

Toxicity of Emamectin Benzoate and Two IGR Compounds against Egg Masses and Different Larval Instars of Cotton Leafworm

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ABSTRACT

The present work was carried out to assess the ovicidal activity and the toxicity of emamectin benzoate against the 2nd, 3rd and 4th instar larvae of cotton leaf worm (CLW), *Spodoptera littoralis* compared with lufenuron and flufenoxuron. The joint action between emamectin benzoate and lufenuron or flufenoxuron was also carried out. Results revealed that, 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 residual toxicity of the same concentrations caused 100% mortality for all neonates. The highest concentration of lufenuron (200 ppm) and flufenoxuron (400 ppm) revealed 89.6 and 81.3% mortality of treated eggs, respectively. Both lufenuron and flufenoxuron had no residual toxicity against the neonates. Concerning the larvicidal activity, toxicity of emamectin benzoate against the different larval instars of *S. littoralis* is increased with the increasing in the exposure time and decreased by the increasing in the insect instars. Regarding lufenuron and flufenoxuron, it is also clear that the toxicity of both IGR compounds is increased with the increasing in the exposure time and decreased with the increasing in the insect instar. Regarding the joint toxic action, mixtures of emamectin benzoate and lufenuron or flufenoxuron showed antagonistic effects. From these data, the emamectin benzoate / lufenuron or flufenoxuron mixtures should not be used for controlling the larval instars of *S. littoralis*.

INTRODUCTION

Cotton is one of the major economic crops in Egypt. Throughout cotton growth season, it is attacked by many pests, from the seedling stage to harvest causing different degrees and types of damage. Among these pests, cotton leafworm (CLW), *Spodoptera littoralis*, (Lepidoptera: Noctuidae) (El-Deeb, 2004). Cotton leafworm is one of the most destructive agricultural lepidopterous pests.

Cotton leafworm is a serious pest of several important crops, such as cotton, tobacco and corn (Balachowsky, 1972; Sneh *et al.*, 1981; Sannino *et al.*, 1996). The control of this insect relies heavily on the use of chemical insecticides. The development of insecticide resistance often leads to

failure in crop protection and severely increases economic losses. To counteract resistance problems, compounds belonging to new insecticide classes are needed.

The availability of several new insecticides with different chemistry and mode of action would allow the implementation of schemes designed to slow down the selection for resistance to any insecticide. Emamectin benzoate (MK-244, 4"-deoxy-4"-epi-Nmethylamine avermectin B1, one of many 4 " – substituted analogs) is a new avermectin insecticide developed at Merck Resrarch Laboratories targeted for control of lepidopterous pests on a variety of corps (Leibee *et al.*,1995). The mode of action of emamectin benzoate is similar to abamectin (a GABA and glutamate-gated chloride channel agonist) according to (Dunbar *et al.*,1998). Emamectin benzoate is very effective against a broad spectrum of lepidopteran pests, with translaminar movement, good field efficacy and lack of cross-resistance with other commercially-used pesticides (White *et al.*,1997).

Benzoylphenyl ureas (BPUs) constitute a class of the IGRs that interfere with insect growth and development by inhibiting chitin synthesis in insects (Post and Vincent, 1973). Many institutions have engaged for searching about different derivatives of the optimum molecule of BPUs "diflubenzuron", which are considerably more potent than it on various serious pests (Ascher and Nemny, 1984). Moreover, insect growth regulators are considered an environmentally acceptable because they only affect systems unique to insects and certain other arthropods (Ghoneim *et al.*, 2003).

The aim of this work was to assess the ovicidal activity and the toxicity of emamectin benzoate against the 2nd, 3rd and 4th instar larvae of CLW compared with lufenuron and flufenoxuron. The joint action between emamectin benzoate and either lufenuron or flufenoxuron was also investigated.

MATERIALES AND METHODES

Insects: Cotton leaf worm larvae used for testing program was reared in the laboratory on castor bean leaves. When the larvae pupated they were sexed and 12 pupae put into a clean and sterilized jar. When the moths emerged they were supplied with a piece of cotton moistened with 10% sugar solution and two fresh leaves of *Nerium oleander* leaves, on which they deposited their eggs. The egg masses were collected daily and as they hatched on the oleander leaves the larvae were transferred to fresh castor oil leaves. The colony was kept at a temperature of 25±2 °C and 65±5 % RH (Eldefrawi *et al.*, 1964).

Tested insecticides: Emamectin benzoate (Proclaim® 5%SG), lufenuron (Match® 5% EC) and flufenoxuron (Cascade® 10% EC) were kindly obtained from Syngenta Company.

Larvicidal activity: Toxicity of emamectin benzoate (Proclaim® 5%SG) compared with lufenuron (Match® 5% EC) and flufenoxuron (Cascade® 10% EC) against the 2nd, 3rd and 4th instar larvae of *S. littoralis* was evaluated. Homogenous pieces of castor oil leaves were dipped in a series of the each insecticide concentrations for 10 sec., held vertically to allow excess solution to drip off and dried at room temperature. Treated castor oil leaf pieces were transferred to a plastic cups, and the appropriate number of starved larvae were added. Each concentration was replicated four times. Mortality percentages were recorded after 1, 2, 3 and 4 days post treatment for emamectin benzoate and 1, 2 and 3 days for lufenuron and flufenoxuron. Mortality counts were recorded (Eldefrawi *et al.*, 1964), corrected according to Abbott's equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971).

Ovicidal activity: Ovicidal activity of emamectin benzoate, lufenuron and flufenoxuron against *S. littoralis* egg masses was determined. The upper layers of each egg mass (0-24 hr old) were removed gently with a fine hair brush. The lower layer in each egg mass was counted by the binocular. Counted egg masses were dipped (5 seconds) in different concentrations of each tested compound, while the control was dipped in water (Dittrich, 1967). Each treatment was replicated three times. Treatments and control were held in a plastic cups (9 × 4 cm) at 27± 2°C, 65-75% RH and they were observed until hatching. The number of un-hatched eggs and dead neonates after 24 hrs of hatching were recorded and mortality percentages were calculated.

Joint toxic action of emamectin benzoate with lufenuron or flufenoxuron against the 4th instar larvae: Joint toxic action of the emamectin benzoate with lufenuron or flufenoxuron against 4th instar larvae was investigated. Larvae were treated with emamectin benzoate at LC₂₅ (0.0002 ppm), while lufenuron at LC₂₅ or LC₁₀ (0.40 or 0.13 ppm) and flufenoxuron at LC₂₅ or LC₁₀ (0.04 or 0.014 ppm). Co-toxicity factors (CTFs) were calculated for each mixture according to the equation of Mansour *et al.*, (1966).

$$\text{Co-toxicity factor} = \frac{\text{observed \% mortality} - \text{expected \% mortality}}{\text{expected \% mortality}} \times 100$$

RESULTS AND DISCUSSION

Ovicidal activity: Several studies had been conducted to evaluate the ovicidal activity of certain compounds against many insect species (Wells and Guyer, 1962; Dittrich, 1967; Mitri and Kamel, 1970; El-Guindy *et al.*, 1983; Renkleff *et al.*, 1995; Canela *et al.*, 2000;..... etc.). In the present study we focused mainly on the possibility of controlling *S. littoralis* by emamectin benzoate, lufenuron and flufenoxuron at other stages (eggs), when it may be more susceptible. According to the results in Table (1), 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 residual toxicity of the same concentrations caused 100% mortality for all neonates. The highest concentration of lufenuron (200 ppm) and flufenoxuron (400 ppm) revealed 89.6 and 81.3% mortality of treated eggs, respectively. Both lufenuron and flufenoxuron had no residual toxicity against the neonates. In respect with emamectin benzoate (semi-synthetic of abamectin), Bueno and Freitas (2004) reported that abamectin has no effect on the *Chrysoperla externa* egg viability. On the other hand, concerning the residual toxicity of emamectin benzoate our results are in accordance with Abou-Taleb (2010), who reported a high residual toxicity of emamectin benzoate against neonates of *S. littoralis*. Regarding lufenuron, although it is known that among the diverse actions of IGRs on the life cycles of insects are ovicidal and larvicidal effects (Ascher *et al.*, 1987), it has a low ovicidal activity compared with methomyl and chlorpyrifos.

Larvicidal activity:

A complete regression lines were established for the tested compounds on the 2nd, 3rd and 4th instar larvae. Susceptibility of 2nd, 3rd and 4th-larval instars from the laboratory strain, to emamectin benzoate, lufenuron and flufenoxuron is presented in Tables (2, 3 and 4 respectively). Toxicity of emamectin benzoate against the 2nd, 3rd and 4th instars laboratory strain of *S. littoralis* by dipping technique after different exposure times is shown in Table (2). The LC₅₀ values of emamectin benzoate against the 2nd instar larvae were 0.0042, 0.002 and 0.0007 ppm after 48, 72 and 96 hrs of treatment, respectively. In the case of 3rd instar, the LC₅₀ values of emamectin benzoate were 0.0067, 0.0025 and 0.0012 ppm after 48, 72 and 96 hrs of treatment, respectively. The LC₅₀ values of emamectin benzoate against the 4th instar larvae were 0.016, 0.012 and 0.011 ppm

after 48, 72 and 96 hrs of treatment, respectively. From these data, it is clear that the toxicity of emamectin benzoate against the different larval instars of *S. littoralis* is increased with the increasing in the exposure time and decreased by the increasing in the insect instars. Regarding lufenuron and flufenoxuron, it is also clear that the toxicity of both IGR compounds is increased with the increasing in the exposure time and decreased with the increasing in the insect instar (Tables 3 and 4). The median lethal concentrations (LC₅₀) values of lufenuron against the 2nd instar were 5.39, 1.05 and 0.98 ppm after 48, 72 and 96 hrs of treatment, respectively. In the case of 3rd instar, the LC₅₀ values of lufenuron were 15.26, 10.08 and 9.27 ppm after 48, 72 and 96 hrs of treatment, respectively. The LC₅₀ values of emamectin benzoate against the 4th instar larvae were 24.30, 16.45 and 14.96 ppm after 48, 72 and 96 hrs of treatment, respectively (Table 3). Concerning flufenoxuron, the LC₅₀ values against the 2nd instar larvae were 6.34, 2.75 and 2.00 ppm after 48, 72 and 96 hrs of treatment, respectively. In the case of 3rd instar, the LC₅₀ values were 10.69, 5.75 and 4.94 ppm after 48, 72 and 96 hrs of treatment, respectively. The LC₅₀ values against the 4th instar larvae were 18.87, 12.36 and 10.62 ppm after 48, 72 and 96 hrs of treatment, respectively.

Similar results were obtained by Corbitt *et al.*, (1989) with abamectin on *S. littoralis*. They demonstrated that the residual toxicity of abamectin on Chinese cabbage was 15 and 30-fold greater to 1st instar larvae than to 3rd and 4th instar larvae. They, also, recorded that the relative toxicity of abamectin against *S. littoralis* decreased from the third to the 4th and 5th larval instars. In another study, Scarpellini (2001) found that the susceptibility of different larval instars of cotton leafworm, *Alabama argillacea*, was decreased with the increasing in the larval instar. Also, Abou-Taleb *et al.* (2009) reported an increase in the toxicity of emamectin benzoate against *S. littoralis* larvae with the increasing in the exposure time and a decreasing by the increasing in the larval instar.

Joint toxic action of emamectin benzoate with lufenuron or flufenoxuron against the 4th instar larvae: Certain pesticides being used in pest control are hazardous. In order to reduce these hazards and the development of resistant populations, insect control should be accomplished with fewer applications at far lower doses. This aim might be realized, for example, by combining acute toxicants with other chemicals, such as insect growth regulators (El-Guindy *et al.*, 1983). Also, Clark *et al.* (1998) stated that the performance of the organic and low input systems indicate that pesticide use could be reduced by 50% or more in

maize with little or no yield reduction. Table (5) represents the joint action of binary insecticide mixtures on the 4th instar larvae of CLW. Generally, mixtures of emamectin benzoate and lufenuron or flufenoxuron showed antagonistic effects. Co-toxicity factors of the emamectin benzoate (LC₂₅) and lufenuron (LC₂₅ and LC₁₀) mixture were -44.4 and -67.9 after 96 hrs of exposure. On the other hand, the CTF of the emamectin benzoate (LC₂₅) and flufenoxuron (LC₂₅ and LC₁₀) mixture were -65.7 and -80.0 after 96 hrs of exposure. From these data, the emamectin benzoate / lufenuron or flufenoxuron mixtures should not be used.

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Table (1): Ovicidal and residual toxicity of emamectin benzoate, lufenuron and flufenoxuron against *S. littoralis* egg masses:

Insecticide	Conc ppm (a.i.)	No. Tre. eggs	No. hatched	No. Killed eggs	No. Killed larvae	% Mortality 24 hrs after Hatching		
						eggs	larval	total
Control	-	300	297	3	-	1.0	-	-
	3.75	280	180	100	180	35.7	100	100.0
Emamectin benzoate	7.5	226	113	113	113	50.0	100	100.0
	15	382	176	206	176	53.9	100	100.0
	30	374	124	250	124	66.9	100	100.0
	25	425	204	221	0	52.0	0	52.0
Lufenuron	50	444	146	298	0	67.1	0	67.1
	100	443	99	344	0	77.7	0	77.7
	200	346	36	310	0	89.6	0	89.6
	50	409	240	169	0	41.3	0	41.3
Flufenoxuron	100	460	195	265	0	57.6	0	57.6
	200	398	120	278	0	69.9	0	69.9
	400	400	75	325	0	81.3	0	81.3

Table (2): Toxicity of emamectin benzoate against different larval instars of *S. littoralis* at different exposure times:

Larval instar	Time (hrs)	LC ₅₀ (ppm)	Confidence limits	Slope ± SE
2 nd instar	48	0.0042	0.0033-0.0062	1.92 ± 0.34
	72	0.002	0.0016-0.0026	1.29 ± 0.15
	96	0.0007	0.0006-0.0008	1.40 ± 0.12
3 rd instar	48	0.0067	0.0060-0.0075	3.49 ± 0.44
	72	0.0025	0.0022-0.0029	3.15 ± 0.25
	96	0.0012	0.0010-0.0014	2.44 ± 0.27
4 th instar	48	0.016	0.015-0.017	4.46 ± 0.49
	72	0.012	0.011-0.013	3.61 ± 0.33
	96	0.011	0.010-0.012	3.43 ± 0.43

Table (3): Toxicity of lufenuron against different larval instars of *S. littoralis* at different exposure times:

Larval instar	Time (hrs)	LC ₅₀ (ppm)	Confidence limits	Slope ± SE
2 nd instar	48	5.39	3.11 – 8.37	1.01 ± 0.23
	72	1.05	0.79 – 1.41	0.89 ± 0.09
	96	0.98	0.58 – 1.52	0.98 ± 0.09
3 rd instar	48	15.26	13.44 – 17.55	2.09 ± 0.29
	72	10.08	8.12 – 12.16	1.57 ± 0.29
	96	9.27	8.21 – 11.65	1.90 ± 0.24
4 th instar	48	24.30	20.69 – 28.04	3.41 ± 0.33
	72	16.45	14.94 – 22.92	3.22 ± 0.46
	96	14.96	12.42 – 18.46	3.30 ± 0.41

Table (4): Toxicity of flufenoxuron against different larval instars of *S. littoralis* at different exposure times:

Larval instar	Time (hrs)	LC ₅₀ (ppm)	Confidence limits	Slope ± SE
2 nd instar	48	6.34	4.22 - 9.35	1.44 ± 0.22
	72	2.75	2.11 – 3.85	1.86 ± 0.26
	96	2.00	1.44 – 2.82	1.67 ± 0.19
3 rd instar	48	10.69	8.24 - 13.36	2.00 ± 0.34
	72	5.75	3.83 - 7.69	1.96 ± 0.24
	96	4.94	3.28 – 7.04	2.07 ± 0.14
4 th instar	48	18.87	14.28 – 24.12	2.92 ± 0.34
	72	12.36	9.86 – 16.77	2.77 ± 0.25
	96	10.62	8.42 – 14.28	2.89 ± 0.37

Table (5): Joint toxic action of emamectin benzoate with lufenuron and flufenxuron against the 4th instar larvae of *S. litoralis*

Insecticide Mixutre	Time after exposure (hrs)								
	48			72			96		
	Expected %M	Observed %M	CTF*	Expected %M	Observed %M	CTF	Expected %M	Observed %M	CTF
Emamectin benzoate LC ₂₅ + Lufenuron LC ₂₅	16	8	-50.0	32	22	-31.3	54	30	-44.4
Emamectin benzoate LC ₂₅ + Lufenuron LC ₁₀	28	4	-85.7	48	8	-83.3	56	18	-67.9
Emamectin benzoate LC ₂₅ + flufenxuron LC ₂₅	15	4	-73.3	25	6	-76.0	35	12	-65.7
Emamectin benzoate LC ₂₅ + flufenxuron LC ₁₀	10	0	-100.0	28	4	-85.7	40	8	-80.0

CTF= Co-toxicity factor

الملخص العربي

سمية مبيد إيمامكتين بنزوات ومركبين من مثبطات النمو الحشري ضد البيض والاعمار اليرقية المختلفة لدودة ورق القطن.

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أجريت دراسة معملية لتقدير سمية مبيد الإيمامكتين بنزوات على البيض وعلى العمر اليرقى الثانى والثالث والرابع لدودة ورق القطن مقارنةً بمركبات تثبيط النمو الحشري اللوفينيرون والفلوفينوكسيرون. مع تقدير الفعل المشترك بين الإيمامكتين بنزوات وكلا من اللوفينيرون والفلوفينوكسيرون. كانت نسبة تثبيط قفس البيض لمبيد الإيمامكتين بنزوات على هي 35.7 ، 50.0 ، 53.9 و 66.9 % عند التركيزات 3.57 ، 7.50 ، 15.0 و 300 جزء بالمليون على الترتيب، وحدثت نفس التركيزات نسبة موت 100% على القفس الحديث. أحدثت أعلى تركيزات من كل من اللوفينيرون (200 جزء بالمليون) والفلوفينوكسيرون(400 جزء بالمليون) نسبة تثبيط قدرت ب 89.6 و 81.3% للبيض المعامل على التوالي ، ولم يكن لهذه التركيزات أى تأثير إبادى على اليرقات حديثة القفس.

زاد تأثير الإيمامكتين بنزوات على الأعمار اليرقيه المختلفة لدودة ورق القطن كلما قل العمر اليرقى وزادت فترة التعرض للمبيد. أيضا إزداد التأثير السام لمبيدات مثبطات النمو الحشريه (اللوفينيرون والفلوفينوكسيرون) على الأعمار اليرقيه المختلفة لدودة ورق القطن كلما قل العمر اليرقى وزادت فترة التعرض للمبيد.

وعند دراسة الفعل الإبادى المشترك وجد ان خلط الإيمامكتين بنزوات مع كلا من اللوفينيرون أو الفلوفينوكسيرون أحدث تأثير تضاد على يرقات العمر اليرقى الرابع لدودة ورق القطن ولذلك يمكن القول انه لا يمكن استخدام مخاليط مبيدات الإيمامكتين بنزوات وكلا من اللوفينيرون أو الفلوفينوكسيرون فى عمليات مكافحه الأعمار اليرقية لدودة ورق القطن.