

Evaluation of Some Insecticides Against Citrus Flower Moth, *Prays citri* (Millière) (Lepidoptera : Hyponomeutidae) on Lime Trees

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Abstract: A field experiment was conducted to evaluate and compare the effectiveness of two bioinsecticides, *Bacillus thuringiensis kurstaki* and *Beauveria bassiana*, and three chemical insecticides, belonging to two recently introduced and widespread groups, avermectin (emamectin benzoate and spinosad) and oxadiazine (indoxacarb), against the citrus flower moth, *Prays citri* (Millière), on lime trees. The results showed that, based on the general mean reduction percentages of *P. citri* immature stages, there were significant reductions in tree infestation and significant differences among the five insecticides. According to Chi square analysis, these insecticides could be separated into two groups; the first group included indoxacarb, which showed the highest mortality (77.6%), emamectin benzoate (74.9%), *B. thuringiensis kurstaki* (72.5%) and spinosad (71.3%). The second group was *B. bassiana*, which resulted in a low mortality (63.6%). A general trend was observed in the second year (2023), which was also divided into two groups. The first one included indoxacarb (74.1%), spinosad (70.2%) and emamectin benzoate (68.2%). The second group, *B. thuringiensis kurstaki* and *B. bassiana*, showed low mortality (57.2% and 50.1%, respectively). In addition, all the applied insecticides significantly enhanced the yield based on the fruit weight and the number of fruits per tree.

1 Introduction

The citrus flower moth, *Prays citri* Millière (Lepidoptera: Hyponomeutidae), is a serious pest that attacks citrus trees. It is a significant economic insect that infests several citrus species and varieties throughout the Mediterranean area (Silva et al 2006, Conti and Fiscaro 2015, Badr et al 2018). *P. citri* larvae feed on the flower buds, flowers and

newly emerged fruits of lemon trees (Carimi et al, 2000, EFSA, 2008, Badr et al, 2018).

The bioinsecticide *B. thuringiensis* var. *kurstaki*, an entomopathogenic bacterium, is a good example of biological pest management. This bacterium has been demonstrated to be highly successful in controlling specific agricultural insect pests (Dent 2000). Shehata and Nasr (2000) investigated the effects of *B. bassiana* (Bio-fly) on *P. citri* larvae in both laboratory and field conditions.

The new insecticides indoxacarb, spinosad and emamectin benzoate are used to manage lepidopteran pests chemically. Spinosad and emamectin benzoate belong to the new natural insecticide group called Avermectin (Liguori et al 2008). Spinosad has a positive eco-toxicological profile and is compatible with many insect natural enemies. It is particularly powerful against lepidopteran insect pests (Racke 2006, Bonni et al 2020).

Several environmental problems have been caused by the irresponsible use of chemicals, including environmental pollution, an imbalance between pests and their natural enemies, the buildup of pesticide residues in water and on plants, and harm to human health and domestic animals. Therefore, estimating the effectiveness of control agents on lime trees is fairly fruitful. The new philosophy in Integrated Pest Management (IPM) programs utilizes all available and feasible methods to achieve effective pest control while minimizing environmental impacts. The current study aimed to assess the effectiveness of two bioinsecticides, *Bacillus thuringiensis kurstaki* and *Beauveria bassiana*, and three recently introduced and worldwide widespread chemical insecticides, emamectin benzoate, spinosad and indoxacarb against the *P. citri* on lime trees.

2 Materials and Methods

2.1 Insecticide treatments

The field trials were conducted during the spring season of lime plots in Egypt for two consecutive years, 2022 and 2023. The spraying application of five insecticides, i.e., *Bacillus thuringiensis kurstaki* (Dipel®), *Beauveria bassiana* (Biosect®), emamectin benzoate (Speedo®), spinosad (Shirolin®) and indoxacarb (Flax®), was performed to evaluate the efficacy against the citrus flower moth, *P. citri*, on lime trees. **Table 1** contains the tested insecticides in the study, their chemical groups, active ingredients, application rates, mode of action and chemical structures. An area of approximately one feddan of lime trees was selected and divided into six plots, including one for each insecticide and one left untreated (control). Each plot consisted of 12 lime trees. Trees are spaced six meters apart in the row and six meters between trees. All insecticides were applied on the same day. The spraying

was performed using a 20-liter motor sprayer with a 100 Lb/in² pressure. There were four sprays in the spring season (1 April, 20 April, 10 May and 1 June). The evaluation of insecticides against the citrus flower moth on lime trees was applied according to the pest control protocol for the Egyptian Ministry of Agriculture. The number of trees in each plot was 12 to match the number of trees in the orchard for all treatments. The spraying started early (April 1) since the pest infestation rate exceeded 5%. The sample size was increased to 90 plant parts, including the plant parts susceptible to infestation, to determine which of these parts is most infested by the immature stages of this pest.

2.2 Population densities of immature stages of *P. citri*

The population density was determined by the mean number of immature stages of *P. citri* (eggs, larvae and pupae) in 30 lime flower buds, 30 flowers and 30 newly formed fruits. The reduction percentages of immature stages of *P. citri* were evaluated after 3, 5, 7 and 10 days of spraying. The reduction in the mean number of immature stages was an indicator of pesticide effectiveness.

2.3 Assessment of the yield crop

Lime fruits were picked on 1 August in both seasons. The number and weight of fruits produced by the wooden square (25cm×25cm) were recorded. The average weight of yield and the number of fruits for each treatment were calculated.

2.4 Data analysis

The reduction percentage in immature stages of *P. citri* due to treatments was calculated according to the Henderson and Tilton (1955) equation as follows:

$$\% \text{ Reduction} = \left[1 - \frac{Ta \times Cb}{Tb \times Ca} \right] \times 100$$

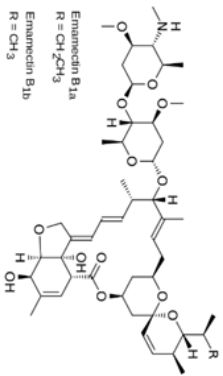
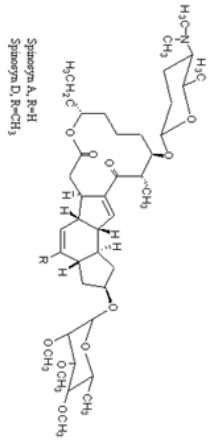
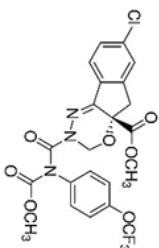
Where: Ta is the number of insect stages after treatment.

Tb is the number of insect stages before treatment.

Ca is the number of insect stages in the control after treatment.

Cb is the number of insect stages in the control before treatment.

Table 1. List and description of the tested insecticides

Insecticide group	Active ingredient	Trade name	Rate of spray/feedan (g or ml)	Mode of action	Chemical structure
Bacteria	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	Dipel 6.4% DF	200 g/feedan	Microbial disruptors of insect midgut membranes	--
Fungi	<i>Beauveria bassiana</i>	Biosect mg/32×10 ⁶ WP	200 g/100 L. water	Control insects by growing on them secreting enzymes that weaken the insect's outer coat	--
Avermectin	Emamectine benzoate	Speedo 5.7% WG	80 g/feedan	Glutamate-gated chloride channel (GluCl) allosteric modulators	 Emamectin B1a R = CH₂CH₃ Emamectin B1b R = CH₃
Avermectin	Spinosad	Shirolin 10% WDG	150 g/feedan	Nicotinic acetylcholine receptor (nAChR) allosteric modulators-Site I	 Spinosyn A, R=H Spinosyn D, R=CH₃
Oxadiazine	Indoxacarb	Flax 15% SC	25 cm ³ /100 L. water	Voltage Sodium channel blockers	

The χ^2 method of chi-square analysis was used to test the homogeneity of control applications (Snedecor and Cochran 1989).

$$\chi^2 = \frac{\sum(ai \times pi) - C1 \times \bar{P}i}{\bar{P}i \times \bar{q}i}$$

Whereas: $\bar{P}i = C1/G$ $\bar{q}i = 1 - \bar{P}i$,
C1= Total of % reduction immature stages,
G = Total of samples.

All data are the mean of six determinations. One-way ANOVA was applied for the analysis of variance. LSD test at a p -level of 0.01 was used to determine the significance of differences among the means using CoStat Statistical Software (2005) version 6.451.

3 Results and Discussion

Evaluation of the five tested insecticides against immature stages of *P. citri* on lime trees in two years, 2022 and 2023, was recorded as follows:

3.1 Population densities of *P. citri* immature stages on lime trees

The effectiveness of the tested insecticides against the immature stages of *P. citri* on lime trees during the spring season in 2022 was recorded in **Table 2**. According to the general mean reduction percentages of the *P. citri* population, it is clear that indoxacarb was the most potent one, followed by emamectin benzoate, *B. thuringiensis* and Spinosad (1.7, 2.0, 2.0 and 2.1 immature stages/30 samples, respectively). The least effective one was *B. bassiana* with a population of 2.6, compared with the control of 8.6 immature stages/30 samples. Thus, all insecticides significantly reduced the immature stages of *P. citri* under field conditions compared to the control. The "F" value between treatments was 131.0, highly significant at 0.01% and L.S.D. 0.63 for the immature stage.

Data in **Table 3** indicated significant differences between the five treatments, whereas χ^2 was 22.2, significant at the 0.01 level. These insecticides could be classified into two groups. The first group (a) contains indoxacarb, emamectin benzoate, *B. thuringiensis*, and spinosad, with general mean percentages of reduction in immature stages of 77.6, 74.9, 72.5 and 71.3%, respectively. The second group (b) was *B. bassiana*, resulting in a low mortality (63.6%).

The effectiveness of the tested insecticides against *P. citri* during the spring season in the second year, 2023, was recorded in **Table 4**. According to the general mean of reduction percentages in the *P. citri* population, it shows again that indoxacarb was the most potent, followed by spinosad, emamectin benzoate and *B. thuringiensis* as 2.0, 2.4, 2.6 and 3.3 immature stages/30 samples, respectively. The least effective was *B. bassiana*, with 4.1 immature stages per 30 samples compared to the control (4.1 and 8.9 immature stages per 30 samples, respectively). Thus, all insecticides significantly reduced the immature stages of *P. citri* under field conditions compared to the control. Meanwhile, in the immature stage, the "F" value between treatments is 117.8, which is highly significant at the 0.01% level and L.S.D. 0.64.

The data in **Table 5** showed significant differences between the five treatments, with $\chi^2 = 30.1$, significant at the 0.01 level. The insecticides were divided into two groups. 1st group (a) was indoxacarb, followed by spinosad and emamectin benzoate, with a general mean of population reduction percentages of 74.1, 70.2 and 68.2%, respectively. The 2nd (b) group contains *B. thuringiensis kurstaki* and *B. bassiana*, which showed a low mortality of 57.2% and 50.1%, respectively, compared to the control. The slight difference in the categories of the tested insecticides against *P. citri* during the first and second years could be attributed to changes of the main weather factors, where the daily temperature average was 24.7 °C and 23.6 °C, while the relative humidity average (R.H.%) was 53.1% and 53.5% during the spring seasons of 2022 and 2023, respectively.

3.2 Assessment of the yield crop

In addition to the effective pest control, the applied insecticides significantly enhanced the yield and the number of lime fruits per tree at the end of the season; the results were tabulated in **Table 6**. During the spring season of the first year of 2022, the untreated (control) trees produced 379.4±9.3 lime fruits/tree while in the experimental plots with the insecticides indoxacarb, emamectin benzoate, spinosad, *B. thuringiensis* and *B. bassiana*, the estimated number was 1059.2±29.3, 1014.4±29.6, 953.6±29.8, 898.4±13.2 and 841.6±33.7 lime fruits/tree, respectively. The F value between treatments was 6.25, highly significant at the 1% level, and L.S.D. was 72.4 fruits/tree. In the second year (2023), the estimated number of lime fruits in the control plot was 564.8±8.5 lime fruits/tree. While in the experimental plots of the same insecticides, the estimated numbers of lime fruits were 1041.6±23.1, 963.2±18.3, 948.8±32.9, 926.4±15.9, and 913.6±18.5 lime

fruits/tree, respectively. Whereas the F value between treatments was 4.1, significant at the level of 0.01% and L.S.D. was 61.2 fruit/trees.

The present results are in agreement with some previous reports. Shehata and Nasr (2000) reported that *B. thuringiensis* at a dose of 2 mL/L decreased the larval infestation of *P. citri* by 89.8–93.4%, while *B. bassiana* at a dose of 4 mL/L. decreased the infestation of eggs, larvae and pupae only by 65.8, 68.5, and 72.6%, respectively. Sabbour et al (2012) reported reducing pest infestation of *P. oleae* trees by *B. bassiana*, with enhancing the yield from 2319 to reach 2998 kg/feddan. Pankaj (2020) found that the greatest decrease in the populations of *Brevicoryne brassicae* and *Pieris*

canidia on cauliflower was observed with 45% spinosad application (82.70 to 89.94%), followed by 5% emamectin benzoate (80.15 to 83.28%) and 14.5% indoxacarb (74.60 to 72.87%). Divekar et al (2023) recorded a significant decrease in *Plutella xylostella* following the application of indoxacarb, spinosad, and emamectin benzoate, which were 89.91, 86.72 and 74.88, respectively. When compared to untreated, all of the insecticides were found to be effective in suppressing these pests. Moustafa et al (2023) found that emamectin benzoate was the most effective insecticide which reduced *Tuta absoluta* population by 78.05 and 87.11%, followed by indoxacarb (77.01% and 79.52%) and spinosad (69.65% and 80.44%) in two different cites.

Table 2. Efficacy of five tested insecticides in reduction of *Prays citri* immature stages on lime trees during spring season (2022)

Treatments	Mean no. $\pm$ S.E. immature stages of <i>Prays citri</i> during spring season in first year (2022)							
	Flower buds		Flowers		Newly formed fruits		General mean	
	Immature stages	% Reduction	Immature stages	% Reduction	Immature stages	% Reduction	Immature stages	% Reduction
<i>Bacillus thuringiensis kurstaki</i> (Dipel)	2.5 $\pm$ 0.2bc	72.4	2.3 $\pm$ 0.1bc	73.7	1.3 $\pm$ 0.2bc	71.5	2.0 $\pm$ 0.3bc	72.5
<i>Beauveria bassiana</i> (Biosect)	3.1 $\pm$ 0.2b	64.7	3.0 $\pm$ 0.1b	65.6	1.6 $\pm$ 0.1b	60.6	2.6 $\pm$ 0.5b	63.6
Emamectine benzoate (Speedo)	2.5 $\pm$ 0.2bc	73.4	2.4 $\pm$ 0.2bc	77.4	1.3 $\pm$ 0.2bc	74.0	2.0 $\pm$ 0.4bc	74.9
Spinosad (Shirolin)	2.5 $\pm$ 0.1bc	73.8	2.4 $\pm$ 0.2bc	75.3	1.5 $\pm$ 0.1bc	64.7	2.1 $\pm$ 0.3bc	71.3
Indoxacarb (Flax)	2.2 $\pm$ 0.3c	76.8	2.0 $\pm$ 0.2c	78.2	1.0 $\pm$ 0.1c	77.7	1.7 $\pm$ 0.3c	77.6
Untreated (Control)	9.8 $\pm$ 0.7a	--	10.8 $\pm$ 0.7a	--	5.3 $\pm$ 0.5a	--	8.6 $\pm$ 1.7a	--
"F" value between treatments=	131.0*** highly sig.							
L.S.D. at 0.01%=	0.63 immature stage							

Table 3. Chi square (χ^2) analysis for the changes of control applications by the immature stages of *Prays citri* on lime trees during spring season in 2022

Treatments	% Corrected (ai)	R 100%	Pi (ai/R)	ai * Pi	Group
Indoxacarb (Flax)	77.6	100	0.776	60.2	a
Emamectine benzoate (Speedo)	74.9	100	0.749	56.1	
<i>B. thuringiensis kurstaki</i> (Dipel)	72.5	100	0.725	52.6	
Spinosad (Shirolin)	71.3	100	0.713	50.8	
<i>B. bassiana</i> (Biosect)	63.6	100	0.636	40.4	b
Total	C1= 359.9	G= 500	Pi= 3.599	$\Sigma ai*Pi= 260.1$	

$\chi^2= 22.2$ sig. at 0.01 (value of chi-square tabular at degree of freedom n-1 and a significance level at 0.01= 13.28)

Table 4. Efficacy of five tested insecticides in reduction of *Prays citri* immature stages lime trees during spring season (2023)

Treatments	Mean no. $\pm$ S.E. immature stages of <i>Prays citri</i> during spring season in second year (2023)							
	Flower buds		Flowers		Newly formed fruits		General mean	
	Immature stages	% Reduction	Immature stages	% Reduction	Immature stages	% Reduction	Immature stages	% Reduction
<i>Bacillus thuringiensis kurstaki</i> (Dipel)	3.9 $\pm$ 0.2c	58.6	4.2 $\pm$ 0.1b	58.9	1.9 $\pm$ 0.1c	54.1	3.3 $\pm$ 0.8c	57.2
<i>Beauveria bassiana</i> (Biosect)	5.3 $\pm$ 0.4b	47.9	4.7 $\pm$ 0.2b	55.6	2.4 $\pm$ 0.2b	46.7	4.1 $\pm$ 0.9b	50.1
Emamectine benzoate (Speedo)	3.3 $\pm$ 0.2cd	66.5	3.2 $\pm$ 0.2c	69.8	1.4 $\pm$ 0.1d	68.2	2.6 $\pm$ 0.6cd	68.2
Spinosad (Shirolin)	2.8 $\pm$ 0.2de	72.9	2.7 $\pm$ 0.3cd	75.3	1.6 $\pm$ 0.1cd	62.4	2.4 $\pm$ 0.3cd	70.2
Indoxacarb (Flax)	2.4 $\pm$ 0.3e	74.1	2.2 $\pm$ 0.2d	77.4	1.3 $\pm$ 0.1d	71.1	2.0 $\pm$ 0.3d	74.1
Untreated (Control)	10.3 $\pm$ 0.7a	--	11.4 $\pm$ 0.8a	--	5.0 $\pm$ 0.4a	--	8.9 $\pm$ 2.0a	--
"F" value between treatments=	117.8*** highly sig.							
L.S.D. at 0.01%=	0.64 immature stage							

Table 5. Chi square (χ^2) analysis for the changes of control applications by the immature stages of *Prays citri* on lime trees during spring season in 2023

Treatments	% Corrected (ai)	R 100%	Pi (ai/R)	ai * Pi	Group
Indoxacarb (Flax)	74.1	100	0.741	54.9	a
Spinosad (Shirolin)	70.2	100	0.702	49.3	
Emamectine benzoate (Speedo)	68.2	100	0.682	46.5	
<i>B. thuringiensis kurstaki</i> (Dipel)	57.2	100	0.572	32.7	b
<i>B. bassiana</i> (Biosect)	50.1	100	0.501	25.1	
Total	C1= 319.8	G= 500	Pi= 3.198	$\Sigma ai*Pi= 208.5$	

$\chi^2= 30.1$ sig. at 0.01 (value of chi-square tabular at degree of freedom n-1 and a significance level at 0.01= 13.28)

Table 6. The lime yield per tree after treating *P. citri* by the examined insecticides

Control applications	Numbers and weighted fruits per/ one lime tree					
	Spring season, 2022			Spring season, 2023		
	Mean no. of lime fruits/ Tree	Mean weight/one fruit in gram	Weight lime fruits Kg/Tree	Mean no. of lime fruits/ Tree	Mean weight/one fruit in gram	Weight lime fruits Kg/Tree
<i>B. thuringiensis kurstaki</i> (Dipel)	898.4±13.2ab	20.2±0.3	18.148	926.4±15.9a	24.4±0.8	22.604
<i>B. bassiana</i> (Biosect)	841.6±33.7ab	19.8±0.6	16.664	913.6±18.5ab	24.8±0.8	22.657
Emamectine benzoate (Speedo)	1014.4±29.6a	19.5±0.6	19.781	963.2±18.3a	25.0±0.6	24.080
Spinosad (Shirolin)	953.6±29.8a	20.4±0.5	19.453	948.8±32.9a	25.6±0.3	24.289
Indoxacarb (Flax)	1059.2±29.3a	19.1±0.6	20.231	1041.6±23.1a	24.8±0.1	25.831
Untreated (Control)	379.4±9.3c	19.6±0.3	7.436	564.8±8.5c	23.4±0.6	13.216
"F" value between treatments=	6.25*** highly sig.			4.1** sig.		
L.S.D. at 0.01=	72.4 fruit at 0.01			61.2 fruit at 0.01		

4 Conclusion

The current research examined the effectiveness of two bioinsecticides, *Bacillus thuringiensis kurstaki* and *Beauveria bassiana*, and three recently introduced and widespread chemical insecticides, emamectin benzoate, spinosad and indoxacarb, to manage the citrus flower moth in lime orchards under field conditions. Additionally, the used insecticides increased both the fruit weight and fruit number per tree significantly. Thus, they can be involved in IPM pest control programs to reduce the excessive use of pesticides.

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