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Studies on the Lethal Effects of Spinosad on Adults of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) with Two Bioassay Methods

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Abstract: Lethal impacts of spinosad were determined against adults of *Leptinotarsa decemlineata* (Say), with two bioassay methods. Adult insects were exposed to different concentrations of spinosad for 24, 48 and 72 h. After the exposure intervals, mortality was scored in both bioassay techniques. Complete mortality was obtained 3 days post-exposure in contact and dipping bioassay methods at 240 and 7920 ppm of test chemical, respectively. A significant linear relationship between spinosad concentrations and mortality rates was detected. The estimated LC_{50} values were 6.59 and 12.50 ppm by contact and dipping methods at 72 h post-treatment, respectively. Overall, results indicated that CPB adults were highly susceptible to spinosad. Contact method, however, exhibited less variability in LC_{50} estimates and showed a higher degree of sensitivity than the dipping method. Contact method is simple and sensitive test bioassay technique for measuring susceptibility of CPB adults to spinosad.

Key words: Spinosad, Colorado potato beetle, bioassay methods

INTRODUCTION

The potato is an important crop worldwide. The amount of potato yield is depended on many factors, especially degree of the damage caused by the Colorado potato beetle *Leptinotarsa decemlineata* Say, (Coleoptera: Chrysomelidae) (Igrc *et al*., 2006). This pest requires specific annual control measures to prevent economic damage (Kalushkov and Batchvarova, 2005). CPB belongs to the group of insects in which the development of resistance is very fast and treatment with a range of broad spectrum insecticides has resulted in high resistance level in CPB and variety of untoward effects on untarget organisms and environment (Scott *et al*., 2003).

Some insecticides for example aldicarb (Timik®) and methomyl (Lannate®) which have been used for a long-time, have high toxicity not only for target organisms but also for untargeted organisms and resulted undue contamination of ecosystem (Igrc *et al*., 2006). In Iran, no insecticides that effectively control this insect are registered. Igrc *et al*. (1999) demonstrated that insecticides belonging to the new classes, such as imidacloprid, hexaflumuron, spinosad, tiametoxam and lufenuron, showed a satisfactory residual action against CPB and ensured good leaf protection and high yields.

One approach, in which we could retard resistance development phenomenon, is to correct use of the insecticides belonging to the new classes. Spinosad, a neurotoxin that acts on both nicotinic acetylcholine receptors and a novel site in the GABA system could be an obvious of choice (Bjorksten and Robinson, 2005). Little information is currently available about the susceptibility of populations of *L. decemlineata* to spinosad. However, due to novel action on nerve system, this compound seems to be more suitable for the CPB control than conventional chemical insecticides. The application of

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spinosad as a component of an IPM program could reduce pollution dilemma, the deleterious impact on beneficial entomofauna and expression of resistance development through retardation of this phenomenon (Nault *et al*., 2000).

Diverse methods have been used to estimate resistance in the pests and CPB, include of topical application (Zhao *et al*., 2000; Choo *et al*., 2000), filter paper disc method (Alyokhin *et al*., 2006), leaf dipping method (Erdogan and Toros, 2007), glass jar method (Pourmirza, 2005), diet bioassay (Olson *et al*., 2000).

The choice of appropriate exposure method can often improve discrimination between susceptible and resistant genotypes (Pourmirza, 2005). Due to applied nature of this research, we used contact and dipping methods.

The current study was designed to determine the extent of CPB adult susceptibility to spinosad as a potential safe alternative for conventional insecticides; also the responses of CPB adults in contact and dipping bioassay methods were compared for potential use as simple and sensitive bioassay techniques to determine of CPB adult susceptibility.

MATERIALS AND METHODS

Insects

Colorado potato beetle were collected from April to July 2007 in several fields of Urmia (37.39°N 45.4°E), a town is West Azarbijan County, Iran. For each test, depending on availability, 500-600 first generation CPB adults were collected from the fields. The insects were returned to the laboratory and reared on greenhouse grown untreated fresh potato foliage inside plastic boxes with screen lids. The boxes were maintained in the rearing room for 2 d before initiation of each test.

Insecticide

A commercial formulation of spinosad (Tracer® 240SC) which was a gift from Dow AgroScience Inc, was used in all bioassays.

Bioassays

On the basis of preliminary tests, five or six concentrations of spinosad diluted with water and used in each test to produce ≈ 25-75% mortality at the lowest and the highest concentration, respectively (Robertson and Preisler, 1992). Fresh preparations of spinosad were made for each bioassay. No mortalities were observed in control groups of tested insects. In each bioassay method, results of all replicates were pooled.

Dipping Method

In dipping method on the basis of preliminary tests, five concentrations of spinosad were used in each test. A vial was used to dip groups of 15 insect in each insecticide concentration for 10 sec. A control group of 15 insects was dipped in water for the same period of time. To absorb excess moisture, insects were placed on paper towel in 190 mL glass jar with screen lids. Vials were kept in a dark environmental chamber at 27±2°C and 60±10% RH. Mortality was recorded after 24, 48 and 72 h. Insects were scored dead if no movement was observed after they were prodded with hot dissecting needle. Bioassay was replicated three times.

Contact Method

In contact method the bioassay procedure involves continuous exposure of insect within 190 mL glass jars coated with a known quantity of insecticide solutions. Five doses of spinosad were used per each jar. The jar treated only with water served as a control treatment. After treatment, the insects were kept in jars (15 insects per jar) and jars were sealed with screen lids and kept in dark environmental chamber at 27±2°C and 60±10% RH. Mortality was recorded after 24, 48 and 72 h. Insects were considered dead if they did not move their legs or antenna in response to probes with a hot dissecting needle. The experiment was replicated three times.

Data Analysis

Mortality data from bioassays were analyzed with SPSS software. Assuming the probit model. Median lethal concentrations (LC_{50} values) and their corresponding 95% fiducial limits (FLs) were estimated. Two LC_{50} values were significantly different if the corresponding 95% FLs did not overlap (Gerber and Finn, 2005). The data were analyzed by using analysis of variance (ANOVA) and to equalize variance, mortality percentage was transformed using the square-root of arcsin (SAS, 1999).

RESULTS

Spinosad was relatively fast-acting against adults of CPB. Based on the dynamic of intoxication of given insects on different days after treatment, the best time to evaluate the effects of spinosad on adults of CPB was the second and third days after treatment (Table 2, Fig. 1). After days exposure interval the main effects for adults of CPB in contact and dipping bioassay methods were all significant (Table 1). In addition, interaction between dose and exposure time was also significant (Table 1). Figure 1 displays mortality percentage for CPB adults after 24, 48 and 72 h of exposure to different

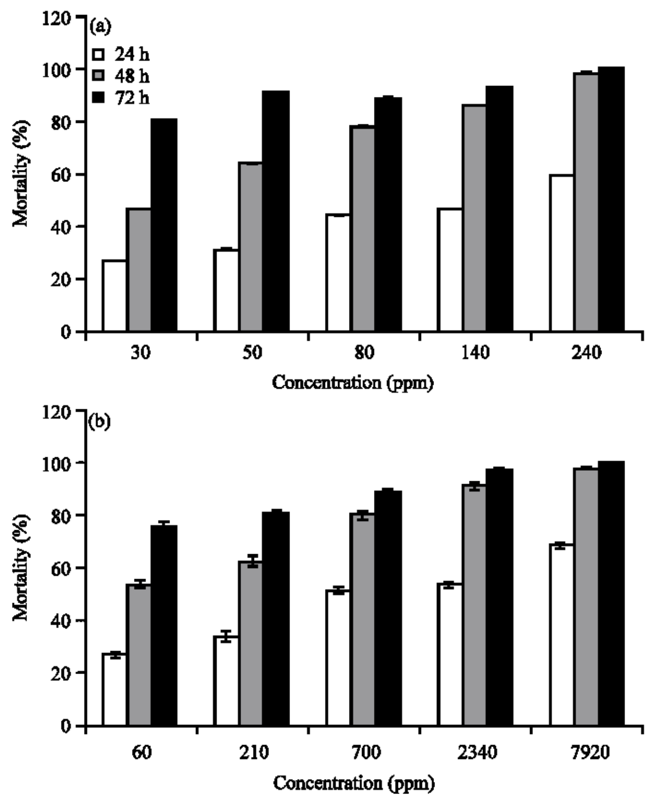

Fig. 1: The mortality percentage±SE of CPB adults exposed to different doses of spinosad after 24, 48 and 72 h in contact method (1) and dipping method (2)

Table 1: ANOVA parameters for main effects for mortality of *L. decemlineata* with two bioassay methods

	Contact			Dipping		
SOV	df	F-value	p-value	df	F-value	p-value
Dose of spinosad	4	688.12	<0.000	4	39.28*	<0.000
Exposure interval	2	543.79	<0.000	2	26.83*	<0.000
Dose×exposure interval	8	29.96	<0.001	8	1.34^{ns}	<0.000

ns: No. Significant difference, *: Significant difference at p<0.05

Table 2: The LC_{50} values (ppm) for adults of *L. decemlineata* exposed to spinosad with two bioassay methods

Test method	Exposure interval (h)	LC_{50} (ppm)	Confidence limit (95%)	Slope±SE	df	Chi-square	Probability
Contact	24	140.62	93.60-328.49	0.95±0.27	3	0.58*	0.89
	48	33.49	21.98-43.24	2.03±0.34	3	0.85*	0.83
	72	6.59	0.10-16.80	1.29±0.42	3	2.81*	0.42
Dipping	24	1038.50	480.56-2673.27	0.51±0.11	3	0.84*	0.83
	48	64.52	21.53-122.47	0.86±0.15	3	1.12*	0.77
	72	12.50	0.73-38.25	0.80±0.19	3	2.31*	0.50

*: Significant difference at p<0.05

doses of spinosad for contact and dipping bioassay techniques. The mortality percentage obtained with the high doses of spinosad in 24 h exceed 60% in contact method and 78% in dipping method.

Insecticidal efficacy of spinosad based on LC_{50} value is presented in Table 2. In each bioassay, in contact technique LC_{50} value was lower than corresponding LC_{50} value in dipping method but it was non-significant.

DISCUSSION

Spinosad is a macrocyclic lactone that causes involuntary muscle contractions, tremors and eventually paralysis of treated insects (Galvan *et al*., 2005). The application of spinosad has some advantages over other measures for control of CPB. For instance, spinosad is naturally derived compound and has low level of toxicity to mammals (Thompson *et al*., 2000). Spinosad has very fast biodegradation rate with no or low impact on beneficial fauna and very low impact on the resistance development. These characteristics are important issues in human health and pest control measures; render spinosad as an appropriate candidate in insect control program. Michaud and Grant (2003) reported that, spinosad fit very well into IPM potato programs. The results of current study indicate that spinosad is relatively fast-acting against adults of CPB and are consistent with results of later authors. Intoxication began to appear 4-5 h after treatment, whereas several reports is in this instance: spinosad is slower acting than many conventional chemical insecticides (Williams *et al*., 2003) and ignored the 1 h observations suggested in the WHO protocol specifically to account for the speed of action of spinosad (Bond *et al*., 2004).

An inverse relationship between LC_{50} values with exposure time was determined. In contact and dipping methods after 72 h LC_{50} values for CPB adults were 6.59 and 12.5 ppm, respectively. Results showed spinosad has high insecticidal efficacy on CPB adults. Mota-Sanchez *et al*. (2005) evaluated the resistance to neonicotinoid insecticides and spinosad in the CPB and emphasized the importance of spinosad for control of CPB.

Results indicated a direct positive relationship between mortality of CPB adults with spinosad exposure time. Jiang and Mulla (2005) evaluated the susceptibility of the adult eye gnat *Liohippelates collusor* to spinosad and stated that mortality increased as the exposure interval increased. In contact method with longer time insects walk over the treated surface and chemical receptors in tarsal have more contact with insecticide (Pourmirza, 2005). Spinosad in high doses provide a satisfactory level of protection but it maybe have effects toxicity on honeybee and untarget insect. Therefore, we compared toxicity of spinosad with two bioassay methods in this research.

The contact method (tarsal exposure method) is a common route of insecticide uptake by adults of CPB in the field. This technique is more realistic than dipping method and compared with the dipping test, it is sufficiently sensitive to determine the magnitude of resistance because it always produced smaller fiducial limits (Pourmirza, 2005). In contact method LC_{50} value is lower than LC_{50} in dipping method despite 100% mortality achieve with low level doses of spinosad and in short period of time (Azimi *et al*., 2008).

The main conclusions of current study are:

- Spinosad is toxic to CPB adults
- Both bioassay methods are suitable for assessing magnitude of CPB adult susceptibility to spinosad
- Contact method is superior to dipping method because it is simple and labor-efficient, with acceptable precision

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