THE BIO-EFFECT OF NPV VIRUS, DELTAMETHRIN AND THEIR BINARY MIXTURE ON SOME BIOLOGICAL FEATURES OF THE COTTON LEAFWORM

Spodoptera *littoralis* (Boisd.) (Lepidoptera : Noctuidae)

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Abstract

The present work was carried out under laboratory conditions in order to evaluate resistance building up of a cotton leafworm *Spodoptera littoralis* (Boisd.) laboratory susceptible strain to commercial formulations of Deltamethrin, Nuclear Polyhedrosis Virus and their binary mixtures as indicated by certain biological aspects.

The obtained results showed that no resistance have been achieved after 8 successive generations for the three tested treatments. The three formulations displayed latent effect which took place during the larval stages up to moth emergence. The data reveals that larval duration, pupal duration, pupal weight, adult longevity, mortality rates, sex ratio and reproductive potentiality were strongly affected. The simultaneous effect of these tested formulations was greatly governed; the binary mixture demonstrated mild effect in this respect.

INTRODUCTION

The bio-effect of applied control agents is seriously governed by the feeding habits of the target insect (McKinley, *et al.*, 1989). Several field and laboratory studies reveal that pathogens may be of value when integrated pest management programs are considered and biocides were used as an early season treatment. It is a well known fact that combinations of pathogen with certain chemical insecticides when used in pest control programs as a mixture may demonstrates better control figures for a complex of survivors showing varying susceptibility levels to its different components. A highly effective insecticide might be used at a diluted rate with a less expensive pathogen giving satisfactory control measurements. Moreover, a mixture of an insecticide and a pathogen may be used as an important component of an integrated control strategy of certain pests.

The present investigation was carried out for 8 successive generations to contribute some knowledge on the effect of polyhedrosis virus (NPV), a pyrethroid insecticide (deltamethrin) and their binary mixture on certain biological features of the cotton leafworm, *Spodoptera littoralis* (Boisd.) and on building up of resistance.

MATERIALS AND METHODS

A. Biological and chemical materials

1. Origin of *S. littoralis* strain: The strain of *S. littoralis* used in the present study was originated from Fayoum governorate since 15 years ago and continuously reared on semi-artificial diet (Poitout and Bues, 1970) in the laboratory under constant conditions of 25± 2°C and 65±10 % R.H.

2. Origin of isolated biocide [Nuclear Polyhedrosis Virus (NPV)]: The powder of *S. littoralis* NPV bodies was provided from diseased larvae of the same species of insect which originated from Maroc and isolated after multiplication in "INRA - La Miniére, France" laboratory under the code name of SLNPV 287-86. The concentration of polyhedral inclusion body (PIB) was 39 x 10^9 polyhedra per 1 gram of powder, counted by R.B.C.'s Malasse cells

3. Chemical insecticide used: The synthetic pyrethroid deltamethrin (Decis, 25 g/l), a product of Procida firm was used. Its chemical structure is (S)-α-cyano-m-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2-dimethyl-cyclopropane carboxylate.

B. Laboratory procedures

The susceptible strain of *S. littoralis,* which reared on semi-artificial diet, was divided into four groups; susceptible standard (untreated), larvae treated with nuclear polyhedrosis virus (treatment A), larvae treated with deltamethrin (treatment B) and larvae treated with a combination of NPV + deltamethrin (treatment C).

To investigate the bio-effect of a combination of NPV and deltamethrin on cotton leafworm larval mortality, water suspensions of each were prepared (6 to 8 dilutions) to obtain the applicable concentrations. The semi-synthetic diet for tests was prepared without the formaldehyde in regard to its antiseptic property. The experiments were realized into the same boxes of rearing *S. littoralis* strain. Surface of diet was treated by "Eppendorf combitips" micropipette (25 µl/square of 165 mm^2), the boxes were left to dry under room temperature before transferring the larvae. Selected healthy larvae at the end of the 1st instar were placed individually in $square^2$. Three replicates of 20 larvae each were used for each concentration. Number of dead larvae was recorded daily until the 6th instar. Percentages of larval mortality were corrected by Abbott's formula (1925). Data were subjected to probit analysis (Finney, 1952). The LC_{50} of each product was also estimated (Joly and Giner, 1990).

In order to estimate the changes in the percentage of larval mortality, 1000 larvae were treated at the end of the 1st instar in each treatment and in each generation.

To calculate fluctuations in the percentage of pupation, 264 alive 6th instar larvae in each treatment and generation were considered. Finally, the percentage of adult emergence (male and female) was calculated from the available survivor pupae in each treatment and in each generation.

RESULTS AND DISCUSSION

The LC_{50} at 95% confidence limits for NPV at parent generation was 33.79 pol./mm^2 (17.80-64.16) and the slope was 0.96±0.08, whereas the LC_{50} for deltamethrin was 2.74 x 10^{-11} g. a.i./mm^2 (1.26-5.93) and the slope was 0.97±0.10.

The simultaneous effect on some biological aspects of the cotton leafworm, *S. littoralis*

1. Percentage of mortality and pupation of treated larvae and total percentages of emerged adults: The results in Table 1 show that larval mortality of *S. littoralis* increased gradually generation after generation in treatments A and B, thus giving 92% and 100% mortality figures, treatment C showed mild larval mortality % (61%), when compared to the untreated control (3%).

The percentage of pupation decreased during the following successive generations in A and B treatments giving 8% and 0%, respectively at the 8th generation, whereas treatment C demonstrates mild percentage for the successive 8 generations (39%), on an average duration of the check (97%) treatment was considered..

As shown in Table 1, the percentage of adult emergence decreased during the following successive generation for A and B treatments giving 7% and 0%, respectively at the 8th generation, while treatment C showed mild trend when corrected percentages of adult emergence after 8 generations (24%), as compared to the check (98%) was considered. These results are in agreement with those of Watson and Kelly (1995) and Tabashnik and Lui (1999).

2. Larval and pupal durations: The results in Table 2 indicate that treatments in general, increased the period of both larval and pupal durations as compared to the check. Data also reveal that the chemical insecticide deltamethrin has the superior efficiency in elongation of both larval and pupal durations in all tested generations, as compared to the other 2 treatments (A and C).

The present data is in harmony with those of Watson and Kelly (1995) and Al-Kazafy (2001).

Table 1.: The changes in larval, pupal mortality and adult emergence percentages during 8 successive generations of a susceptible strain of *S. littoralis* larvae in three different treatments, compared to the untreated control.

Generations	% Mortality of larvae				% Pupation of larvae				% of emerged adults			
	Untreated control	NPV (A)	Delta-methrin (B)	NPV + Delta-methrin (C)	Untreated control	NPV (A)	Delta-methrin (B)	NPV + Delta-methrin (C)	Untreated control	NPV (A)	Delta-methrin (B)	NPV + Delta-methrin (C)
Parent	4	40	45	45	95.88	59.84	54.92	54.92	92.88	89.87	86.89	91.03
1*st*	8	68	70	65	92.04	31.81	29.92	34.84	88.06	88.09	69.62	92.39
2*nd*	10	79	77	71	90.15	20.83	23.1	29.16	78.15	81.81	78.68	81.81
3*rd*	7	79	60	92	93.18	20.83	40.15	7.95	89.83	90.91	88.67	85.71
4*th*	6	61	76	53	93.93	39.01	23.86	46.96	87.9	69.9	82.53	78.22
5*th*	9	64	71	77	90.9	35.98	29.16	23.1	50	47.36	70.13	31.14
6*th*	3	62	79	18	96.96	37.87	20.83	81.81	85.15	44	67.27	37.96
7*th*	7	82	85	75	93.18	18.18	15.15	25	82.11	37.5	32.5	30.3
8*th*	3	92	100	61	96.96	7.95	0.00	39.01	98.04	4.76	0.00	24.27
Mean	6.33 b	69.67 a	73.67 a	61.89 a	93.69 a	30.26 b	26.35 b	38.04 b	83.57 a	61.58 a	64.04 a	61.43 a
F value	37.8438				38.0616				1.46			
LSD 5%	14.7129				14.6812				25.4611			

Means with the same letter are not significantly different.

3. Weight of male and female pupae: The results in Table 3 show, in general, that the simultaneous effect of the tested treatments expressed as average weight of pupae (both sexes) developed from treated larvae in treatments B and C was less than that of untreated (control) especially that of the 8th generation. Treatment "A", however, demonstrates high average weight of pupae (both sexes) at the 8th generation as compared to treatments B and C. Results indicate the superior efficiency of deltamethrin in decreasing pupal weight either when deltamethrin was used alone or mixed with NPV.

The integration of the data in Table 3 reveal that the average pupal weight for the treated larvae (A, B and C) decreased gradually in both sexes during the successive generations till the 8th generation.

This is in agreement with Muller-Cohn *et al.* (1996) and Trisyono and Whalon (1997).

4. Sex ratio of the emerged moths: Data in Table 4 reveal in general that the percentages of emerged female moths were more than males in B and C treatments. The case in treatment A, was the other was round when the 2nd, 4th, 6th, 7th and 8th generations figures were considered.

Reviewing the fore obtained results, it can be concluded that, in general, in the 3 treatments (A, B & C) the same trend of effect on some tested biological aspects was obtained. An elongation in larval and pupal durations was recorded. The larvae which tolerated the NPV, the deltamethrin and their binary mixtures, (i.e. the survivors) suffered from ailment. The effect increased during the successive generations. This phenomenon could be elucidated based on the sick individuals need longer periods to recover and complete their life cycle than the untreated (Al-Kazafy, 2001).

The simultaneous effect of the tested 3 treatments on pupal weight expressed as corrected averages increased obviously in A and C treatments during the last tested generations. For treatment "B", these averages decreased obviously during the successive generations.

Data also show a strong bio-effect of the treatments on the percentage pupation of larvae and adults emergence.

In general, treatment "C" demonstrates mild effect when certain biological features were considered.

In general, during the course of the present study, no evidence was obtained revealing that resistance to the use of NPV and deltamethrin was found. This was very clear when 8 generations were tested.The percentages of larval mortality in A and B

Table 2.: Corrected larval and pupal duration figures (in days) during 8 successive generations in a susceptible strain of *S. littoralis* larvae in three different treatments, compared to the untreated control.

	Larval duration in days ± S.E.				Pupal duration in days ± S.E.			
	Untreated control	NPV (A)	Deltamethrin (B)	NPV + Deltamethrin (C)	Untreated control	NPV (A)	Deltamethrin (B)	NPV + Deltamethrin (C)
Parent	24.1±3.2	26.3±3.3	27.1±3.6	25.8±4.2	16.3±1.3	19.8±1.2	18.6±5.0	17.5±4.7
1*st*	24.4±4.5	26.5±3.8	26.4±3.2	25.6±4.1	15.6±2.3	20.0±2.1	19.0±0.7	18.5±1.8
2*nd*	25.6±4.1	27.4±3.6	28.1±3.4	27.1±3.1	16.1±1.2	19.6±2.2	18.2±4.1	18.0±1.0
3*rd*	26.3±3.3	28.6±3.2	26.9±3.5	26.2±3.4	14.9±2.1	20.4±2.1	20.9±5.0	20.2±4.3
4*th*	24.7±4.5	30.1±4.6	30.8±4.7	27.8±3.4	16.3±2.4	19.9±1.2	20.1±2.3	19.2±1.2
5*th*	27.0±3.6	31.8±6.5	31.5±5.6	30.1±3.2	15.8±3.1	18.6±4.1	19.2±3.6	18.5±3.1
6*th*	26.5±3.6	34.2±2.8	35.0±1.0	30.2±3.1	16.2±3.2	19.4±2.3	20.1±3.4	19.9±3.7
7*th*	27.8±3.3	30.3±3.2	32.0±1.0	29.0±2.0	15.5±3.1	20.9±2.6	23.3±3.1	20.2±2.1
8*th*	24.6±3.4	32.5±3.9	35.8±3.8	30.9±3.9	14.9±2.1	20.1±3.1	21.8±2.7	20.0±1.0

Table 3.: Corrected mean weight of *S. littoralis* male and female pupae (in mg.) during 8 successive generations in a susceptible strain in three different treatments, compared to the untreated control.

Generations	Untreated larvae		Treatment "A"		Treatment "B"		Treatment "C"	
	Weight of male pupae±SE	Weight of female pupae±SE	Male±SE	Female±SE	Male±SE	Female±SE	Male±SE	Female±SE
Parent	398.53±16.59 b	453.13±13.02 b	461.52±15.46 a	499.40±29.77 a	419.10±23.10 a	495.70±15.90 a	469.62±16.20 a	521.50±24.40 a
1*st*	349.87±13.45 c	434.87±20.59 c	388.17±12.99 b	462.83±13.47 b	465.03±21.42 a	516.90±14.93 a	433.67±16.39 a	511.73±17.31 a
2*nd*	389.76±14.12 b	460.76±14.52 b	368.55±14.55 b	432.30±16.50 c	412.60±18.35 a	471.30±18.40 a	417.50±12.70 a	486.45±19.55 b
3*rd*	462.53±15.42 a	504.93±17.27 a	390.35±12.90 b	452.35±13.30 b	328.45±20.25 b	392.30±25.75 b	409.05±23.90 b	516.30±24.10 a
4*th*	345.36±20.00 c	385.03±19.69 d	336.50±12.90 c	387.60±19.35 d	300.35±16.40 b	342.25±21.70 bc	363.60±14.70 c	449.60±16.55 c
5*th*	295.23±16.05 d	347.60±14.61 e	309.25±12.80 c	363.95±18.35 d	289.21±15.39 b	341.30±24.80 bc	352.40±14.70 c	404.95±18.60 d
6*th*	341.43±10.95 c	375.70±10.17 d	318.75±16.10 c	352.50±19.90 d	285.60±13.20 b	324.65±13.25 c	408.55±15.65 b	472.50±15.60 b
7*th*	355.83±13.99 c	418.83±15.85 c	409.65±12.40 a	482.55±17.15 a	267.80±24.80 c	305.92±19.95 d	332.10±18.75 c	413.40±15.55 c
8*th*	390.30±15.14 b	432.30±19.89 c	398.80±25.85 b	464.65±23.45 b	231.71±19.78 c	291.60±22.95 d	333.40±15.95 c	383.40±18.65 d
Mean	369.81	423.68	375.72	433.12	358.11	386.88	391.09	462.2
F	40.49	37.52	22.27	29.17	39.08	35.99	22.67	20.78
LSD 5%	46.26	42.35	37.79	46.06	37.76	42.16	38.59	49.71

Table 4.: Percentages of emerged male and female moths and sex ratio during 8 successive generations in a susceptible strain of *S. littoralis* in three different treatments, compared to the untreated control.

Generations	Untreated larvae			Treatment A			Treatment B			Treatment C		
	Moth emergence		Sex ratio (R)*	Moth emergence		Sex ratio (R)*	Moth emergence		Sex ratio (R)*	Moth emergence		Sex ratio (R)*
	Male %	Female %		Male %	Female %		Male %	Female %		Male %	Female %	
Parent	91	94	1:1.03	86	93	1:1.08	86	88	1:1.02	89	92	1:1.03
1*st*	86	90	1:1.04	80	95	1:1.18	62	75	1:1.21	91	93	1:1.02
2*nd*	74	81	1:1.09	85	79	1:0.92	78	80	1:1.03	85	79	1:0.92
3*rd*	90	89	1:0.98	85	95	1:1.11	89	89	1:1	79	93	1:1.17
4*th*	88	87	1:0.98	77	62	1:0.81	79	84	1:1.06	78	78	1:1
5*th*	45	55	1:1.22	41	53	1:1.29	67	73	1:1.08	19	42	1:2.21
6*th*	91	79	1:0.86	46	42	1:0.91	67	66	1:0.98	34	41	1:1.21
7*th*	79	84	1:1.06	46	30	1:0.65	30	34	1:1.13	25	34	1:1.36
8*th*	99	96	1:0.96	11	2	1:0.18	0	0	0	19	28	1:1.47

where

R* = Sex ratio between male and female adult moths

$$= \frac{\text{\% of adult female moths}}{\text{\% of adult male moths}}$$

treatments were 92% and 100%, respectively, whereas in untreated control, it was 3%. For the larvae of treatment "C" slight tolerance was obtained expressed as percentage of both larval and pupal mortalities. The corrected percentages of emerged moths for A or B and to untreated treatments demonstrate similar trend. These results in agreement with Kaomini and Rough (1988).

It is a well known fact that development of resistance depends greatly on a package of factors, including selection intensity, duration of selection and presence of genetic variations for resistance.

In this study, moderately intense selection pressure has been used for 8 successive generations which seems quite adequate for producing reliable increase in resistance in the presence of genetic variations. This study, however, seems not quite enough for evaluating resistance owing to that of *S. littoralis* treated with NPV alone or deltamethrin alone, the genes responsible for resistance must at least be rare or absent in the wild populations sampled for our base culture.

Although resistance of some species of Noctuidae to *Bacillus thuringiensis* toxins became frequently recorded (Sims and Stone, 1991; Frutos *et al.*, 1995; Al-Kazafy, 2001), resistance of Noctuidae to other microbial agents (virus or fungus) are not frequently published.

Before we can confirm the presence or absence of response to selection for resistance to NPV in *S. littoralis,* more studies on other susceptible strains and on more number of successive generations have to be conducted.

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تأثير فيروس البولى هيدروزس النووى ومبيد الدلتامثرين وخليط بينهما على بعض المظاهر البيولوجية والحيوية ليرقات دودة ورق القطن

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أظهرت النتائج المعملية عدم تكون مقاومة بعد ٨ أجيال متتابعة ليرقات سلاله حساسة لدودة ورق القطن عوملت تحت الظروف المعملية بالفيروس النووى (NPV) والمبيد البيرثرويدى (deltamethrin) و الخليط بينهما.

أوضحت النتائج حدوث إطالة فى العمر اليرقى وكذلك أعمار العذارى والفراشات الناتجة. كما أظهرت إزدياداً ملحوظاً فى نسبة الموت لليرقات المعاملة وإنخفاضاً فى نسبة التعذير ونسبة خروج الفراشات. بأخذ غير المعامل فى الإعتبار كانت هناك زيادة واضحة فى وزن العذارى ذكوراً وإناثاً وخاصة فى الأجيال الأولى للسلالة المعاملة بالفيروس والسلالة المعاملة بالخليط وذلك فى الأجيال الخمسة الأولى ثم إنخفض الوزن بعد ذلك. أما بالنسبة للسلالة المعاملة بالمبيد منفرداً فقد لوحظ إنخفاض مضطرد فى وزن العذارى سواء الإناث أو الذكور مقارنة بأوزان العذارى غير المعاملة.

وعموما، دلت النتائج أن تأثير المبيد البيرثرويدى على المظاهر الحيوية هو الأقوى يليه المبيد الفيروسى ثم الخليط بين المبيد الكيميائى والمبيد الحيوى.