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Research Article

Effect of azadirachtin, chlorantraniliprole and some insect growth regulators on vegetable leafminer, *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae)

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Abstract: The aim of this study was to evaluate the efficacy of some insecticides with novel modes of action on *Liriomyza sativae* Blanchard and their sublethal effects on pupal mortality, pupal weight and sex ratio in adult insects. The effects of hexaflumuron, chromafenozide, chlorfluazuron, cyromazine, lufenuron + fenoxycarb and azadirachtin and chlorantraniliprole were evaluated on 1st instar larvae of *L. sativae* using a leaf dip method. Dose - response lines were constructed for insecticides which caused higher mortality of the larvae at field recommended doses. LC_{50} values for chlorantraniliprole, cyromazine, azadirachtin and hexaflumuron were 0.24, 0.49, 8.51 and 67.6 mg ai/l, respectively. A significant reduction in pupal weight and adult emergence was observed in all of the treatments except chromafenozide; but the adult sex ratio did not change significantly compared with control. Most of the insecticides used in this study are fairly new compounds with unique modes of action and had considerable lethal and sublethal effects on *L. sativae*. If these results also hold true in the field and commercial greenhouse conditions, these compounds could be suitable candidates in management of vegetable leafminer.

Keywords: *Liriomyza sativae*, azadirachtin, chlorantraniliprole, insect growth regulators, sublethal effect

Introduction

The vegetable leafminer, *Liriomyza sativae* (Blanchard) is a polyphagous and cosmopolitan insect. This pest has several host plants, but mainly attacks crops in Solanaceae, Liliaceae and Fabaceae families (Capinera, 2001). It is one of the key pests of ornamentals and agricultural crops especially in greenhouses. Both larvae and adults damage the host plants. The larvae feed on leaf mesophyll and reduce photosynthesis. The adult flies also puncture the leaves for feeding and oviposition. High density of these stipples causes some dryness on the leaf surface and results in decreased yield (Bethke and Parrella, 1985; Parella, 1987). Mainly chemical insecticides are used for controlling this pest. Sometimes the impact of insecticides on beneficial organisms (e.g. natural enemies) can be more severe than on target pests (Oatman and Kennedy, 1976). Frequent application of conventional pesticides results in elimination of natural enemies, displacement of secondary pests and development of resistance to many of the insecticides (Mason *et al*., 1987; Sharma *et al.* 1980). The effect of abamectin, spinosad and cyromazine (Saberfar *et al*., 2012), azadirachtin, lufenuron and pyriproxyfen

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(Kücükakyüz *et al*., 2012), cartap, profenophos and cypermethrin (Johnson *et al*., 2003) and several other pesticides (Hara, 1986; Mason *et al*., 1987) has been investigated on *L. sativae*.

Effective management of this pest requires some new insecticides with novel mechanisms of action. Chlorantraniliprole, is a new insecticide from ryanodine receptor modulators. It activates ryanodine receptors which stimulate the release of calcium and resulting in cessation of feeding, lethargy, muscle paralysis and, ultimately death of the insect (yu, 2008). Azadirachtin is a tetranortriterpenoid and the active ingredient of neem seed oils. It has proven to be one of the most important plant ingredients for integrated pest management (yu, 2008). Insect growth regulators are chemicals or substances that disturb normal growth and development of target insects and finally can kill them (yu, 2008).

The objectives of this study were to assess the toxicity of some fairly new insecticides with novel modes of action on *L. sativae* and their sublethal effects on certain bioparameters of this insect.

Materials and Methods

Insects

The insects used to establish the colony for this study were obtained from a culture maintained in the research greenhouse of the Department of Plant Protection of the University of Tabriz. The insects were reared on common bean plants *Phaseolus vulgaris* cv Khomein in greenhouse conditions at 26 ± 2 °C, 60 ± 10% RH and 16:8 h (L: D) photoperiod for *ca*. 25 generations without insecticide pressure. The adult flies were fed with 10% honey solution.

Insecticides

Azadirachtin (NeemAzal® -T/S 1 EC, Trifolio-M GmbH, Lahnau, Germany), Cyromazine (Trigard® 75 WP, Syngenta, Basel, Switzerland), Chlorfluazuron (Caprice® 5 EC), Chromafenozide (Virtu® 5 SC, Nippon Kayaku, Tokyo, Japan), Lufenuron + fenoxycarb (Lufox® 10.5 EC, Syngenta, Basel, Switzerland), Hexaflumuron (10 EC, Golsam, Gorgan, Iran) and Chlorantraniliprole (Coragen® 18.4 SC. Dupont, USA) were used in this study.

Bioassays

Larval synchronization and bioassays were carried out according to Cox *et al*. (1995). Briefly, about 10-12 plants (10 day old) with 2-3 true leaves were placed in an infestation cage with 100-150 female and male adult leafminer flies. After three hours, the adult flies were removed from the plants and the plants were transferred to a fly-free cage. After 3-4 days, the number of mines (representing the number of 1st instar larvae) was counted using a 10× hand lens and marked by a permanent marker. The plants were divided into groups with approximately equal number of larval mines. After doing preliminary experiments the range of concentrations for each of the insecticides was determined. The leaves were dipped into different concentrations (0.15-1.13 mg ai/l for cyromazine, 0.09-0.51mg ai/l for chlorantraniliprole, 5-20mg ai/l for azadirachtin and 37.5-125 mg ai/l for hexaflumuron) of the insecticides for five seconds. These concentrations were prepared with distilled water plus 0.05% Tween®-80 as a wetting agent. The control consisted of distilled water and Tween®-80. The treated plants were kept in greenhouse for 24 h and then their leaves were excised. The leaves were placed in plastic containers (23 × 23 × 12 cm) lined with tissue paper for absorbing excess moisture. The results were recorded after nine days in chlorantraniliprole treatment and after 11 days in the rest of the insecticide treatments. The reason for this difference was that the insecticides with IGR effects (including azadirachtin) caused a delay in molting after which the effect was evaluated and recording of the results was possible. Based on preliminary tests, five concentrations of each insecticide were used. All treatments were replicated three times at different days. Larval mortality was calculated using the formula described by Leibee (1988). Sublethal effects of cyromazine, chlorantraniliprole, azadirachtin and hexaflumuron were assessed using LC_{25}s of these insecticides. Altered pupal weight, adult emergence and adult sex ratio were monitored as the sublethal effects. Since lufenuron + fenoxycarb, chromafenozide and chlorfluazuron did not have considerable lethal effect on immature stages of *L. sativae* at concentrations as high as twice the recommended field doses, possible sublethal effects of these compounds on adults were investigated. Based on some preliminary testing, the concentrations used for this purpose were 2.25, 50 and 2.5 mg ai/l of lufenuron + fenoxycarb, chromafenozide and chlorfluazuron, respectively.

Statistical analysis

Percent mortalities were corrected using Abbott's formula (Abbott, 1925). The bioassay data were subjected to probit analysis of SAS (SAS Institute, 2004). Dose - response lines were plotted using Excel. Comparison of LC_{50} values was done based on overlapping of their confidence limits (Wheeler *et al*., 2006). The hypothesis of parallelism of the dose-response lines were tested using PoloPlus (Robertson *et al*., 2007).

Variations in bioparameters were analyzed using a completely randomized design. One way ANOVA (SAS Institute, 2004) was used for analyzing these sublethal effects. Separation of the means was done using Tukey's HSD test at 0.05 level.

Results

Toxicity symptoms

Most of the larvae treated with IGR effects showed symptoms at molting. In the larvae treated with azadirachtin, some of the last instar larvae were stuck in leaves and could not get out of the mines (Fig. 1). Larvoids were seen on the leaf surfaces in some other cases (Fig. 2). Some of the hexaflumuron treated larvae turned black and squashy (Fig. 3). The pupae in cyromazine treatment were malformed and smaller in size; and some of the adults were trapped in the pupation exuviae and finally died (Fig. 4).

Lethal effects

LC_{50} and LC_{90} values for the first instar larvae of *L. sativae* are shown in Table 1. Chlorantraniliprole and cyromazine were more effective than the other insecticides. The toxicities of the insecticides tested can be ranked in the following order (Fig. 5): Chlorantraniliprole > cyromazine> azadirachtin > hexaflumuron.

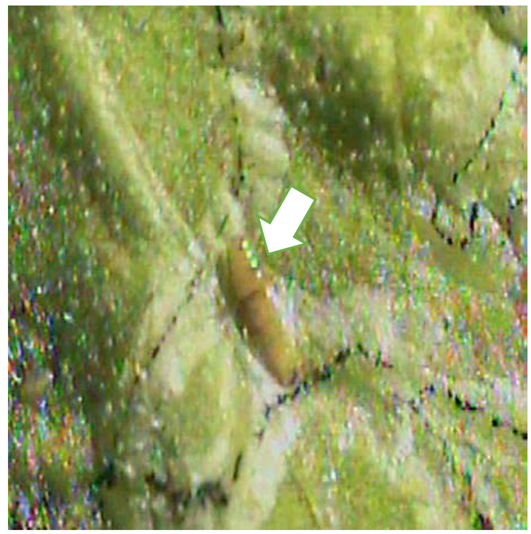

Figure 1 Azadirachtin treated larva of *Liriomyza sativae* stuck in leaf mine.

Figure 2 Larvoid pupa of *Liriomyza sativae* in azadirachtin treatment.

Figure 3 Normal (a) and abnormal (b) pupa of *Liriomyza sativae* in hexaflumuron treatment.

Figure 4 Adult fly trapped in pupal exuviae of *Liriomyza sativae* (resulted from cyromazine treated larva).

Sublethal effects

Changes in some bioparameters were considered as sublethal effects and are shown in Table 2. Larval exposure to LC_{25} of the insecticides resulted in a significant reduction in pupal weight ($F_{4,\ 140}$ = 33.34; P < 0.0001) compared with control. Pupal mortality was significantly affected by the insecticides tested and the control had lower mortality compared with the treatments ($F_{4,\ 695}$ = 8.15; P < 0.0001). The sex ratio in the treatments was not different from that of control. Pupal mortalities in chlorantraniliprole and cyromazine were not significantly different. There was a significant difference in pupal mortalities in hexaflumuron and azadirachtin. The insecticides lufenuron + fenoxycarb, chromafenozide and chlorfluazuron in doses higher than the recommended dose did not have significant effects on the larvae. Hence, LC_{50} values were not calculated for these insecticides. Since these compounds are IGRs and have delayed effects on insects, their possible delayed effects on some bioparameters of *L. sativae* were assessed. The results are shown in table 3. The pupal mortality in Lufenuron + fenoxycarb and chlorfluazuron was significantly higher than that of the control. Pupal weight in all treatments was also reduced compared with control ($F_{7,\ 1367}$ = 39.56; P < 0.0001).

Discussion

Most of the larvae treated with these compounds showed symptoms at molting. The symptoms observed in azadirachtin treatment were similar to those reported by Martinez and Van Emden (2001) and Hossain and Poehling (2009). Karimzadeh *et al*. (2007) also reported a change in color of *Leptinotarsa decemlineata* larvae treated with hexaflumuron. Robinson and Scott (1995) also reported decrease in body size of larvae and pupae, malformation and discoloration of the larvae of two species of mosquitoes after treatment with cyromazine.

The higher effectiveness of chlorantraniliprole and cyromazine on leafminer flies has been reported by several researchers (Conroy *et al.*, 2008; Schuster *et al.*, 1991; Saito, 2004). The LC_{50} value for chlorantraniliprole reported by Conroy *et al*. (2008) was 0.21 mg ai/l for first instar larvae of *L. sativae*, which compares favorably with our estimate of 0.24 mg ai/l. This may show similar sensitivities of the populations tested and the experimental procedures used. The LC_{50} value for cyromazine in this study was slightly lower than the results obtained by Johnson *et al*. (2003). This may be due to the differences in sensitivities of the populations tested (the population tested in this study was in culture for more than 25 generations in the greenhouse).

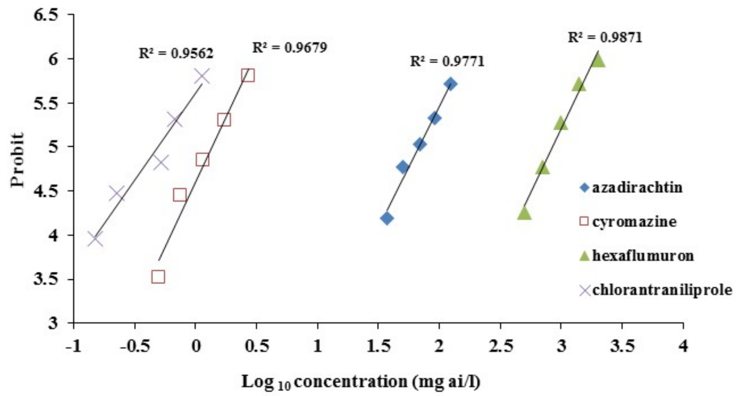

Figure 5 Concentration- response lines for the 1st instar larvae of *Liriomyza sativae.*

Table 1 Toxicity of the tested insecticides on 1st instar larvae of *Liriomyza sativae.*

Insecticides	Total number of insect	LC_{50} (mg ai/l) (95% CL)	Slope ± SE	LC_{90} (95% CL) (mg ai/l)	χ^2	P_r
Azadirachtin	909	8.51 (7.82-9.18)	2.91 ± 0.24	23.46 (20.38-28.34)	1.18	0.76
Hexaflumuron	941	67.61 (61.35-74.22)	2.69 ± 0.30	202.44 (164.32-277.26)	2.37	0.49
Cyromazine	606	0.49 (0.43-0.56)	2.95 ± 0.32	2.21 (1.67-3.31)	6.11	0.11
Chlorantraniliprole	1009	0.24 (0.22-0.26)	2.77 ± 0.23	0.69 (0.59-0.87)	3.64	0.30

CL: confidence limits.

Table 2 Effects of LC_{25}s of the tested insecticides on some biological parameters of *Liriomyza sativae.*

Treatment	C[1] (mg ai/l)	No. of larvae tested	No. of pupae formed	No. of adults emerged	No. of females	No. of males	%larval mortality ± SE	%Pupal mortality ± SE	Pupal weight ± SE (mg)
Chlorantraniliprole	0.15	140	94	66	27	39	32.9 ± 4.7a	29.8 ± 4.7b	0.29 ± 0.015c
Cyromazine	0.25	140	105	74	38	36	25.0 ± 3.7a	29.5 ± 4.5b	0.38 ± 0.018b
Azadirachtin	5	137	91	8	3	5	33.6 ± 4a	91.2 ± 2.9a	0.33 ± 0.016bc
Hexaflumuron	36	140	100	0	-	-	28.6 ± 3.8a	100 a	0.28 ± 0.018c
Control	0	143	131	119	61	58	8.4 ± 2.3b	29.0 ± 2.5c	0.51 ± 0.015a

[1] C: Insecticides concentrations

Table 3 Effects of the reduced field recommended doses of tested insecticides on some biological parameters of *Liriomyza sativae.*

Treatment	C[1] (mg ai/l)	No. of larvae tested	No. of pupae formed	No. of adults emerged	No. of females	No. of males	%Larval mortality ± SE	%Pupal mortality ± SE	Pupal weight ± SE (mg)
Lufenuron + fenoxycarb	2.25	187	150	81	46	35	19.8 ± 2.9 a	46.0 ± 3.8 a	0.401 ± 0.015a
Chromafenozide	50	181	158	110	59	51	12.7 ± 2.5 a	30.4 ± 3.7 b	0.378 ± 0.015 a
Chlorfluazuron	2.5	180	171	73	26	47	5.0 ± 1.6 a	57.3 ± 3.8 a	0.415 ± 0.015 a
Control	0	178	170	125	70	57	4.5 ± 1.6 a	26.5 ± 3.4 b	0.512 ± 0.013 b

[1] Insecticides concentrations.

The slopes of the dose-response lines of the insecticides tested were quite steep. Although the difference between the slopes for the dose-response lines of the insecticides under study do not differ substantially, the hypothesis of parallelism and equality was rejected when the data were analyzed by PoloPlus. Due to steep slopes, the differences between the highest and lowest effective concentrations were low. That is, the population tested, was homogeneous, and with a fairly small increase in insecticide concentration, the mortality would increase considerably. This necessitates more careful use of these insecticides in the fields and greenhouses because of higher chance for development of resistance.

Similar to our results, Weintraub and Horowitz (1998) found that while low concentrations of azadirachtin had a minor effect on the larvae, they caused higher mortality in the pupae. Similar results were also found in studies done by other researchers (Parkman and Pienkowski, 1990; Hossain, 2005; Das *et al*., 2006).

Kandil *et al*. (2012) also reported that fecundity, adult emergence and pupal weight were reduced when the eggs were treated with lufenuron, chlorfluazuron and chromafenozide. Perveen (2000) reported that in *Spodoptera litura* larvae, chlorfluazuron treatment caused high mortality of pupae, failure in adult emergence or emergence of deformed adults.

Most of the insecticides used in this study were fairly new compounds with unique modes of action and had considerable lethal and sublethal effects on *L. sativae*. If these results also hold true in the field and greenhouse conditions, these compounds could be suitable candidates for implementation in management programs for vegetable leafminer.

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تأثیر آزادی راختین، کلران ترانیلی پرول و برخی سموم تنظیم‌کننده رشد حشرات روی مگس مینوز سبزیجات(*Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae

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چکیده: مگس مینوز سبزی و جالیز *Liriomyza sativae* (Blanchard) حشره‌ای چندین‌خوار و همه‌جازی بوده و از مهم‌ترین آفات محصولات گلخانه‌ای مخصوصاً خیار و گوجه‌فرنگی می‌باشد. در این پژوهش اثرات کشندگی حشره کش‌هایی با نحوه‌ی اثر جدید روی این آفت و اثرات غیرکشندگی آن‌ها روی مرگ‌ومیر شفیره، وزن شفیره و نسبت جنسی حشرات کامل مورد بررسی قرار گرفت. اثرات هگزافلوموران، کرومافنوزاید، کلرفلوآزوران، سایرومازین، لوفنوزان + فنوکسی کارب، آزادیراکتین و کلرانترانیلی پرول روی لاروهای سن اول مگس مینوز سبزی و جالیز با روش غوطه‌ور کردن برگ در محلول سمی بررسی شد. برای چهار تا از حشره‌کش‌ها که مرگ‌ومیر بالایی در مرحله‌ی لاروی نشان دادند خطوط دوز اثر رسم شدند. مقادیر LC_{50} برای کلرانترانیلی‌پرول، سایرومازین، آزادیراکتین و هگزافلوموران به‌ترتیب ۰/۲۴، ۰/۴۹، ۸/۵۱ و ۶۷/۶ میلی‌گرم ماده‌ی مؤثر در لیتر تخمین زده شد. در همه‌ی تیمارها غیر از کرومافنوزاید کاهش معنی‌داری در وزن شفیره و ظهور حشرات کامل مشاهده شد، اما نسبت جنسی حشرات کامل تفاوت معنی‌داری با شاهد نداشت. استفاده از حشره‌کش‌های جدید و مناسب برای کنترل این آفت و کاهش بروز مقاومت ضروری به‌نظر می‌رسد. بسیاری از حشره‌کش‌های استفاده شده در این پژوهش ترکیبات جدیدی با نحوه‌ی اثر جدید هستند که اثرات کشنده و غیرکشنده‌ی قابل ملاحظه‌ای روی *L. sativae* دارند. بنابراین اگر آزمایش‌های گلخانه‌ای و مزرعه‌ای هم نتایج مشابهی نشان دهند، این ترکیبات می‌توانند جایگزین‌های مناسبی برای کنترل این آفت باشند.

واژگان کلیدی: *Liriomyza sativae*، آزادیراکتین، کلرانترانیلی‌پرول، حشره‌کش‌های تنظیم کننده‌ی رشدی، اثرات غیرکشنده