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Compatibility of insecticides in IPM of citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

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ABSTRACT

Citrus leaf miner (CLM), Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) is a serious pest of citrus trees throughout the citrus-growing areas in Egypt. The objectives of the present study were to assess the efficacy of the insecticides; abamectin, hexythiazox, emamectin benzoate, thiamethoxam, triflumuron, lufenuron+fenoxycarb, and imidacloprid against CLM, as well as to evaluate their selectivity on CLM parasitoids. The results revealed highly significant differences between the tested treatments in mortality rates of CLM, abamectin was the most effective insecticide, however it was IPM-incompatible. Thiametoxam was the most selective for CLM parasitoids with a selectivity index of 1.73, and an IPM compatible product on the control of this pest. These results might help on the development of integrated pest management plans for citrus orchards in Egypt.

Keywords: Citrus leaf miner – IPM-selectivity of parasitoids.

INTRODUCTION

Citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton, is an important pest of citrus and related Rutaceae in Southeast Asia, Australia, East and West Africa and California [1-2]. It also occurs in the Mediterranean coast of Europe, the Middle East, North Africa, Caribbean Island, Central America, Mexico and South America [3-5]. CLM moths attack newly formed leaves, nurseries and flushing of citrus trees; the first larval instar attacks them making mines in the under and upper surfaces of leaves, confined to the epidermal layer. The infested leaves are frequently distorted and may be abscised [6]. Nowadays, natural enemies are a useful tool for suppressing populations of the CLM [7]. In case of a heavy infestation, indirect effects affect quality and quantity of citrus fruits, and control measurements must be taken. Chemical control is an important measure [8]; however, larvae and pupae are protected by the leaf cuticle and the rolled leaf margins, and effectiveness of the chemical control on CLM is not always granted. Therefore, it is essential to apply an effective insecticide against the target pest, but it must be selective on natural enemies. Hence, the objectives of the present study were to determine the efficacy of certain novel insecticides against larvae and pupae of CLM, as well as their selectivity on native parasitoids, as potential biological control agents useful on integrated pest management programs.

MATERIALS AND METHODS

Experimental orchard

Field evaluation of insecticides against CLM was carried out from April to August 2016 in an isolated orchard of 10 Feddans. The orchard was planted with 7-years-old navel and sour orange trees, in Damanhour, El-Beheira Governorate, Egypt.

Insecticides used

Seven commercial insecticides, 2 organic- and 5 bio-pesticides were used. Their type and name are provided in Table 1; abamectin and imidacloprid were tested at the recommended field concentration for CLM. Hexythiazox, emamectin benzoate, thiamethoxam, triflumuron and lufenuron+fenoxycarb were tested at the recommended field concentration against the grape fruit worm, *Lobesia botrana*.

Procedures

Eight groups of citrus trees, 40 trees per a group, were set in the present study. Ten trees were considered a replicate in a completely randomized design, with four replicates per treatment. Samples of 16 new flushes (15-20 cm) were selected randomly per group (≈360 leaves), flush samples were cutoff directly from each treatment into paper bags and transported to the laboratory of fruit flies at El-Beheira Governorate (25°C, 80 ± 5% Relative Humidity and a photoperiod of 12 h). Samples were inspected under the binocular microscope, and dead and alive larvae were recorded.

Seven groups of the trees were sprayed with the insecticides (Table 1), one per group.The control treatment was sprayed with water. Sprayer motor (Agramondoo, 3WF-3C(20L)) for foliar spray was used in all cases. Samples from each treatment were taken 7-days post-treatment, and carried to the laboratory. Samples were inspected, dead and alive, larvae and pupae were recorded. Pesticide's efficacy in suppressing the CLM population (CLM Efficacy Index, CEI) was estimated according to Villanueva-Jimenez and Hoy [9].

Extended Laboratory study

Samples containing alive larvae and/ pupae of CLM of each treatment were retained in plastic jars under laboratory conditions, until adults of CLM or parasitoids emerged. Dead pupae, parasitized pupae and adult emergence of both CLM and parasitoids also were recorded.

Pesticide selectivity, efficacy and its compatibility for IPM

The effects of the tested insecticides on parasitoids were categorized according to the principles of the IOBC (International Organization for Biological Control) classification [10]. Four categories to classify percentage of mortality were used: 1= harmless (< 25%), 2 = lightly harmful (25-50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%).

The selectivity of the tested pesticides for CLM parasitoids (selectivity index, SI), and its pesticide compatibility (compatibility index, IPM CI) for CLM-IPM programs were assessed according to the method of Villanueva-Jimenez and Hoy [9]. SI was ranked as non-selective if the SI value was 0 to < 0.5, moderately selective if the SI value was 0.5 to <1.0, and selective if the SI was1.0 (up to 2.0)

Statistical analysis

Mortality rates of the tested insecticides against CLM-larvae were corrected according to Henderson and Tilton [11], CLM-pupae according to Sun-Shepard's (Ref. Püntener)[12] and parasitoids according to Abbott's formula [13], using LdP line® software. Data was analyzed as one-way ANOVA using Costat software (version, 2008), the mean values were separated by Student-Newman-Keuls test, $p<0.05$ and Spearman's of rank correlation was used for non-parametric.

RESULTS

Efficacy of insecticides against larval and pupal CLM

Field assay results on effectiveness of the seven insecticide against CLM-larvae (Table2), revealed highly significant differences ($p<0.05$) between treatments ($F=121.15$, $df=6$, $p=0.0001$). Abamectin exhibited a high reduction of the CLM-larvae 7-days post-treatment with 97.47% mortality. Imidacloprid was the second in efficacy (96.55% mortality) with no significant difference with abamectin. Larval reduction decreased to 83.46 % with hexythiazox, followed by lufenuron + feoxycarb, emammectin benzoate, triflumuron and thiametoxam with mortalities of 77.24, 75.26, 71.61 and 68.1%, respectively. They were significantly different not only with the first group, abamectin and imidacloprid, but also between them, except between lufenuron + feoxycarb and emammectin benzoate, there was no significant difference.

Effects of the tested insecticides extended to the pupal stage with 73.85 to 100 % mortality (Table 2), with significant differences ($p<0.05$) between the treatments ($F=125.76$, $df=6$, $p=0.0001$). Abamectin, imidacloprid and hexythiazox recorded the highest reduction on the pupae with 100 % mortality, being no significantly different, followed by triflumuron, emamectin benzoate, lufenuron + fenoxycarb and thiametoxam with a mortality of 89.29, 88.23, 85.29 and 73.85 %, respectively, with no significant differences between them, except with thiametoxam.

According to the general mean mortality of larvae+pupae, abamectin, imidacloprid and hexythiazox, with 98.73, 98.27 and 91.72% mortality, respectively, were more effective than lufenuron + fenoycarb, emamectin benzoate and triflumuron, with a mortality of 81.74, 81.27 and 80.46%, respectively, while thiametoxam was the least efficient of the tested insecticides with 70.99 % mortality. Highly significant differences in the mortality of larvae+pupae were obtained among the tested insecticides ($F=308.13$, $df=6$, $p<0.0001$). Results showed that triflumuron and lufenuron + fenoxycarb were the most efficient insecticides, with a CLM efficacy index (CEI) of 1.44, while abamectin was the least efficient compound with a CEI value of 0.97. In between, hexythiazox, emamectin benzoate, thiametoxam and imidacloprid were arranged descendingly with corresponding CEI values of 1.41, 1.40, 1.36 and 0.99.

Table 1: Insecticides used in the experiment.

Trade name	Active ingredient	Type	Formulation	Application rate
Abanteen	abamectin	Bio-Insecticide	1.8% EC	30 cm/100 L
Shoshi	hexythiazox	MGR[a]	10% WP	50 gm/100 L
Catch	Emamectin benzoate	Bio-Insecticide	3%WDG	20 GM/100 L
Voliam Flexi	thiamethoxam	Insecticide	40% WG	80 gm/ F
Cysten	triflumuron	IGR[b]	48% SC	26.25 cm/100 L
Lufox	lufenuron+fenoxycarb	IGR	10.5%EC	100 CM/100 L
Aquador	imidacloprid	Insecticide (neonicotinoid (niectonid	35% SC	75 CM/100 L

[a]Mite growth regulator
[b]Insect growth regulator

Side effects of pesticides and their selectivity to CLM parasitoids

Results in Table 3 showed that abamectin, imidacloprid and hexythiazox were the most harmful (Class, 4) insecticides against the CLM parasitoids with 100% mortality. Lufenuron + fenoxycarb and emamectin benzoate were less harmful than the previous insecticides with 80% mortality, and classified as harmful (Class, 4), as well. While, the effect of triflumuron on parasitoids, with 72% mortality, was considered as a moderately harmful product (Class, 3). On the other hand, thiamethoxam caused 24 % mortality in parasitoids and was classified as harmless (Class, 1).

Table 2: Mortality percentages of the larval and pupal of CLM, *P. Citrella*, treated with insecticides

Pesticide	Corrected Mortality (%) (±S.E.)			CLM Efficacy Index (CEI)
	larvae	pupae	Larvae + pupae	
Abamectin	97.47±00.31[a]	100.00±00.00[a]	98.73±00.09[a]	0.97
Imidacloprid	96.55±00.49[a]	100.00±00.00[a]	98.27±00.24[a]	0.99
Hexythiazox	83.45±01.28[b]	100.00±00.00[a]	91.72±00.64[b]	1.41
Lufenuron + fenoxycarb	77.24±01.30[c]	85.29±01.11[c]	81.74±01.69[c]	1.44
Emamectin benzoate	75.25±01.31[c]	88.23±01.59[b]	81.27±00.21[c]	1.40
Triflumuron	71.61±01.07[d]	89.29±01.51[b]	80.46±01.25[c]	1.44
Thiamethoxam	68.12±01.17[e]	73.85±01.54[d]	70.99±00.56[d]	1.36
F	121.15	125.76	308.13	-
LSD	3.12	2.58	1.73	-
P	0.000	0.000	0.000	-

Means followed by the same letter(s) are not significantly different (Student-Newman-Keuls test, p<0.05).

According to the SI of parasitoids abamectin, imidacloprid and hexythiazox had 0.0 SI and ranked as non-selective compounds for CLM parasitoids. Triflumuron, lufenuron + fenoxycarb, emamectin benzoate and thiamethoxam ranked as selective compounds for CLM parasitoids with some discrepancy in SI values of 1.11, 1.26, 1.45 and 1.73, respectively.

Compatibility of the tested insecticides for CLM IPM

Results in Table 4 revealed that the tested insecticides varied in their CLM IPM-compatibility, according to CI value. Abamectin was the least compatible, with a CLM IPM CI of 0.97, followed by imidacloprid with 0.99, ranked as in-compatible for CLM IPM. While, hexythiazox ranked as a semi–compatible compound with CI value of 1.41. On the other hand, triflumuron, lufenuron + fenoxycarb, emamectin benzoate and thiamethoxam ranked as compatible compounds for CLM IPM with corresponding values of 2.55, 2.7, 2.85 and 3.09, respectively.

DISCUSSION

Although, it is known that some insecticides are not very effective controlling the CLM, caused by the inaccessibility of the larvae inside the mines, our results on effectiveness of the tested insecticides against CLM larvae showed that abamctin and imidacloprid had the potential to effectively control the larvae of CLM. Furthermore, their potential effect extended to the pupal stage and hexathiazox shared with them the same effect. On the other hand, thiamethoxam was the least toxic product to both larvae and pupae of the CLM, with 1.43- and 1.33-fold less than abamectin. Supported results revealed that abamectine+petroleum oil, lufenuron and thiamethoxam were arranged descendingly in reduction of both larvae and larvae+pupae of CLM, respectively; abamectin caused 87.2% (7-days post-treatment in the first spray) and 95.0 % reduction (5-days post-treatment in the second spray) in the larvae and larvae+pupae of CLM, lufenuron caused 75.4% reduction in the larvae+pupae and thiamethoxam (0.5) caused 74.0 % reduction (7-days post-treatment in the first spray) in the larvae [14]. Other studies revealed that biorationals insecticides, such as abamectin and azadirachtin with Triton X-100 were effective against CLM on Lime seedlings [15]. As well as, abamectin and mineral oil had a comparable effect against the three larval instars of CLM under field conditions [16]. Furthermore, treated leaves with abamectin were completely free of CLM damage along 3 and 4weeks post-treatment (0.05 and 0.03 alive larvae)) [17]. The present results revealed that thiamethoxam was the least efficient of the tested insecticides on both larvae and pupae of the CLM. However, thimethoxam exhibited a reduction of CLM larvae by weak 4-posttrearment [18].

Table 3: IOBC class and selectivity of the insecticides for CLM, *P. Citrella*Parasitoids in a citrus orchard treated and untreated with insecticides.

Pesticide	Corrected Mortality (%) (±S.E.)	IOBC Class*	Parasitoids Selectivity Index (SI)	Rank**
Abamectin	100.00±00.00[a]	4	0.0	Non-selective
Imidacloprid	100.00±00.00[a]	4	0.0	Non-selective
Hexathiazox	100.00±00.00[a]	4	0.0	Non-selective
Lufemuron	80.00±02.12[b]	4	1.26	Selective
Emamectin benzoate	80.00±01.29[b]	4	1.45	Selective
Trifumuron	72.00±02.06[c]	3	1.11	Selective
Thiametoxam	24.00±01.15[d]	1	1.73	Selective
Control	-	-	-	-
F	380.04	-	-	-
LSD	4.08	-	-	-
P	0.000	-	-	-

*Means followed by the same letter(s) are not significantly different (Student-Newman-Keuls test, p<0.05).*1= if mortality (m) < 25%, 2 = 25-50%, 3 =51-75% and 4 = >75%.**Non-selective: 0 to 0.49; moderately selective: 0.5 to 0.99; selective: ≥1.0.*

Table 4: Insecticides ranked for parasitoids selectivity and IPM compatible of CLM, *P. Citrella*, in a citrus orchard treated.

Insecticide	Abmectin	Imidacloprid	Hexythiazox	Lufenuron + fenoxycarb	Emamectin benzoate	Triflumuron	Thiamethoxam
Index (CI)	0.97	0.99	1.41	2.70	2.85	2.55	3.09
IPM compatible Rank[a]	IC	IC	SC	C	C	C	C

[a]Incompatible (IC): 0 to 0.99; Semi-compatible (SC): 1.0 to 1.99; Compatible (C): ≥2.0.

The present results showed that triflumuron and lufenuron + fenoxycarb had the highest efficacy index of CLM, while abamectin followed by imidacloprid had the lowest efficacy index with 0.97 and 0.99, because they had high toxicity, they were able to eliminate the non-damaged-stage of CLM. These results seemed to agree with efficacy indexes of the treated insecticides against CLM in Florida; abamectin had 0.1, imidacloprid 0.4 drenched and 0.0 as a spray and fenoxycarb had 1.0 [9]. In relation the insecticidal effect on CLM parasitoids, the present results showed that abamectin, imidacloprid and hexythiazox eliminated the native parasitoids, followed by lifenuron + fenoxycarb and emamectin benzoate, that were less toxic than the previous group. However, they all classified as harmful insecticides (class, 4) for parasitoids according to IOBC, because they caused mortality of parasitoids grater than 75%. On the other hand, triflumuron was partially safe to CLM parasitoids and moderately harmful (class, 3), followed by thiamethoxam, which was the most safely insecticide to parasitoids. In relation to pesticides effects on citrus parasitoids in Italy, abamectin was classified as a harmful insecticide for the predator, *Orius laevigatus* up to 14-d after the treatment, while emamectin was moderately harmful until 7-d after the treatment [19]. In Louisiana, USA, using regularly insecticides in citrus orchards, might have limited the performance of the CLM parasitoid, *Ageniaspis citricola* [20]. As well as the biorational product, fenoxycarb (Eclipse) + 0.4% oil did not disrupt the CLM parasitoid, *Semielacher petiolatus* [21]. However, in another study including insecticide treatments with abamectin + PO, showed a very low level of parasitism [14].

Our findings showed that abamectin, imidacloprid and hexythiazox are not selective for CLM parasitoids, while lufenuron + fenoxycarb, emametin benzoate, triflumuron and thamethoxam were selective insecticides for the CLM parasitoids, and they had a selectivity index of parasitoids > 1. These results were partially in agreement with the results of Villanueva-Jimenez and Hoy [9]; they mentioned that imidacloprid (spray) and fenoxycarb were non-selective for CLM parasitoids, conversely they mentioned that a low dose of abamectin was selective to the parasitoids.

Also in the present study, abamectin and imidacloprid did not exhibit compatibility for CLM parasitoids. Similarly, abamectin and abamectin at the lowest recommended field rate +0.4% of petroleum oil were considered an incompatible pesticide in IPM of CLM [22]. In another study, abamectin+ oil and imidacloprid were considered as IPM-incompatible insecticides in citrus orchards [17]. While, hexythiazox was semi-compatible, on the other hand lufenuron + fenoxycarb, emamectn benzoate, triflumuron and thiamethoxam were compatible for CLM IPM [9],[22].

These results might facilitate the development of integrated pest management plans in citrus orchards in Egypt.

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