxicity of spinosad on 2nd, 3rd, 4th instar larvae and adults of Colorado potato beetle.

Developmental stages	n	Slope ± SE	LC_{50} (95% CI)[a]	LC_{90} (95% CI)[1]	χ^2 (df)[*]	p-value
2nd instar larva	357	2.24 ± 0.25	4.75 (3.8-5.38)	16.98 (13.16-24.64)	2.393 (3)[*]	0.495
3rd instar larva	358	1.91 ± 0.22	6.46 (5.31-7.82)	30.09 (21.71-49.20)	1.943 (3)[*]	0.584
4th instar larva	362	2.24 ± 0.27	20.24 (17.06-35.4)	67.83 (53.94-94.58)	1.540 (3)[*]	0.673
Adult	327	3.18 ± 0.36	11.97 (10.56-34.8)	30.21 (24.62-40.21)	0.899 (3)	[*]0.826

1. Concentration of insecticide (based on commercial formulation) quoted in parts per million.
2. χ^2 (df) values marked by [*] indicate good fit of the data to the probit model ($P < 0.05$).

Table 3 Toxicity ratio and different susceptibility of developmental stages of Colorado potato beetle.

Developmental stages	Relative tolerance[1]	Toxicity ratio[2]	Susceptibility ratio[3]
Neonate	-	62.6	9.9
1st instar larva	1.55	40.4	6.4
2nd instar larva	2.31	27.1	4.3
3rd instar larva	3.13	19.9	3.1
4th instar larva	9.82	6.3	-
Adult	5.81	10.7	1.7

1. In comparison with neonates (LC_{50} of each stage/ LC_{50} of neonates).
2. Highest recommended field rate (ppm)/ LC_{50} value (ppm).
3. In comparison with 4th instar larva (LC_{50} of each stage/ LC_{50} of 4th instar larvae).

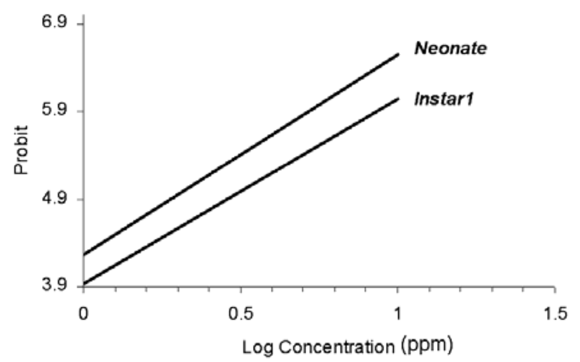

Figure 1 Dose-response lines of spinosad bioassay against *Leptinotarsa decemlineata* neonates and first instar larvae.

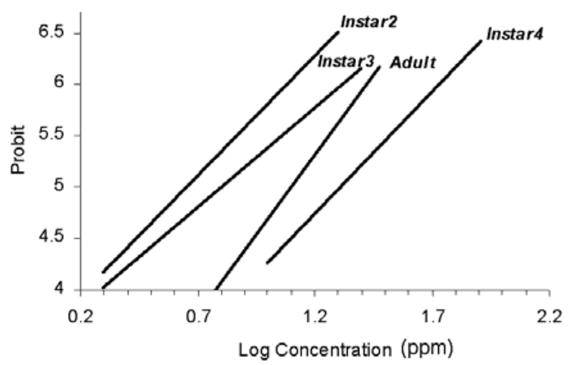

Figure 2 Dose-response lines of spinosad bioassay against *Leptinotarsa decemlineata* adults and second, third and fourth instar larvae.

Discussion

Spinosad has potent insecticidal activity against some insect pests of Lepidoptera, Diptera, Coleoptera and some others (Bond *et al*., 2004; Huang and Subramanyam, 2007; Morishita, 2001; Razaq *et al*., 2007; Saito, 2004). Some investigations have confirmed its effectiveness for controlling stored product insect pests (Daglish *et al*., 2008; Huang and Subramanyam, 2007; Vayias *et al*., 2009). Effectiveness of the same formulation of spinosad (Spintor® 2 SC) against eggplant flea beetle, *Epitrix foscula* Crotch, another species of Chrysomelidae, was similar. LC_{50} values were 25.9 and 9.8 ppm two and four days after treatment, respectively (McLeod *et al*., 2002).

Effectiveness of spinosad has only been studied in a few researches against CPB. Osman (2010) investigated the effect of field recommended dose (0.5 ml/L) of a commercial formulation of spinosad (12% SL) against the eggs, 1st, 2nd, 3rd, 4th larval instars and adults of CPB by dipping potato leaves into the solutions. No ovicidal effect was observed and more than 90% of treated eggs were hatched. Mortalities of the larval stages and adults at 3 days after treatment were 86.6, 84.4, 73.3, 57.8 and 86.6%, respectively. He demonstrated 4th larval instar to be the most tolerant stage which is in accordance with our findings. Spinosad effectiveness were somewhat increased at 7 days after treatment. Effect of temperature (15, 20 and 25 ºC) was evaluated on efficacy of spinosad (Biospin®, 120 gr a.i./L) against larvae (combination of 3rd and 4th instars) and adults of CPB through feeding the insects by sprayed potato leaves (Kowalska, 2010). The mortalities caused by concentrations of 0.2, 0.1 and 0.05% were higher at 15 ºC after 6 days of treatment (about 100% for the highest concentration against the larvae and adults). However, the mortality level was about 60% for both stages at 25 ºC.

Control of CPB lean heavily upon the use of insecticides and due to its high insecticide resistance ability; we have to include resistance management tactics in IPM programs. Determining the appropriate application time and using new insecticides with different mode of action are some practical approaches. In the field condition, different developmental stages are overlapped but 4th instar larvae cause the major damage (Hare, 1990). Most CPB management programs emphasize the need for early and midseason suppression and determining the most susceptible stage (s). LC_{50}s of neonates and 1st instar larvae were 9.8 and 6.3 times less than that for the 4th instar larvae, respectively. Although 1st instar larvae (including neonates) was the most susceptible stage, but non-ovicidal property of spinosad limits the window of time for its application. Therefore, determining CPB first instar larva population peak is one of the key factors for maximizing insecticide application efficacy and perhaps minimizing insecticide resistance evolution.

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سمیت حشره‌کش اسپینوساد روی مراحل مختلف زیستی سوسک کلرادوی سیب‌زمینی *Leptinotarsa decemlineata* (Say) (Col: Chrysomelidae)

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چکیده: سوسک کلرادوی سیب‌زمینی *Leptinotarsa decemlineata* یکی از مهم‌ترین آفات حشره‌ای سیب‌زمینی است. در این تحقیق سمیت اسپینوساد که یک حشره‌کش سازگار با محیط‌زیست است روی مراحل مختلف رشدی این آفت مورد بررسی قرار گرفت. آزمایش‌های زیست‌سنجی با استفاده از تخم‌ها، لاروهای نئونات، سن یک، دو، سه و چهار و همچنین حشرات کامل انجام شد. برای زیست‌سنجی مراحل مختلف لاروی و حشرات کامل از برگ‌های سیب‌زمینی آغشته به غلظت‌های مختلف حشره‌کش استفاده شد. مقادیر LC_{50} محاسبه شده برای لاروهای نئونات، سن یک، دو، سه، چهار و حشرات کامل به‌ترتیب ۲/۰۶، ۳/۱۹، ۴/۷۵، ۶/۴۶، ۲۰/۲۴ و ۱۱/۹۷ پی‌پی‌ام (بر اساس فرمولاسیون تجارتی) برآورد شد. این حشره‌کش هیچ‌گونه اثر تخم‌کشی روی سوسک کلرادو نشان نداد. حساس‌ترین مرحله، لارو نئونات و متحمل‌ترین، لارو سن چهارم بود. علاوه بر این حساسیت لاروهای نئونات (لاروهای ۲۴ ساعته) به‌طور معنی‌داری بیش از لاروهای سن یک (۲۴ تا ۴۸ ساعت پس از تفریخ) بود. نتایج این تحقیق نشان داد که حساسیت مراحل مختلف زیستی سوسک کلرادو به اسپینوساد متفاوت است. ازآنجاکه کنترل موثر این آفت وابسته به مبارزه با مراحل حساس آن در اوایل فصل زراعی است، براساس نتایج حاصل، به‌خصوص عدم تخم‌کشی اسپینوساد، برای اجتناب از لاروهای متحمل به حشره‌کش، بازه زمانی محدودی وجود دارد.

واژگان کلیدی: سوسک کلرادوی سیب‌زمینی، زیست‌سنجی آزمایشگاهی، اثر تخم‌کشی، اسپینوساد

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