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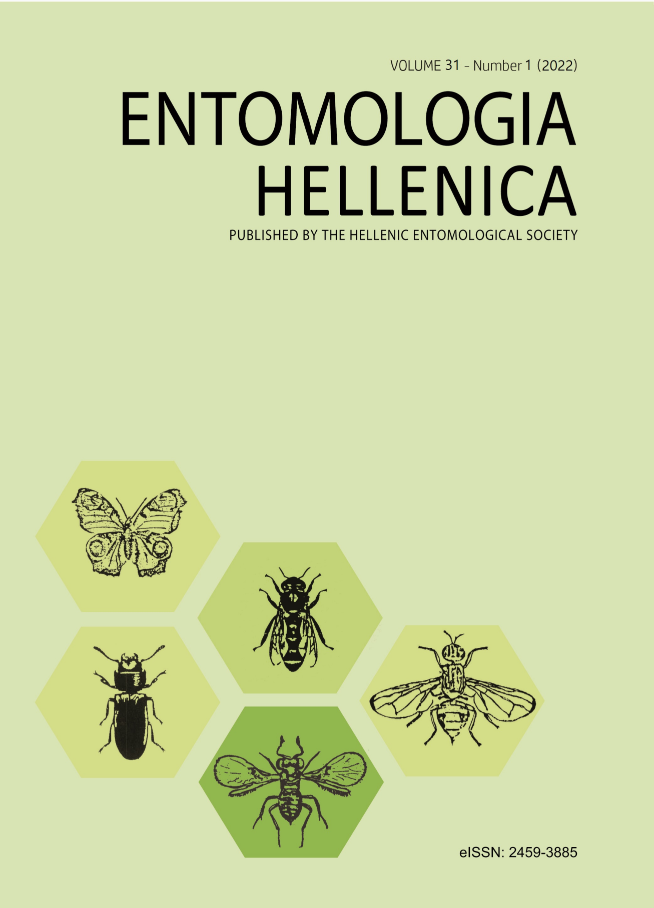

Efficiency of Some Insecticides in Controlling Citrus Leafminer, Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae)

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Efficiency of Some Insecticides in Controlling Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

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ABSTRACT

The toxicity of four commercial insecticides (mineral oil, azadirachtin, phenthoate and abamectin) in addition to mint oil (variety of terpenoids), against the citrus leaf-miner, *Phyllocnistis citrella*, larvae, was tested under laboratory conditions using the leaf-dipping method. The bioassay data indicated that abamectin was the most toxic insecticide against *P. citrella* larvae, followed by azadirachtin, phenthoate, mint oil and finally mineral oil. On the other hand, field evaluation showed that the highest larval mortality was 94.79 and 83.87% induced by abamectin and azadirachtin, respectively, five days after the 2nd application. Mineral oil and phenthoate showed a 72.43 and 61.4% mortality respectively, at 11 days after the 2nd application, and finally mint oil, 49.82% three days after the 1st application.

KEY WORDS: *Phyllocnistis citrella*; Toxicity; Insecticides; Mint oil; Egypt.

Introduction

The citrus leaf miner (CLM), *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) is an important insect in many regions of the world (Hoy and Nguyen, 1997). This insect has become the most important pest of citrus in Egypt (El-Saadany and Wahed, 2002). *Phyllocnistis citrella* goes through four developmental stages: eggs, larvae, pupae, and adults, with total developmental duration from eggs to adult emergence at 13- 52 days and has 6-13 generations per year depending on weather conditions (Heppner, 1993). It attacks the new leaves of seasonal flushes and in severe cases causes complete loss of new foliage. Although *P. citrella* is an indirect pest, the nature of its attack makes it a serious threat for young trees. Infested leaves curl down and may drop prematurely (Sponagel and Diaz, 1994).

CLM control practices comprise application of insecticides as well as biological control. Effective chemical control is difficult because the larvae are protected from insecticides by the leaf cuticle (Legaspi et al., 1999). Furthermore, CLM has a long history of resistance development against many insecticides, which makes it difficult to obtain sufficiently effective control (Mafi and Ohbayashi, 2006). Moreover, due to its multiple generations per year, there is a need for frequent applications of insecticides to improve control, due to the multiple generations of the insect per year (Yumruktepe et al., 1996). Mineral oil can

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be used as a surfactant and can be very beneficial for managing *P. citrella* by reducing the protective effect of the leaf epidermis to the insect (Dias et al., 2005). Mineral oils are the most common alternative to managing *P. citrella* and are recommended for use in home gardens, nurseries and orchards (Khalid et al., 2012). However, petroleum oils are commonly sprayed in summer to control CLM in many countries, but oil sprays after late July should be avoided owing to the decrease of the Brix content of fruits (Ujiye, 2000). Several insecticides, such as indoxicarp, buprofezin, pyriproxifen, diflubenzuron over phosalone and etrimfos have been used (Jafari, 1996; Amiri- Besheli, 2006). However, chemical insecticides have toxic effects on natural enemies and environment (Huang and Li 1989).

Although biological control is generally a promising option against major insect pests and there is an increasing trend of using it as an alternative to conventional insecticides (Lacey et al., 2001), such effective control of CLM is complicated because of its high migration ability from the periphery of orchards and its high fertility potential, as well as the fact that the citrus leaf epidermis offers substantial protection for the insect (Sarada et al., 2014). Bioinsecticides comprise several types of pesticides deriving from different natural materials and include biochemical pesticides, microbial pesticides, and plant-incorporated-protectants. These are inherently less harmful to non-target organisms and more specific to target pests (EPA, 2011; Isman, 2006), while additionally they are effective in small doses and decompose quickly, without leaving undesirable residues (EPA, 2011), thus they can reduce the use of conventional insecticides as a major component of IPM programs (Bravo et al, 2011). For example, Spinosad and azadirachtin have high efficacy in controlling *P. citrella* on young citrus plants, within seven days post treatment (Perovic and Hrncic, 2008a). Also, imidacloprid, thialoprid, acetamiprid and thiametoxam have successfully achieved effective control against these species (Perovic and Hrncic, 2008b). Therefore, the present study aims to evaluate the efficacy of specific insecticides against citrus leaf miner larvae, in the laboratory as well as at the field.

Materials and Methods

Bioassay: Toxicity of the tested insecticides against *P. citrella* larvae was conducted at the Toxicology Laboratory, Faculty of Agriculture, Ain Shams University, Qalyubia Governorate, Egypt, using commercial insecticides: mineral oil (kZ 95% EC Kafr El Zayat pesticides and chemicals co), azadirachtin (Achook 0.15% EC Godrej Agrovet Ltd), phenthoate (Elsan 50% EC, Arysta LifeScience Corporation), abamectin (Bermectin 1.8% EC, Agromen Chemicals Co. Ltd) and mint oil 100% (raw material, from Agriculture Research Center, Cairo, Egypt), containing l-menthol, menthone, isomenthone, neomenthone and a variety of other terpenoids. 53-year-old citrus seedlings were placed in the center of a *P. citrella* infested orchard (untreated during the latest years), pruned, fertilized and watered to promote the flush of new leaves until infection occurred. Infected leaves were further used for the present bioassay.

The tested concentration series were determined following a preliminary study using (1/100) of the field application rate according to the recommendations of the Ministry of Agriculture (Table 1). For the mint oil, a preliminary assessment of different concentrations (10, 5, 2, and 1 ppm) emulsified with Tween 80 was conducted. Based on the mortality rates obtained from this preliminary study, the tested concentrations were as follows: mineral oil: 142.5, 285, 570, 1000 and 1425 ppm; abamectin: 0.072, 0.144, 0.252, 0.324 and 0.432 ppm; azadirachtin: 0.22, 0.33, 0.44, 0.55 and 0.66 ppm; phenthoate: 2.25,

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3.75, 5.25, 6.75 and 8.25 ppm and mint oil: 0.25, 0.5, 1, 2 and 5 ppm.

The bioassay method was applied as described by Amiri-Besheli (2008) with some modifications. The leaf-dip technique was used to test insecticide toxicity. Only leaves with actively feeding 2nd stage leaf miner larvae (because the response to insecticides is higher at this stage) were completely excised from the petioles from Washington citrus trees and used for bioassays. To keep leaves turgescent during the bioassay, each petiole was covered with wet cotton. Leaves were dipped individually, for approximately 10 sec into each dilution, air-dried for approximately 2h and placed at the bottom of plastic Petri dishes (9 cm ø), previously lined with wet filter paper. The experiment for each concentration was replicated five times and each replicate included 10 leaves, along with a control group, for which the leaves were treated with distilled water. All Petri dishes were incubated at 26±1°C, 65± 5% RH with 16:8 h (L:D) photoperiod. 24, 48 and 72 h post treatment, the numbers of living and dead larvae for each replicate were counted under a stereomicroscope. Abbott's (1925) formula was used to correct the mortality in larval density of P. citrella.

Effect of the tested insecticides on the reduction of the target insect: The efficacy of the tested insecticides against P. *citrella* under field conditions was estimated according to the method used by Raga et al (2001) with some modifications, i.e., a randomized complete block design with 4 replicates, one tree plot, at a navel orange orchard (*Citrus sinensis* L. var. Washington), at El Qualubia Governorate, season 2011. The commercial insecticides used are summarized in Table 1. The insecticides were applied using a knapsack sprayer (20L capacity, nozzle diffuser). About 4–5L were sufficient to ensure complete coverage of all the parts of a tree till run-off. Each tested insecticide was applied separately at the rate recommended by the Ministry of Agriculture in Egypt. The products were applied on the 6th and 16th of September. Control trees were sprayed with water. Ten immature leaves from each replicate were randomly picked from different sides of the treated trees on the 6th (pre-treatment), 9th, 13th, 21st and 27th of September. The leaves were kept in plastic bags and transferred to the laboratory where they were examined with a stereomicroscope. Infestation reduction levels were calculated using the formula of Henderson and Tilton (1955).

Table 1. Insecticides used in the bioassay experiment

Insecticide	Active ingredient	Chemical class	MOA	a.i.%	FAR[a] (/100L)
KZ oil	Mineral oil	Aliphatic hydrocarbons	Suffocation	95%	1.5 l
Bermectin	Abamectin	Avermectin	Glutamate-gated chloride channel (GluCl)	1.8%	40 ml
Achook	Azadirachtin	Biochemical	Antifeedant and Insect Growth Disruptor	0.15%	750 ml
Elsan	Phenthoate	Organophosphate	AChE inhibitor	50%	45 ml
Mint oil	Variety of terpenoids	Plant extract	Unknown	100%	0.5 g

[a] FAR= Field application rate

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Statistical analysis: The data were analyzed using the program of the SAS Institute (1999) with a 5% significant difference. Comparison between the effectiveness of the tested insecticides against the insect under laboratory and field conditions was analyzed by ANOVA. Means were separated by Tukey's honestly significant difference (HSD). Lethal concentration (LC_{50}) values of the tested stage were calculated according to Finney (1971). From these concentrations the corresponding toxicity lines were estimated (LDP line program) and the relative efficiency of LC_{50} values and slope values of the tested insecticides was determined, using the following two equations (Sun, 1950):

Toxicity index = LC_{50} of the most effective compound / 100 x LC_{50} of the tested compound

Relative toxicity = LC_{50} of the lowest effective compound / LC_{50} of the other compound.

Results

Bioassay: Leaf-dip technique: the toxicity of the five insecticides was determined by using five different concentrations of each test compound against *P. citrella* larvae and recording mortality after 24, 48 and 72h. The LC_{50} and LC_{90} values and regression coefficient (slope) after 72h were calculated. Results are represented in Table 2. The comparison between the *P. citrella* larval LC_{50} for the tested insecticides shows that abamectin was the most toxic by a.i. weight unit (0.26 ppm) followed by azadirachtin (0.46 ppm), phenthoate (5.91 ppm), mint oil (7.16 ppm as value expected by Ldp Line program) and mineral oil (944.85). The LC_{50} values differed significantly at 0.05 level of probability (x^2= 1838.8, df= 2, P= 0.0001). On the other hand, the LC_{90} values of these insecticides were 0.74, 0.93, 11.95, 102.44 and 3784.88 ppm for abamectin, azadirachtin, phenthoate, mint oil and mineral oil respectively. As for slope values, the steepest toxicity line of azadirachtin possessed the highest slope value (4.22), which indicates higher homogeneity of the tested population, whereas the flattest line of mint oil possesses the lowest slope value (1.11). The remaining values were 2.78 for abamectin, 4.19 for phenthoate and 2.13 for the mineral oil.

Regardless the concentrations, the obtained data presented in Table 3 show clearly that the lowest mean number of living larvae was the observed for abamectin, at all time lapses, with significant difference to the other tested insecticides. At 24h post treatment, abamectin was the most effective (mean=8.84) followed by the mineral oil (mean=9.08), then mint oil, phenthoate, and finally azadirachtin, with means 9.28, 9.52, 9.6852 respectively. At 48h post treatment, again abamectin was the most effective (mean=7.24) followed by the mineral oil (mean=7.68), then phenthoate, mint oil and azadirachtin with means 8.16, 8.2 and 8.8 respectively. Also, at 72h post treatment, abamectin was the most effective (mean=4.4) followed by mineral oil, phenthoate and azadirachtin (means = 5.36, 5.08 and 5 respectively), with no significant difference to each other, whereas mint oil was the least effective (mean= 6.52). Regardless the number of days, the results showed that abamectin was significantly more effective (mean number of living larvae = 6.83), followed by mineral oil, phenthoate, azadirachtin then mint oil (mean number of living larvae = 7.37, 7.59, 7.83, 8 respectively).

These results show that there is a correlation between the LC_{50} value and the recommended application rate. More specifically, when the application rate decreases, the insecticide LC_{50} value decreases. The toxicity of abamectin was found to be the highest, with a toxicity index 100% compared to the other insecticides. On the other hand, the relative efficiency showed that abamectin toxicity was 3634-fold greater than the mineral oil toxicity. The application

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rate of azadirachtin was 18 times higher than that of abamectin, but the toxicity of abamectin was 1.8- fold greater than that of azadirachtin. The application rate of phenthoate was close to that of abamectin, but the toxicity of abamectin was 22.7-fold greater than of phenthoate.

Table 2. LC_{50} and LC_{90} values in ppm (ug/ ml) for *P. citrella* larvae treated with the insecticides 72h post treatment, under laboratory conditions.

Insecticide	LC_{50} ppm	LC_{90} ppm	Slope± SE	*Toxicity index (%)	** Relative toxicity (fold)
Abamectin	0.26	0.74	2.78± 0.43	100	3634.04
Azadirachtin	0.46	0.93	4.22± 0.61	56.52	2054.02
Phenthoate	5.91	11.95	4.19± 0.61	4.4	159.87
Mint oil	7.16	102.44	1.11± 0.25	3.63	131.96
Mineral oil	944.85	3784.88	2.13± 0.34	0.03	1

*= index compared with abamectin; **= Relative toxicity compared with mineral oil

Table 3. Mean of living *P. citrella* larvae exposed to insecticides (dipping method bioassay) at 24, 48 and 72h regardless of the series of concentrations

Treatment	*Mean living larvae			Total of mean
	After 24 h.	After 48 h.	After 72 h.	
Abamectin	8.84±0.6a	7.24±1.6 a	4.4±2.9 a	6.83 a
Mineral oil	9.08±0.7 ab	7.68±1.6 ab	5.36±3 b	7.37 b
Phenthoate	9.52±0.3 c	8.16± 1.2 bc	5.08±2.9 b	7.59 bc
Azadirachtin	9.68±0.3 cd	8.8±0.7 d	5±2.8 b	7.83 cd
Mint oil	9.28±0.5 bc	8.2± 1.2 c	6.52± 2 c	8 d
Control	10 d	10 e	9.96 d	9.99 e
Mean **	9.4 c	8.35 b	6.05 a	

* Means with the same letter within a column are not significantly different (Tukey HSD test, at 5%); ** Means with the same letter within a row column are not significantly different (Tukey HSD test, at 5%)

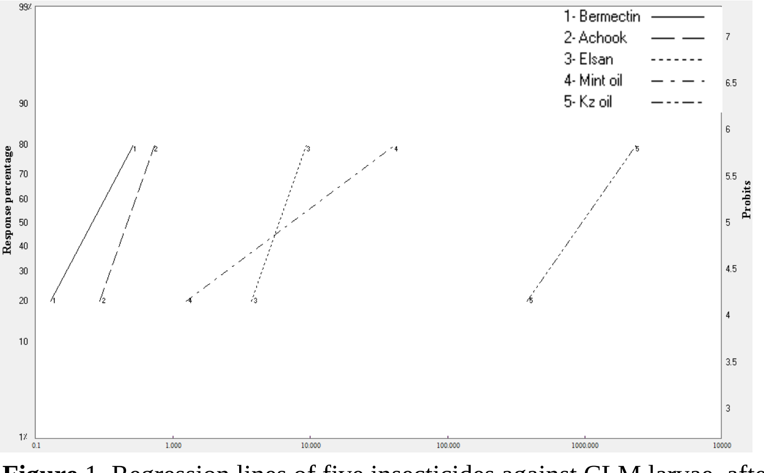

Figure 1. Regression lines of five insecticides against CLM larvae, after 72 h., under laboratory conditions (leaf- dip technique).

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Efficiency of the tested insecticides in controlling *P. citrella*: The field experiment against CLM was conducted on navel orange var. Washington autumn flushes during 2011. The insecticides and respective concentrations applied were: abamectin - 40ml/100L, azadirachtin - 750ml/100L, phenthoate - 45ml/100L, mineral oil - 1.5L/100L and mint oil - 0.5g/100L. The results showed that the mean number of larvae before application varied between 7.5 and 8.5, where no significant difference was observed amongst treatments (Table 4).

At 3 days after the 1st application, abamectin significantly increased the mortality of *P. citrella* larvae, with a 93.06 % reduction rate (Table 5). No significant differences were recorded between the other treatments and the control.

When compared with the unsprayed control, all treatments differed significantly 7 days after the 1st application, with a reduction rate ranging from 31.32 (mint oil) to 84.88% (abamectin). In this evaluation, abamectin caused the highest reduction; azadirachtin provided good control (75%), while mint oil was the least effective. No significant difference was observed in the number of living larvae between mineral oil and phenthoate.

At 5 days after the 2nd application, abamectin and azadirachtin provided significant reduction in the larval population (94.79 and 83.87% respectively). In the same evaluation, mineral oil, phenthoate and mint oil showed significantly lower larval density (50.98, 50.98 and 40.86% of reduction respectively) compared to the control.

At 11 days after the 2nd application, abamectin, mineral oil and phenthoate reduced the larval population by 76.56, 72.43 and 61.4% respectively. No significant difference was observed between azadirachtin and mint oil treatments.

On the other hand, the reduction rate for each treatment, with respect to the reading date, indicated that the reduction rate differences induced by abamectin were not significantly different. For mineral oil, the highest reduction rate was recorded 11 days after the 2nd application, while there were no significant differences between the first three readings. Azadirachtin provided a significant larval population reduction 5 days after the 2nd application, while its effectiveness clearly decreased after 11 days. Finally, the effectiveness of phenthoate increased slowly after each application, whereas mint oil provided larval population reduction immediately after each application, which then decreased.

Discussion

Amiri-Besheli (2009) showed that the efficacy of Tracer (spinosyn) + oil, Runner (methoxyfenozide) + oil and Tondixer (pepper extract) + oil (after 72 hours after, against the 2nd and 3rd stage larvae of *P. citrella*) was 98 ± 3.2, 98 ± 8.1 and 93 ± 5%, respectively, compared to mineral oil alone, Tondixer and Sirinol (insecticidal emulsion)+ oil with death rates of 85 ± 8.3, 81 ± 7.2 and 78.25 ± 8.2%, respectively. Also, the overall death rate after 72 hours was more significant compared to the death rate after 24 and 48 hours. However, the biocides penetrate the tunnels and kill larvae, as reported by Shapiro et al. (1998). George et al. (2017) reported that among six insecticides tested against the 2nd instars of *P. citrella* larvae collected from Nagpur mandarin/ acid lime cultivars, during 2013–2016, abamectin was the most toxic insecticide at the initial year (LC50 values ranged from 20.99 to 49.00ppm), while dimethoate (LC50 of 36.57–160.95ppm) and thiamethoxam (39.90–71.96ppm) were consistently effective against P. citrella larvae for the rest of the period.

On the other hand, Halawa et al. (2007) indicated that an increase of the concentration of peppermint oil from 0.13 to 1% increased larval mortalities of *Sesamia cretica* Led. (Lepidoptera: Noctuidae) from 40 to 93% and the LC50 value was 2.1. However, mortality of target insects may be due to the effect of

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allelochemicals of the family Lamiaceae, such as essential oils which were demonstrated to be behaviorally active against several insect pests (Isman, 2000).

Furthermore, the results of field evaluation indicated that abamectin and azadirachtin provided a significant reduction of the larval population with a reduction percentage of 94.79 and 83.87%, respectively, 5 days after the 2nd application, whereas mineral oil and phenthoate provided a reduction of 72.43 and 61.4% respectively and mint oil reduced the population by 49.8% 3 days after the 1st application. Raga et al. (2001) indicated that ten days is the maximum period of efficacy for all tested insecticides at field conditions. P. citrella larval stage can occupy only 4-5 days and the period from egg to adult is as short as 14 days (Browning et al., 1999). Also, Peña (1994) observed that, two weeks after application, all insecticide treatments had a similar effect as the untreated control on the larvae. Thus, they suggested the use of alternative insecticides in order to prevent the development of pesticide resistance in *P. citrella*. Van de Veire et al. (2002) found that abamectin was much more persistent in spring than in summer. Spray deposits (at the recommended rate of 10 ppm a.i. for leafminer control) were toxic for 1 month in spring, whereas they were no longer toxic after 2 weeks in summer.

Table 4. Efficacy of insecticides against citrus leaf miner, *P. citrella*, (Mean± SE)

Treatments	Mean number of living larvae/ 10 leaves /replicate					Mean
	1 DBFS*	3 DAFS	7 DAFS	5 DASS	11 DASS	
Abamectin	8 ± 0.41 a (b)	0.5 ± 0.29 a (a)	1.25± 0.41 a (a)	0.25± 0.25 a (a)	1± 0.41 a (a)	2.2± 1.4 a
Azadirachtin	7.75± 0.75 a (c)	3.25± 0.48 b (b)	2± 0.41 ab (ab)	0.75± 0.25 a (a)	2.5± 0.29 bc (ab)	3.25± 1.2 b
Mineral oil	8.5± 0.5 a (c)	4± 0.41 b (b)	3± 0.41 b (b)	2.5± 0.29 b (ab)	1.25± 0.25 ab (a)	3.85± 1.2 bc
Phenthoate	8.5±0.29 a (d)	4.5± 0.29 b (c)	3.5± 0.29 b (bc)	2.5± 0.64 b (ab)	1.75± 0.25 abc (a)	4.15± 1.2 c
Mint oil	7.75± 0.75 a (c)	3.5± 0.29 b (a)	5.5± 0.29 c (b)	2.75± 0.25 b (a)	2.75± 0.25 cd (a)	4.45± 0.9 c
Control	7.5± 0.64 a (b)	6.75± 0.25 c (b)	7.75± 0.25 d (b)	4.5± 0.29 c (a)	4± 0.41 d (a)	6.1± 0.8 d
Mean	8± 0.2 (c)	3.75± 0.8 (b)	3.83± 0.9 (b)	2.21± 0.6 (a)	2.21± 0.4 (a)	4± 1.1
F	0.51	34.73	43.89	18.18	12.27	51.62
Significance	0.76	0.0001	0.0001	0.0001	0.0001	0.0001

*DBFS- Day before first spray, DAFS- Days after first spray, DASS- Days after second spray
Means with the same letter within a column are not significantly different; Letters in parentheses = Means with the same letter within a row are not significantly different.

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Table 5. Reduction (%) of *P. citrella* larvae by insecticides under field condition, on navel orange, at El Qualubia Governorate

Treatments	Reduction %				Mean
	3 *DAFS	7 DAFS	5 DASS	11 DASS	
Abamectin	93.06	84.88	94.79	76.56	87.32
Azadirachtin	53.41	75.03	83.87	39.52	62.96
Mineral oil	47.71	65.84	50.98	72.43	59.24
Phenthoate	41.18	60.15	50.98	61.4	53.43
Mint oil	49.82	31.32	40.86	33.47	38.87

*DAFS Days after first spray; DASS- Days after second spray

Raga et al. (2001) reported that the petroleum oil spray residues reduced infestations of *P. citrella* by preventing oviposition, and its effect depended on the concentration of the oil and the time of spraying. However, the efficacy of petroleum-derived spray oils used as oviposition deterrents to control CLM is related to the time of application, the oil dose and the persistence of oil molecules on sprayed surfaces. Low toxicity of the mineral oil may be due to different factors including cuticle properties, ambient temperature and the size and volume of the oil molecules. Amiri-Besheli (2008) reported that emergence of *P. citrella* is very low in spring, thus its control is not necessary before late June, in most citrus growing regions in Northern Iran, but it is really important to protect the new shoots of the young or top grafting citrus trees against infestation by summer and autumn P. citrella generations. On the other hand, azadirachtin showed significant death rates when used against *P. citrella* larvae compared to others, and this may be due to the rapid penetration of neem oil through the surface tissues (Mujica et al., 2000). Our findings were supported by IIHR (2006) who reported that neem oil showed a death rate of 65.9% of CLM larvae, and the results of Cañarte-Bermúdez et al. (2020) who reported a high mortality rate of CLM, caused by a 77.17% aqueous extract of neem oil, which was observed 48 hours after application, suggesting inhibition of feeding.

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Αποτελεσματικότητα ορισμένων εντομοκτόνων στον έλεγχο του φυλλορύκτη των εσπεριδοειδών, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

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ΠΕΡΙΛΗΨΗ

Η τοξικότητα τεσσάρων εμπορικών εντομοκτόνων (ορυκτέλαιο, αζαδιραχτίνη, phenthoate και αμπαμεκτίνη), καθώς και του ελαίου μέντας (μίγμα τερπενοειδών), εναντίον του φυλλορύκτη των εσπεριδοειδών, *Phyllocnistis citrella*, δοκιμάστηκε σε εργαστηριακές συνθήκες με τη μέθοδο εμβάπτισης φύλλων. Τα δεδομένα των βιοδοκιμών έδειξαν ότι η αμπαμεκτίνη ήταν το πιο τοξικό εντομοκτόνο εναντίον των προνυμφών *P. citrella*, ακολουθούμενη από την αζαδιραχτίνη, το phenthoate, το έλαιο μέντας και τέλος το ορυκτέλαιο. Από την άλλη πλευρά, τα αποτελέσματα της αξιολόγησης πεδίου έδειξαν ότι η υψηλότερη θνησιμότητα προνυμφών ήταν 94,79 και 83,87% οφειλόμενη στην αμπαμεκτίνη και την αζαδιραχτίνη αντίστοιχα, πέντε ημέρες μετά τη 2η εφαρμογή. Το ορυκτέλαιο και το phenthoate έδωσαν θνησιμότητα 72,43 και 61,4% αντίστοιχα, 11 ημέρες μετά τη 2η εφαρμογή, και τέλος το έλαιο μέντας, 49,82% τρεις ημέρες μετά την 1η εφαρμογή.

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