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Comparative toxicity of abamectin, cyromazine and spinosad against the leaf-miner fly, *Liriomyza sativae* (Dip.: Agromyzidae)

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Abstract

The leaf-miner fly, *Liriomyza sativae* (Blanchard), is one of the major insect pests of vegetable crops in Iran and other countries. To determine the toxicity (LC_{50}) of bioinsecticides abamectin 1.8% EC, cyromazine 75% WP and spinosad 24% SC against *L. sativae*, bioassay was done on its larval and adult stages under 25 ± 1°C, 65 ± 5% R.H. and a photoperiod of 16: 8 (L: D). The LC_{50} values found to be 1.5, 1.8, 14.3 ppm for abamectin, 34.8, 38.4, 1295 ppm for cyromazine and 4.4, 12.1, 13.7 ppm for spinosad against the first and last larval stages as well as the adults of *L. sativae*, respectively. The toxicity of the insecticides on the larval stages was higher than on adults. Using a mixture of petroleum oil, as a synergist, and the insecticides significantly increased the mortality of the first instar larvae. The results indicated that cyromazine is ineffective for the control of adults, while it is proved to be effective against the leaf-miner's larval stages. It is found that abamectin and spinosad are more efficient insecticides than cyromazine for the control of all developmental and adult stages of *L. sativae*.

Key words: abamectin, spinosad, cyromazine, LC_{50}, *Liriomyza sativae*, Iran

چکیده

مگس مینوز برگ، *Liriomyza sativae* (Blanchard)، یکی از آفات مهم سبزیجات در ایران و بسیاری از کشورهای جهان می‌باشد. به‌منظور تعیین سمیت (LC_{50}) حشره‌کش‌های آبامکتین (۱/۸ درصد امولسیون)، سیرومازین (۷۵ درصد پودر خیسا) و اسپینوزاد (۲٤ درصد سوسپانسیون کلوئیدی) نسبت به *L. sativae*، زیست‌سنجی روی لارو سن اول، سن آخر و حشره‌ی کامل این آفت تحت شرایط دمایی ۱ ± ۲۵ درجه‌ی سلسیوس، رطوبت نسبی ۵ ± ٦٥ درصد و دوره‌ی نوری ١٦ ساعت روشنایی و ۸ ساعت تاریکی انجام شد. مقادیر LC_{50} آبامکتین برای لاروهای سن اول، سن آخر و حشره‌ی کامل به‌ترتیب ۱/۵، ۱/۸ و ١٤/۳ پی‌پی‌ام و برای سیرومازین به‌ترتیب ۳٤/۸، ۳۸/٤ و ۱۲۹۵ پی‌پی‌ام به دست آمد. نتایج نشان داد که مقدار LC_{50} اسپینوزاد به‌ترتیب ٤/٤۰، ۱۲/۱ و ۱۳/۷ پی‌پی‌ام بود. سمیت سه حشره‌کش مورد استفاده روی مراحل لاروی *L. sativae* بیش‌تر از مرحله‌ی حشره‌ی کامل بود. استفاده از روغن نفتی به‌صورت مخلوط با حشره‌کش‌های مورد آزمایش باعث افزایش مرگ و میر لارو سن اول شد. نتایج نشان داد که سیرومازین برای کنترل حشره‌ی کامل *L. sativae* مؤثر نبوده و فقط جهت کنترل مراحل لاروی آفت مؤثر است. بنابراین می‌توان اذعان نمود که آبامکتین و اسپینوزاد کارآیی بیش‌تری برای کنترل لاروها و حشره‌ی کامل *L. sativae* نسبت به سیرومازین دارند.

واژگان کلیدی: آبامکتین، اسپینوزاد، سیرومازین، LC_{50}، *Liriomyza sativae*، ایران

Introduction

The leaf-miner flies, *Liriomyza trifolii* (Burgess), and *L. sativae* (Blanchard) are important insect pests on a wide variety of vegetable and flower crops in different countries of the world, including Iran (Baniameri, 2003). The adults of *L. sativae* puncture the leaves of their host plants for feeding and oviposition, resulting in leaves stippling. The feeding behavior of larvae reduces the photosynthetic activity of the leaves by mining, ultimately leads to the defoliation of the infested plants (Parrella *et al.*, 1985). The leaf-miner fly, *L. trifolii* is tolerant to many commonly used insecticides especially broad spectrum chemicals (Ferguson, 2004). It is, therefore, becoming more difficult to control leaf-miner flies worldwide (Parrella *et al.*, 1985). The accurate identification of *L. sativae* and *L. trifolii* is important because of their specific resistance to different insecticides (Spencer, 1990). The Neem-based insecticides are effective against *L. huidobrensis* (Branchard) (Weintraub & Horowitz, 1997) but they are not cost-effective in non-organic farming. The impact of chemicals on beneficial organisms (e.g. natural enemies) can be more severe than on target pests, resulting in pest outbreaks (Oatman & Kennedy, 1976). Many pesticides, including pyrethroids and organophosphates, have been reported ineffective against *L. huidobrensis* due to its resistance to them (Weintraub & Horowitz, 1995). Although abamectin and cyromazine are proved to viably control *L. huidobrensis* in several countries (Hammad *et al.*, 2000), these chemicals, particularly abamectin, can be harmful to beneficial organisms including parasitoids (Shipp *et al.*, 2000). The excessive usage of these chemicals has led to occurrence of complete resistance in leaf-miner populations (Ferguson, 2004). Spinosad belongs to a new group of insecticides, which are developed from the fermentation process of the soil bacteria, *Saccharopolyspora spinosa* Larson, that can provide a new alternative for the control of *L. huidobrensis* on dry beans. Due to its unique mode of action, spinosad might be used as an active component in insecticide rotation programs (Bueno *et al.*, 2007).

Because of its low damage threshold on some crops, notably vegetables and ornamental plants, chemical control remains the most common method for the control of leafminer flies (Cox *et al.*, 1995). Some insecticides retain their effectiveness for two years after their first application (Leibee, 1981; Parrella *et al.*, 1985), and in some countries, different insecticides are routinely applied in the control programs of leaf-miner flies (Rauf *et al.*, 2000). Chemical control can reduce the rate of parasitism and predation by indigenous parasitoid wasps and flies. Since mid 1980s the following two insecticides have been registered and successfully used against leafminer pests: cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) (Trigard), an insect growth regulator (IGR) and abamectin (AgriMek), a GABA agonist (Ferguson, 2004). An alternative control strategy involving the application of abamectin led to an effective control of leaf-miner populations without any harmful impact on parasitoids and predators. It has been suggested that the addition of certain penetrating surfactants may increase translaminar movement and insecticidal activity on pests that mine within leaves and feed on lower leaf surfaces (Larson, 1997).

This research was intended to study the efficiency of spinosad at low rates in a mixture with petroleum oil (0.25 %) in comparison with pure spinosad application on *L. sativae*. The efficacy of the mixture increased as its concentration increased. The mixtures of abamectin and cyromazine with petroleum oil provided synergistic effects to the extent that the active ingredient of the insecticides could be reduced (Guedes *et al.*, 1995).

The translaminar pesticides abamectin and cyromazine are widely used against *L. sativae* in Iran. We studied the toxicological influence of the insecticides on both adult and immature stages of the leaf-miner *L. sativae* and evaluated its response to the different applications of cyromazine, abamectin, spinosad and petroleum oil.

Materials and methods

Host plant and rearing of *L. sativae*

Cowpea (var. Negin) was seeded in 10-cm-diam pots (4-6 per pot) with holes in the bottom, containing soil, peat moss, vermiculite and sand. Plants were grown at 25 ± 1ºC, a photoperiod of 14: 10 h (L: D), until their two true leaves were fully expanded.

The larvae of *L. sativae* were collected from infested cucumbers in the greenhouses located in Pakdasht, southeastern Tehran, Iran. They were transferred into a growth chamber at 25 ± 1ºC, 60-65% RH until their pupation and then placed in cages containing bean pots.

Insecticides and bioassay

The insect growth regulator cyromazine (Trigard 75% WP, Syngenta Crop Protection, Greensboro, NC), abamectin (Agrimec 0.15 EC, Agri Evo Crop Protection), a fermentation metabolite of the soil inhabiting actinomycete *Streptomyces avermitilis*, and spinosad (Spinosin 24% SC), a fermentation metabolite of the soil inhabiting actinomycete *S. spinosa* were used. Their specific mode of action provides excellent crop protection with a relatively low toxicity to non-target organisms.

The bioassays were carried out according to Cox *et al*. (1995) method on the larval stages of the pest. A total of 64 young (10-14-day-old) cucumber plants were caged (in lumite-screened cages) and exposed to several hundred 3 to 4-day-old flies for an oviposition access period (OAP) of 4-6 h. The short period for OAP ensured a synchronous egg hatch. The plants were removed and held in the laboratory (25 ± 1ºC and ambient light) for 96 h to allow eggs to hatch and small mines to develop. Cucumber plants were divided into several groups containing equal numbers of mines, 75-200 per dose. The leaves and a part of the stem were treated by 15-second-long submersion into the serial dilution of the insecticides in distilled water.

Five to seven doses were used in each bioassay. The leaf dipping technique was employed against first and last instar larvae. Mortality of the larvae was recorded in 24 h intervals for abamectin and spinosad, and in 72 h intervals for cyromazine. The application of 25 ppm of cyromazine and 9 ppm abamectin and spinosad led to approximately 95% larval mortality. For the second experiment, the same concentrations of abmectin, cyromazine and spinosad plus 25% petroleum oil were used to treat the infested leaves with the first instar larvae, using leaf dipping method. After 24 h mortality of the larvae was calculated. Adults (24-48 h old) were exposed to pesticide residue on glass. Insecticides were diluted with water and an excess of the insecticide suspension was applied to the inside wall of a glass tube (3 cm in diameter and 22.3 cm in length). The glass tubes were then placed upright in a fume hood for at least 6 h., containing ten males and ten females each. The tubes were covered at both ends with pieces of fine gauze and also cotton swabs saturated with honey/water solution were placed outside the tubes next to one end for the feeding of the flies in the course of experiment. Mortality was recorded 24 h after the onset of exposure.

Preliminary experiments were used to determine the range of concentrations over which the mortality occurred. Within this range, five concentrations of each pesticide were tested. Trials were replicated six times and the data was analyzed, using POLO PC software.

Results and discussion

The bioassay data with cyromazine, abamectin and spinosad was based on the LC_{50} values are presented in tables 1-3. The results indicate that the LC_{50} value of abamectin for first and last instar larvae as well as adults were 1.51, 1.81 and 14.3 ppm, respectively with slop ($\pm$ SE) value of 1.6 ± 0.25, 24 ± 1.19, 1.02 ± 0.31 and chi-square value of 0.34, 0.34 and 0.84 (table 1). The toxicity of abamectin was lower in the adults but the slope of the line was identical among the populations. The results are in accordance with the findings by Mujica *et al.* (2000) who reported the LC_{50} value of abamectin at 1.1 ppm and 1.8 ppm for the first and the last instar larvae of *L. sativae*, respectively. The results contradicted with Van de Veire *et al.* (2002) who found abamectin LC_{50} value at 6.7 ppm for first instar larvae of *L. trifolii*. According to Ferguson (2004), the LC_{50} values of abamectin on various populations of the leaf-miner *L. trifolii* were 0.3, 3.7, 5.2 ppm respectively. This disagreement between our results and theirs can be stemmed from the differences in leafminer species or the formulation and concentration of the insecticides.

The LC_{50} values of cyromazine were 34.8, 38.4 and 1295 ppm, the slop (± SE) values 1.4 ± 0.26, 1.09 ± 0.36 and 1.3 ± 0.31 and the chi-square values 0.18, 0.15 and 0.72 for the first and last instar larvae and adults, respectively (table 2). There was a difference in the slope of the line among the first instar larvae. The results showed that cyromazine was only effective on the larval stages of *L. sativae.* The LC_{50} value of cyromazine for the first instar larvae (34.8 ppm) was close to the reported 35.1 ppm by Ferguson (2004) for the first instar larvae of *L. trifolii*, although our results contradicted Prijono *et al.* (2004) who reported the LC_{50} value of 12 ppm for the first instar larvae of *L. huidobrensis*.

Table 1. Toxicity of abamectin against the larval and adult stages of *L. sativae.*

Stage	N*	LC (mg (a.i.)/l) (95% CI)			Slop ± SE	df	Cs.	I. ± SE
		LC_{90}	LC_{50}	LC_{10}				
1st larval	198	8.86 (5.9-14.5)	1.51 (1.19-1.89)	0.26 (0.13-0.39)	1.6 ± 0.25	3	0.34	-2.16 ± 0.42
3rd larval	210	14.4 (3.4-22.3)	1.81 (1.4-2.2)	0.28 (0.1-0.3)	24 ± 1.19	3	0.34	-3.21 ± 0.51
Adult	180	251 (76.1-284)	14.3 (9.5-19.4)	0.81 (0.07-1.3)	1.02 ± 0.31	3	0.84	-2.98 ± 0.84

*The number of tested insects; I. = intercept; Cs. = Chi-square.

Table 2. Toxicity of cyromazine against the larval and adult stages of *L. sativae.*

Stage	N*	LC (mg (a.i.)/l) (95% CI)			Slop ± SE	df	Cs.	I. ± SE
		LC_{90}	LC_{50}	LC_{10}				
1st larval	167	253 (158-392)	34.8 (24.9-46.2)	4.8 (1.7-7.4)	1.4 ± 0.26	3	0.18	-2.48 ± 0.48
3rd larval	192	561 (257-712)	38.4 (30.1-50.8)	6.2 (2.3-10.2)	1.09 ± 0.36	3	0.15	-1.8 ± 0.7
Adult	180	6201 (2571-7713)	1295 (864-1716)	270 (144-360)	1.3 ± 0.31	3	0.72	-6.09 ± 1.5

*The number of tested insects; I. = intercept; Cs. = Chi-square.

The LC_{50} values for spinosad on the first and last instar larvae and adults were 4.4, 12.1 and 13.7 ppm, respectively, the slop (± SE) values for the same larval stages and adults were 1.7 ± 0.23, 1.4 ± 0.2 and 1.3 ± 0.31 and the chi-square values were 0.11, 0.26 and 0.57 (table 3). There was no difference in the slope of the line among the populations. This agrees with the findings of Ferguson (2004), who reported the LC_{50} value of spinosad at 2.53 and 1.59 for the first instar larvae of *L. trifolii*. Our results show that the toxicity was higher for the larval stages, particularly for the first instar than adult stage. It is suggested that abamectin and spinosad are more effective on the larvae than adult of *L. sativae.*

The LC_{50} value of spinosad against first instar larvae was 4.4 ppm and the value was 1.9 ppm when spinosad was mixed with oil and resulted in higher larval mortality (table 4). Bueno *et al.* (2007) reported that the use of adjuvant, as the surfactant polyether-polymethylsiloxanecopolymer (Break Thru®) helped to efficiently control *L. huidobrensis*, and significantly reduce the cost of the pest management program and also lower the risk of harmful effects on the environment. The LC_{50} of abamectin for the first instar larvae was 1.5 ppm and the value for abamectin mixed with oil was 0.5 ppm. Thus the abamectin mixed with oil had a higher toxicity on first instar larvae than abamectin used alone (table 5). The LC_{50} value for cyromazine for first instar larvae was 34.8 ppm and for cyromazine mixed with oil was 32.2 ppm, suggesting an insignificant improvement of efficiency for the mixture of cyromazine with oil (table 6).

The synergistic effect shown by the mixture of oil with abamectin and spinosad leads to a reduction of the commercially registered concentration of these insecticides without any loss of their efficiency. The reduced cost of the treatments by 1.4% will encourage a higher number of farmers to include abamectin in their control program of leaf-miner flies (Mujica *et al.*, 2000). The inclusion of a penetrating synergist (e.g. petroleum oil) sharply increased the larval mortality. Mujica *et al.* (2000) noted that a mixture of oil and abamectin with 1% oil spray concentration improved the efficiency of the control program and reduced the commonly used dosage by 75%. The natural bio-pesticides are believed to be safer than conventional synthetic pesticides for the human and environment as they leave less residues in the biosphere and do not produce resistance in insect pests, including agromyzid leaf-miner flies (Mujica *et al.*, 2000).

Table 3. Toxicity of spinosad against the larval and adult stages of *L. sativae.*

Stage	N*	LC (mg (a.i.)/l) (95% CI)			Slop ± SE	df	Cs.	I. ± SE
		LC_{90}	LC_{50}	LC_{10}				
1st larval	218	23.8 (18-32)	4.4 (3.4-5.3)	0.8 (0.4-1.2)	1.7 ± 0.23	3	0.11	-2.2 ± 0.36
3rd larval	205	81.1 (24-132)	12.1 (2.9-19.2)	1.8 (0.9-2.1)	1.4 ± 0.2	3	0.26	-3.1 ± 0.21
Adult	180	107 (46-253)	13.7 (8.1-20.7)	1.2 (0.38-1.9)	1.3 ± 0.31	3	0.57	-2.8 ± 0.24

*The number of tested insects; I. = intercept; Cs. = Chi-square.

Table 4. The LC_{50} value of spinosad alone and mixed with oil on the first instar larvae of *L. sativae.*

Insecticide	N*	LC (mg (a.i.)/l) (95% CI)			Slop ± SE	df	Cs.	I. ± SE
		LC_{90}	LC_{50}	LC_{10}				
Spinosad	218	23.8 (18-32)	4.4 (3.4-5.3)	0.8 (0.4-1.2)	1.7 ± 0.23	3	0.11	-2.2 ± 0.36
Spinosad + oil	306	15.3 (9.7-22.8)	1.9 (0.9-2.6)	0.2 (0.1-0.3)	1.4 ± 0.2	3	0.15	-1.3 ± 0.29

*The number of tested insects; I. = intercept; Cs. = Chi-square.

Table 5. The LC_{50} value of abamectin alone and mixed with oil on the first instar larvae of *L. sativae.*

Insecticide	LC (mg (a.i.)/l) (95% CI)			Slop ± SE	df	Cs.	I. ± SE
	LC_{90}	LC_{50}	LC_{10}				
Abamectin	8.86 (5.9-14.5)	1.51 (1.19-1.89)	0.26 (0.13-0.39)	1.6 ± 0.25	3	0.34	-2.16 ± 0.42
Abamectin + oil	7.14 (4.5-12.7)	0.5 (0.1-0.8)	0.01 (0.006-0.02)	1.03 ± 0.13	3	0.21	-1.7 ± 0.59

I. = intercept; Cs. = Chi-square.

Table 6. The LC_{50} value of cyromazine alone and mixed with oil on the first instar larvae of *L. sativae.*

Insecticide	LC (mg (a.i.)/l) (95% CI)			Slop ± SE	df	Cs.	I. ± SE
	LC_{90}	LC_{50}	LC_{10}				
Cyromazine	253 (158-392)	34.8 (24.9-46.2)	4.8 (1.7-7.4)	1.4 ± 0.26	3	0.18	-2.48 ± 0.48
Cyromazine + oil	259.6 (170-396.4)	32.2 (25.2-46.2)	3.9 (2.1-5.6)	1.4 ± 0.34	3	0.74	-1.87 ± 0.15

I. = intercept; Cs. = Chi-square.

It is here confirmed that the tested insecticides are effective alone against the larval stages of the leaf-miner fly, *L. sativae*, at the commercially recommended dosages. The addition of petroleum oil to the insecticides heightens their toxicity against *L. sativae* and leads to a reduction of their usage. Future studies are recommended to determine the potential of these insecticides and other registered pesticides against *Liriomyza* species, including *L. sativae*, in fields and greenhouses.

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