

TOXICITY OF SOME CONVENTIONAL AND NONCONVENTIONAL INSECTICIDES AGAINST COTTON LEFWORM, *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The ovicidal action of three novel compounds (emamectin benzoate, methoxyfenozide, pyridalyl) and conventional insecticide (methomyl) against (0-24, 24-48 and 48-72 hours age) eggs of *Spodoptera littoralis* was assessed. The toxic activity of emamectin benzoate, methoxyfenozide, pyridalyl and methomyl against 4th instar larvae of laboratory and field strains of *S. littoralis* were carried out. Also, the build up of resistance of each tested compound in the field strain was calculated. The joint toxic action of emamectin benzoate, methoxyfenozide and pyridalyl with tested insecticides was also studied. The obtained data revealed that emamectin benzoate gave an ovicidal activity higher than methomyl, methoxyfenozide and pyridalyl. Also, emamectin benzoate was a superior potent compound against 4th instar larvae of *S. littoralis* followed by methoxyfenozide, with LC₅₀ values were 0.007 and 0.932 ppm after 72 hrs, while methomyl was the least toxic one, LC₅₀ value was 95.60 ppm. The build up of resistance of tested compounds indicated that the resistance ratio of methoxyfenozide and methomyl were 5.9 and 5.8 fold as the laboratory strain, while the resistance ratio of emamectin benzoate and pyridalyl were 2.7 and 1.9 fold as the laboratory strain. In respect with the joint toxic action, the all mixtures of the tested insecticides showed highly antagonisms effect as calculated by the co-toxicity factors. The present study suggests that emamectin benzoate is very effective in the control of *S. littoralis*.

Keywords: Cotton leafworm, ovicidal activity, larvicidal activity, joint toxic action, insecticides.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd) is one of the major insect pests that cause a considerable damage to many of the important vegetables and field crop in Egypt. The rising consumption of currently used insecticides in developing countries has led to a number of problems such as insect resistance, environmental pollution and the health hazards associated with pesticide residues. It is necessary to complement our reliance on synthetic pesticides with less hazardous, safe and biodegradable substitutes. Emamectin benzoate is a modified isolation of the soil microorganism, *Streptomyces avermitilis*. It affects the nervous system of arthropods by increasing chloride ion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis. Also, it affects on GABA and glutamate-gated chloride channel agonist (Dunbar *et al.*, 1998).

Methoxyfenozide is classified as a diacylhydrazine insecticide. It acts as ecdysone agonists with enormous potential for development as insect

specific control agents with little or no effect on non-target species (Dhadialla and Carlson, 1998). Also, it provides effective control of a wide range of lepidopteran insects. The chemical upon absorption into the haemolymph of the insect, binds to the ecdysone receptor which initiates the moulting process. As the normal process disrupted, the insects prevented from shedding its old cuticle. The larvae die of dehydration and starvation within 2-5 days (Kumar and Santharam, 2008).

Pyridalyl exhibit high insecticidal activity against Lepidoptera (Sakamoto *et al.*, 2003). It posses a certain type of toxicity for insect cells, it inhibited the cell growth (Satio *et al.*, 2006).

The main target of this study is to compare the insecticidal efficacy of novel insecticides (emamectin benzoate, methoxyfenozide, and pyridalyl) with the conventional insecticide (methomyl) through assessment of ovicidal and larvicidal effects on laboratory and field strains of *S. littoralis* and to show the joint action of mixed emamectin benzoate with different groups of insecticides on the laboratory strain under laboratory conditions.

MATERIALS AND METHODS

1. Insect rearing:

- a. The laboratory strain of *Spodoptera littoralis* was provided from the division of cotton leafworm of Plant Protection Research Institute, Dokki, Egypt. The laboratory strain was reared in the laboratory of Sakha Agricultural Research Station on castor bean leaves. The egg masses were collected daily and they were hatched on the oleander leaves. The larvae were transferred to fresh castor oil leaves. The colony was kept at a temperature of $25 \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ relative humidity (El-Defrawi *et al.*, 1964).
- b. The field strain was obtained by the collection of the egg masses from cotton plants at the Sakha Agricultural Research Station before the start of the chemical control programme. The eggs were allowed to hatch and the insect rearing continued for one generation according to the previous method.

2. Insecticides used:

Emamectin benzoate (Proclaim 5% SG) was produced by Syngenta Co., methoxyfenozide (Runner 24% SC) was produced by Dow AgroSciences Co., pyridalyl (Pleo 50% EC) was produced by Sumitomo Chemical Co., and methomyl (Lannate 90% SP) was produced by DuPont Co.

3. Bioassay studies:

a. Ovicidal activity:

Freshly deposited egg-masses from a laboratory strain of *S. littoralis* were collected daily for three successive days to represent egg-masses having the ages (0-24 hrs) for these laid in the third day, (24-48 hrs) in the second day and (48-72 hrs) in the first day, which were all treated at the third day. The egg-masses were dipped for 10 seconds in different water dilutions of the tested compounds. The untreated eggs were dipped in water. Three replicates were used with each concentration. Treated and untreated egg-

masses were left to dry, placed in plastic cups and kept at 25±2°C, 65±5% relative humidity. Hatched and unhatched eggs were examined 2-4 days after the test (after maximum hatch in the control check). The percent of unhatchability for each treatment adjusted by compared with the control treatment by Abbott's formula (1925) and the LC₅₀ values were calculated according to (Finney, 1971) using « LC-p Line » software.

b. Larvicidal activity:

Leaf dip was adopted to estimate the toxicity of methomyl, pyridalyl, emamectin benzoate and methoxyfenozide to *S. littoralis* larvae. Castor bean leaves were dipped for 10 seconds in aqueous dilution of the mentioned formulated toxicants. The treated leaves were offered to the 4th larval instar of field and laboratory strains, after being left to dry. Serial concentrations of each toxicant were used for the establishment of the LC-P Line. Five replicates with 10 larvae were used for each concentration. The larvae were allowed to feed on treated leaves for 24 hours. Meanwhile control larvae were feed on untreated castor leaves for 24 hours. Mortality counts were recorded after 24 hours post-treatment for methomyl and pyridalyl and after 24, 48 and 72 hours post-treatment for emamectin benzoate and methoxyfenozide according to (Anonymous, 1967). Mortality data were corrected by Abbott's formula (1925) and the LC₅₀ values were calculated according to (Finney, 1971) using « LC-p Line » software.

c. Joint toxic action:

To test the joint action effect of paired combinations of the tested pesticides, mixtures of tested insecticides were prepared by blending insecticides in proportion to their toxicity equivalent portions of one insecticide with the complementary portions of the other to give the expected LC₅₀. Leaves of castor bean were immersed in each pesticide mixture for 10 seconds and mortality percentages were assessed after 1, 2 and 3 days post-treatment. Each pesticide formulation was also tested alone at LC₂₅ level. The joint toxic action of the different pairs of insecticides was evaluated by the following equation (Mansour *et al.*, 1966):

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

The factor was used to differentiate the results into 3 categories. A positive factor (+20 or more) meant potentiation, a negative factor (-20 or more) meant antagonism, and intermediate value between (-20 and +20) was considered only an additive effect.

RESULTS AND DISCUSSION

One of our objectives in this investigation was the evaluation of the ovicidal action of emamectin benzoate compared with another compounds from different chemical groups such as; methoxyfenozide, methomyl and pyridalyl against 0-24 hours, 24-48 hours and 48-72 hours old eggs of *S. littoralis*. According to the results on (0-24, 24-48 and 48-72 hours old) eggs

masses (Tables 1, 2 and 3) data of hatchability showed that emamectin benzoate was superior an ovicidal activity where the LC₅₀ values were 10.94, 11.90 and 12.25 ppm followed by methomyl where LC₅₀ values were 27.33, 41.24 and 83.26 ppm. Methoxyfenozide and pyridalyl was the lowest effect (LC₅₀ values were 250.46, 188.33 & 179.82 and 206.30, 202.54 and 175.10 ppm, respectively). These findings are in full agreement with Hassan (2009), who found that three day old eggs are more affected than that of one or two days old in case of indoxacarb and spinetoram while the reverse was in the case of methoxyfenozide. Moreover, Kotb (2011) mentioned that the emamectin benzoate at concentrations of 3.75, 7.50, 15.0 and 30.0 ppm caused 35.7, 50.0, 53.9 and 66.9 % mortality of treated eggs, respectively. In addition, the residues of the same tested concentrations of emamectin benzoate against egg masses caused 100 % mortality for all neonates (the newly hatched larval from those treated eggs). Also, Amer *et al.* (2012) found that the ovicidal action of the two tested compounds (emamectin benzoate and pyridalyl) against different egg ages of the pink bollworm could be ascendingly arranged as follows: 1, 2, 3 and 4 day old. The corresponding LC₅₀ values were, 659.8, 1473.4, 1652.7 and 1788.4 ppm, respectively for emamectin benzoate and 72.1, 362.8, 817.3 and 7772.4 ppm, ppm, respectively for pyridalyl. It is obvious that the ovicidal activity of the two tested products was increased with egg ages. On the other hand, the ovicidal action of the tested compounds was differed with the different egg ages.

Table (1): Ovicidal activity of emamectin benzoate, methoxyfenozide, methomyl and pyridalyl on *S. littoralis* eggs (age, 0-24 hrs).

Insecticides	LC ₅₀ (ppm)	95% FL ^a of LC ₅₀		Slope ± SE
		Lower	Upper	
Emamectin benzoate	10.94	8.55	13.31	1.39 ± 0.18
Methoxyfenozide	250.46	169.42	356.75	0.82 ± 0.23
Methomyl	27.33	24.85	29.74	2.12 ± 0.08
Pyridalyl	206.30	180.69	231.14	2.44 ± 0.25

FL^a: Fiducial limit.

Table (2): Ovicidal activity of emamectin benzoate, methoxyfenozide, methomyl and pyridalyl on *S. littoralis* eggs (age, 24-48 hrs).

Insecticides	LC ₅₀ (ppm)	95% FL ^a of LC ₅₀		Slope ± SE
		Lower	Upper	
Emamectin benzoate	11.90	10.09	13.74	1.94 ± 0.19
Methoxyfenozide	188.33	165.49	209.96	2.69 ± 0.26
Methomyl	41.24	35.72	46.75	1.40 ± 0.08
Pyridalyl	202.54	175.38	228.64	2.27 ± 0.25

FL^a: Fiducial limit.

Table (3): Ovicidal activity of emamectin benzoate, methoxyfenozide, methomyl and pyridalyl on *S. littoralis* eggs (age, 48-72 hrs).

Insecticides	LC ₅₀ (ppm)	95% FL ^a of LC ₅₀		Slope ± SE
		Lower	Upper	
Emamectin benzoate	12.25	10.07	14.48	1.61 ± 0.18
Methoxyfenozide	179.82	160.29	198.32	3.17 ± 0.27
Methomyl	83.26	68.19	101.58	1.37 ± 0.14
Pyridalyl	175.10	146.99	200.71	2.13 ± 0.24

FL^a: Fiducial limit

Concerning the effect of the aforementioned insecticides against the 4th instar larvae of lab. and field strains. Data illustrated in Table (4) showed that emamectin benzoate was the high toxic against 4th instar larvae of laboratory strain. The LC₅₀ values were 0.019 and 0.007 ppm after 48 and 72 hrs, respectively. While, Pyridalyl was the second one, the LC₅₀ value was 18.679 ppm after 24 hrs. Whereas, methoxyfenozide was the third one, its LC₅₀ values were 20.576 and 0.932 ppm after 48 and 72 hrs, respectively. Methomyl was the fourth one, its LC₅₀ value was 95.604 ppm after 24 hrs.

Also, data in Table (5) revealed that emamectin benzoate was the most toxic one against 4th instar larvae of field strain. The LC₅₀ values were 0.039 and 0.020 ppm after 48 and 72 hrs, respectively. While, methoxyfenozide was the second one, the LC₅₀ values were 33.313 and 5.564 ppm after 48 and 72 hrs, respectively. Whereas, pyridalyl was the third one, its LC₅₀ value was 35.549 ppm after 24 hrs. While, methomyl was the fourth one, its LC₅₀ value was 562.073 ppm after 24 hrs. These results are in agreement with those obtained by Adamczyk *et al.* (1999), they reported that novel insecticides chlorfenapyr, methoxyfenozide, spinosad and tebufenozide were more toxic than conventional insecticide thiodicarb toward the third instar larvae of fall armyworm, *S. frugiperda* using diet bioassay. Moreover, Argentine *et al.* (2002) found that the emamectin benzoate was consistently the most toxic insecticide; it was 20-to 64.240-times more toxic than chlorfenapyr, fipronil and tebufenozide. Moreover, El-Aw (2003), mentioned that the bioinsecticide (proclaim) against 2nd instar larvae was more toxic than the 4th instar larvae of *S. littoralis*. Increases in larval mortalities of both instars were clearly observed for 120 hrs posttreatment. Mortalities that recorded at 120 hrs posttreatment with low proclaim concentration (5 ppm) were 70% and 50% for the 2nd and 4th instars, respectively. It is clear that larval mortalities were increased not only by increase proclaim concentrations but also by increase in days posttreatment. Pineda *et al.* (2004), who reported that spinosad and methoxyfenozide were potentially potent compounds for the control of *S. littoralis*. Ahmad *et al.* (2005), mentioned that emamectin benzoate proved to be the best followed by lufenuron, spinosad and indoxacarb, respectively in their time-oriented mortality at three concentration levels tested. Satio *et al.* (2005) mentioned that pyridalyl caused 100 % mortality in the 4th instar larvae of *S. litura* at concentration of 500 mg / L. Also, El-Aw (2006), found that the LC₅₀ values of emamectin

benzoate and spinosad were decreased, in general, by increasing the posttreatment period of times. Abdu-Allah (2010) found that emamectin benzoate had the best toxicity profile in all tested bioassay than spinetoram. In another study, Dahi *et al.* (2011) found that pyridalyl is more effective on 4th instar larvae due to the larval mortality percent estimated by 78.0 %. Also, Kotb (2011) mentioned that emamectin benzoate was more toxic against the 2nd, 3rd and 4th instar larvae of cotton leafworm than lufenuron and flufenoxuron.

Data presented in Table (6) showed that the level of resistance ratio of the 4th instar larvae of *S. littoralis* laboratory and field strains against tested insecticides. The level of resistance on the field strain for methoxyfenozide and methomyl were (1.6, 5.9) and 5.8 fold as the laboratory strain, these results indicated that the field strain became tolerant. On the other hand, the resistance level of emamectin benzoate and pyridalyl on the field strain were (1.9, 2.7) and 1.9 fold as the laboratory strain. These results showed that all tested insecticides are still effective in the control of *S. littoralis*.

Table (4): LC₅₀ values of emamectin benzoate, methoxyfenozide, methomyl and pyridalyl against 4th instar larvae of Lab. strain of *S. littoralis*.

Insecticides	Times (hrs)	LC ₅₀ (ppm)	95% FL of LC ₅₀		Slope ± SE	X ² **
			Lower	Upper		
Emamectin benzoate	48	0.019	0.015	0.036	1.58 ± 0.37	2.43
	72	0.007	0.005	0.009	1.60 ± 0.33	5.55
Methoxyfenozide	48	20.576	8.824	45.729	0.51 ± 0.122	1.95
	72	0.932	0.028	2.839	0.60 ± 0.172	2.24
Methomyl	24	95.604	75.532	123.667	2.25 ± 0.28	3.91
Pyridalyl	24	18.679	12.614	26.005	1.38 ± 0.22	9.43

FL : Fiducial limit. **X² : Chi square.

Table (5): LC₅₀ values of emamectin benzoate, methoxyfenozide, methomyl and pyridalyl against 4th instar larvae of field Strain of *S. littoralis*.

Insecticides	Times (hrs)	LC ₅₀ (ppm)	95% F.L. of LC ₅₀		Slope ± SE	X ² **
			Lower	Upper		
Emamectin benzoate	48	0.039	0.033	0.058	3.52 ± 0.84	1.54
	72	0.020	0.016	0.022	3.49 ± 0.75	1.07
Methoxyfenozide	48	33.313	21.586	49.394	1.11 ± 0.17	1.02
	72	5.564	1.625	10.676	0.09 ± 0.17	1.50
Methomyl	24	562.073	340.521	1216.972	0.92 ± 0.15	0.79
Pyridalyl	24	35.549	21.467	56.018	1.13 ± 0.25	2.02

*FL: Fiducial limit. **X: Chi square.

Table (6): Resistance ratio of 4th instar larvae of *S. littoralis* laboratory and field strains against tested insecticides.

Insecticides	Strain	Time (hrs)	LC ₅₀	95 % FL ^a of LC ₅₀		Slope ± SE	RR ^b
				Lower	Upper		
Emamectin benzoate	Lab.	48	0.020	0.014	0.036	1.58 ± 0.37	1.9
	Field	48	0.039	0.033	0.058	3.52 ± 0.84	
	Lab.	72	0.007	0.005	0.009	1.60 ± 0.33	2.7
	Field	72	0.020	0.016	0.022	3.49 ± 0.75	
Methoxyfenozide	Lab.	48	20.576	8.824	45.729	0.51 ± 0.12	1.6
	Field	48	33.313	21.586	49.394	1.11 ± 0.17	
	Lab.	72	0.932	0.028	2.839	0.60 ± 0.17	5.9
	Field	72	5.564	1.625	10.676	0.90 ± 0.17	
Methomyl	Lab.	24	95.604	75.532	123.667	2.25 ± 0.28	5.8
	Field	24	562.073	340.521	1216.972	0.92 ± 0.15	
Pyridalyl	Lab.	24	18.679	12.615	26.005	1.38 ± 0.22	1.9
	Field	24	35.549	21.467	56.018	1.13 ± 0.25	

FL^a : Fiducial limit. R.R^b: resistance ratio = LC₅₀ of field strain / LC₅₀ of laboratory strain

Results in Table (7) showed the joint toxic action of binary insecticides mixtures on the 4th instar larvae of cotton leafworm after 24, 48 and 72 hrs post-treatment. Generally, all mixtures of the tested insecticides showed highly antagonisms effect except emamectin benzoate + methoxyfenozide after 24 h of exposure was potentiation effect. The calculated Co-toxicity factors (CTF) of emamectin benzoate (LC₂₅) with methoxyfenozide, methomyl and pyridalyl mixture were -65.52, -83.33 and -60.00 after 72 hrs of exposure. The Co-toxicity factors of the methoxyfenozide (LC₂₅) with methomyl and pyridalyl mixture were -65.53 and -57.16 after 72 hrs of treatment. On the other hand, The Co-toxicity factors of the methomyl (LC₂₅) with pyridalyl mixture were -79.6 after 72 hrs of exposure. From these data, the emamectin benzoate, methoxyfenozide, methomyl and pyridalyl mixtures should not be used. These results are confirmed by Kotb (2011), who found that the mixtures of emamectin benzoate and lufenuron or flufenoxuron showed antagonistic effects.

Overall, our results show the emamectin benzoate may be considered a valuable tool for the control of cotton leafworm as a component of IPM programme.

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تم مقارنة الفعل السام لثلاثة مركبات حديثة وهي الإيمامكتين بنزوات، الميثوكسيفينوزايد والبيريداليل مع مركب تقليدي وهو الميثوميل علي ثلاثة أعمار من بيض دودة ورق القطن وهما ٢٤، ٤٨، ٧٢ ساعة. وقد أوضحت النتائج أن مبيد الإيمامكتين بنزوات هو الأكثر فعالية مقارنة بالميثوميل، الميثوكسيفينوزايد والبيريداليل علي الثلاثة أعمار لبيض دودة ورق القطن. كما تم دراسة سمية المبيدات الحديثة وكذلك التقليدية علي يرقات العمر البرقي الرابع للسلالة المعملية والحقلية لدودة ورق القطن. وتشير النتائج إلي أن الإيمامكتين بنزوات هو الأكثر فعالية علي السلالة المعملية يليه الميثوكسيفينوزايد بناءً علي التركيز القاتل لـ ٥٠% من الأفراد المعاملة حيث كانت ٠,٠٠٧، ٠,٩٣٢ جزء في المليون بعد ٧٢ ساعة من المعاملة وكان أقل هذه المركبات تأثيراً الميثوميل حيث كانت قيمة التركيز القاتل لـ ٥٠% من الأفراد المعاملة ٩٥,٦٠ جزء في المليون علي التوالي. وكذلك تم حساب معامل المقاومة التي تكونت لهذه المبيدات علي السلالة الحقلية لمحافظة كفر الشيخ. حيث تشير النتائج المتحصل عليها أن معامل المقاومة لمبيد الميثوكسيفينوزايد والميثوميل كان ٥,٩، ٥,٨ أما مبيد الإيمامكتين بنزوات، البيريداليل فكان ٢,٧، ١,٩ مرة قدر السلالة المعملية وهذا يعني أن السلالة الحقلية مازالت حساسة لهذه المبيدات. وإمكانية خفض التأثير السلبي لهذه المبيدات علي البيئة تم دراسة الفعل المشترك لهذه المبيدات مع بعضها البعض بناءً علي التركيز القاتل لـ ٢٥% من الأفراد المعاملة. وقد أشارت النتائج المتحصل عليها إلي أن التأثير تثبيطي لفعل هذه المبيدات المختبرة. من خلال النتائج المتحصل عليها نجد أن مركب الإيمامكتين بنزوات ذو فعالية عالية ضد بيض ويرقات دودة ورق القطن وبالتالي يمكن استخدامه في برامج مكافحة متكاملة للحشرات.

Table (7): Joint toxic action of tested insecticides against 4th instar larvae of cotton leafworm, *S. littoralis*.

Treatments	After 24 hrs of exposure			After 48 hrs of exposure			After 72 hrs of exposure		
	Expected % Mortality	Observed % Mortality	Co-Toxicity Factor	Expected % Mortality	Observed % Mortality	Co-Toxicity Factor	Expected % Mortality	Observed % Mortality	Co-Toxicity Factor
Emamectin benzoate (LC ₂₅) + Methoxyfenozide (LC ₂₅)	3.33	10	+200.3	26.67	10.34	-61.22	40.00	13.79	-65.52
Emamectin benzoate (LC ₂₅) + Methomyl (LC ₂₅)	13.67	6.67	-51.2	45.24	6.67	-85.25	60.00	10.00	-83.33
Emamectin benzoate (LC ₂₅) + Pyridalyl (LC ₂₅)	16.63	13.33	-19.84	46.67	13.33	-71.43	50.00	20.00	-60.00
Methoxyfenozide (LC ₂₅) + Methomyl (LC ₂₅)	10.34	3.33	-67.79	38.57	3.33	-91.36	60.00	20.68	-65.53
Methoxyfenozide (LC ₂₅) + Pyridalyl (LC ₂₅)	13.33	6.67	-49.96	40.00	6.67	-83.32	50.00	21.42	-57.16
Methomyl (LC ₂₅) + Pyridalyl (LC ₂₅)	23.67	3.44	-85.46	58.57	3.44	-94.12	70.00	14.28	-79.6