DEVELOPMENT OF SUSCEPTIBILITY OF THE COTTON LEAF WORM, *SPODOPTERA LITTORALIS* TO NOMOLT COMPOUND IN RELATION TO SOME BIOLOGICAL AND BIOCHEMICAL CHANGES IN THE INSECT BODY

IBRAHIM, ELSHAIMAA N. [1], AZZA K. EMAM[2], K. WESHAHY[2] and AZIZA E. ABDEL-AAL[1]

1. *Plant Protection Research Institute, ARC, Dokki, Giza, Egypt.*
2. *Plant Protection Dept., Fac. Of Agric., Ain Shams Univ., Shoubra El-Kheima,* Cairo, Egypt.

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Abstract

The susceptibility of the 4th instar larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) to the chitin synthesis inhibitors, nomolt was evaluated in the laboratory for 10 generations. The fourth instar larvae were treated with various concentrations of nomolt insecticide for 48 hrs in each generation until F10 and LC_{50}s were calculated after 7 days post treatment in each generation. The obtained results showed that the resistance ratios of the selected generations were increased to 1.2709, 1.2894, 2.3034 and 3.0117 folds, the F3, F6, F8 and F10 generations, respectively. Based on the biological aspect, larval durations were increased significantly in the selected generations compared to the control. Furthermore, percentage of pupation was reduced in all the treated generations, which being 56.7, 55.0, 43.85, 27.35, 24.66 and 33.56% the adult emergence was significantly affected as it was 82.09, 70.33, 37.71, 55.06, 41.18 and 43.65% in treated F0, F1, F3, F6, F8 and F10 of the selected generations, respectively. The activities of the measured enzymes at 6 days after treatment of the fourth instar larvae showed up and down pattern through the selected ten generations. The activity of α and β esterases as well as protease enzymes was back to normal activity as that of control in the tenth generation meanwhile chitinase enzyme activity remained significantly higher than that of the control.

Key words: IGR's - Teflubenzuron– *Spodoptera littoralis*– α and β esterases- Protease - chitinase.

1. INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Bosid.) is one of the most important pests in Egypt and other countries in Africa and Asia. It attacks several host plants specially the cotton crop which is considered one of the main sources of the economy in Egypt.

Insect growth regulators (IGR's) received great attention as a hope for the future of insect control. Among these insecticides, chitin synthesis inhibitors (CSI's) was Nomolt which interferes with the chitin deposition. Occurrence of chitin is mainly

restricted to arthropods, fungi and nematodes, and does not occur in vertebrates. These compounds cause a qualitative selectivity to both other phyla of invertebrates and vertebrates. Ingestion of chitin synthesis inhibitors by insect larvae disturbed endocuticular deposition during molting process because it blocks chitin synthesis. The blocking of chitin synthesis occurs by disruption the function of connecting N-acetylglucose amine moieties to chitin chain.

The resistance problem is the greatest single challenge facing applied entomologists today, because of the widening circle of cross and multiple resistance among the insect pests, the diminishing number of effective commercial insecticides and the exponentially- increasing costs of development of new insecticides**.** In 1938, there were already 7 species showed resistance to insecticides toxicity and in 1988 there were closed to 500 resistant species. More significantly, insects with multiple resistances are becoming increasingly common. This often occurs when different groups of insecticides interfere with a common target that has changed (Brattsten, 1989).

In this study, the development of resistance in the cotton leaf worm *Spodoptera littoralis* after treating the fourth instar larvae with nomolt compound for ten generations was determined, in addition, the changes in some biological and biochemical aspects in the larval homogenate at six days after treatment was measured.

II. MATERIALS AND METHODS

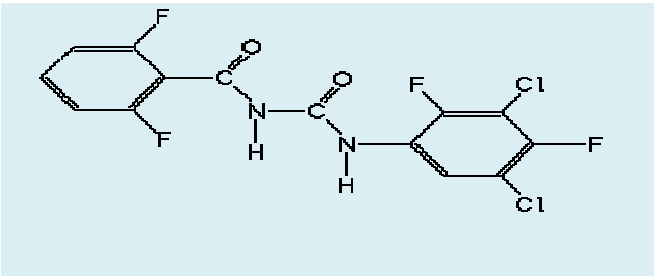
1. Rearing Technique

The colony of the cotton leaf worm *Spodoptera littoralis* (Bosid.) was obtained from the division of the Cotton Leaf Worm, Plant Protection Research Institute, Agricultural Research Center larvae were reared for about 13 generations on castor been leaves (*Ricinus communis* L) before any treatment as the method described by El- Defrawi *et al.* (1964).

2. Insecticide Used:-

Common Name: Chitin synthesis inhibitors. (nomolt, EC 15 %) from Treade Name : Nomolt 15% EC provided from BASF Agri production.

Structural formula:



3. Toxicological studies:

Dipping method was used in this bioassay. Fourth instar larvae were fed on treated castor bean leaves in different concentrations of the tested compounds for 3 seconds. One hundred larvae were divided into four replicates, each 25 larvae were used four concentration (2.0, 1.0, 0.5 and 0.25 ppm) were used in this study. A control experiment was performed using castor been leaves dipped in water. The tested larvae were fed on treated leaves for 48 hrs. then the survived larvae were transferred to other clean jars and supplied daily fresh castor bean leaves for 7 days after treatment. The mortality percentage was recorded daily and corrected according to Abbott's formula (Abbott, 1925). The LC -p lines software program was used to obtain the toxicity regression lines. Percentages of corrected mortalities were statistically analyzed according to Finney (1971) and the LC_{50} value was determined.

Selection of resistance was carried out by rearing the larvae firstly on castor been leaves treated with Lc_{50} value of nomolt. According to the response of treated insect to selection, a higher concentrations of the tested compound was sometimes used in subsequent generations.

In comparisons of the resist abilities as called resistance ratio (LC_{50} of resistance strain / LC_{50} of control), differences of five folds or more (true resistance differences) were considered as indicating positive correlation, those between 1 and 4 folds (tolerance differences) as indicated by Litchfield and Wilcoxon (1949).

4. Biological Studies.

Newly molted 4^{th}instar larvae treated with LC_{50} value of selected generation were examined daily to determine the larval and pupal durations as well as percentage of both pupation and adult emergence.

5. Biochemical studies:

5.1. Preparation of samples for biochemical studies:

Larvae were collected after six days following the treatment of the fourth instar, placed in ice containers and homogenized in appropriate buffer using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 8000 rpm for 10 minutes at 4°C (Biofuge 28RS Heraeus, Sepatech centrifuge). The resulted supernatants were used directly for determination of enzymatic activity.

5.2. Determenation of the enzyme activities:

The activity of both α and β non-specific esterases, protease and chitinase were determined according to the method of Van Asperen (1962), Ishaaya *et al.* (1971) and Ishaaya and Casida (1974), respectively.

6. Statistical analysis procedure:

The significance of the main effects was determined by using analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range tests ($p<0.05$). All analysis was preceded using a software package "Costat", a product of cohort software Ine. Berkley, California. (Duncan, 1955).

III. RESULTS AND DISCUSSION

I-Toxicological studies

The LC_{50} values of nomolt treatment against the fourth instar larvae through the selected generations were determined as 0.1809, 0.2275, 0.2308, 0.4123 and 0.5391ppm, in F1, F3, F6, F8 and F10, respectively. The estimated resistance ratios were 1.0106 folds in F1 generation, then gradually increased in the following selected generations to reach 1.2709, 1.2894, 2.3034, and 3.0117 folds in F3, F6, F8 and F10 generations, respectively compared to the treated parent (F0) [Table 1 and Fig. 1].

Ghoneim *et al.* (2012) studied the toxicity of thirteen insecticides of different classes under laboratory conditions against egg mass and 4thinstare larvae of of *Spodoptera littoralis* (Boisd.) field strain obtained from Gharbia Governorate in Egypt during 2010 season. The field population showed high resistance to pyrethroids and moderate resistance to both organophosphorus and carbamates, however it had low resistance to the chitin synthesis inhibitors used against the 4thinstar larvae.

Table 1. Susceptibility of the 4thinstar larvae of *S. littoralis* (Boisd.) toxicity.

Concentrations (ppm)	% Corrected Mortality					
	Parents (F0)	F1	F3	F6	F8	F10
0.125	43.3	40.1	36.05	37.29	25.14	15.76
0.25	60.0	58.5	56.15	55.48	40.35	29.87
0.5	83.3	79.7	75.44	72.65	58.43	47.94
1.0	93	91.4	89.11	85.94	75.34	66.44
2.0	99	99	96.26	94.02	87.81	81.61
LC_{50} - value	0.179	0.1809	0.2275	0.2308	0.4123	0.5391
Resistance Ratio		1.0106	1.2709	1.2894	2.3034	3.0117
Slope function	1.6513± 0.3102	1.9282± 0.1888	1.8534± 0.3172	1.621±0.2 965	1.6385±0. 2806	1.5825±0 .1516
95% Confidence limits (lower -upper)	0.1034- 0.2568	0.1446- 0.2164	0.1498- 0.3062	0.1416- 0.3204	0.2915- 0.5641	0.3114- 0.9713

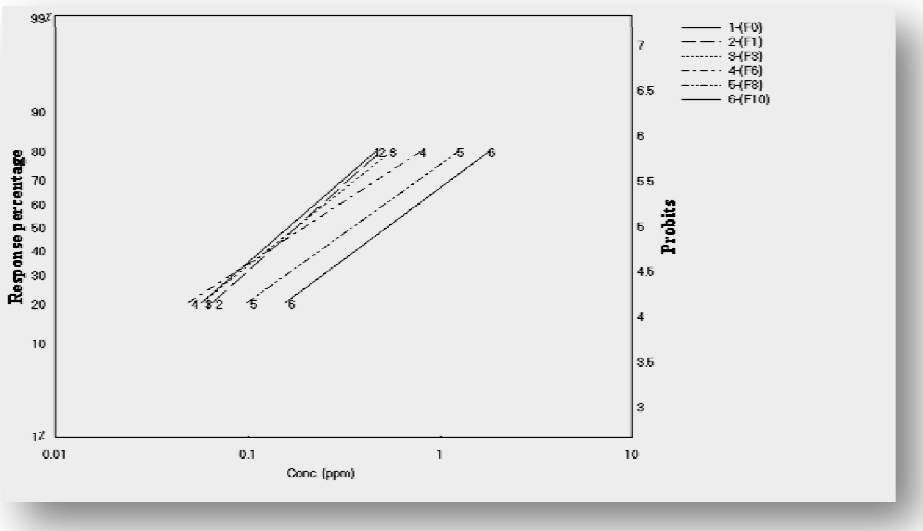


Fig. 1. Toxicity regression lines of the 4th instar larvae of *Spodoptera littoralis* treated with nomolt insecticide during selected generations.

2. Biological Studies.

Effects of the devoloped resistance to nomolt toxicity on some biological aspects associated to the following selected generations of *S. littoralis* treated as 4th instar larvae were recorded in Table 2.

Data indicated that larval duration increased significantly during the following selected generations**,** percentage of the increase in larval duration was12.5 in the treated parent (F0), 20.1, 17.5, 35.5, 45.0 and 20.8 in survived larvae obtained in F1, F3, F6, F8 and F10 generations, respectively.

The percentage of increase in the duration of pupal stage than those of the control was 3.81, 19.05, 9.52, 4.76 and 20.95 days in F0, F1, F3, F6 and F10 generations respectively. On the other hand, F8 had the same duration as in the control.

In addition, treatment of selected larvae with nomolt insecticide caused a reduction in pupation percentage compared with control in all respective treated generations which being 42.3, 43.3, 54.8, 71.8, 74.6 and 65.4.

The highest adult emergence percentage resulted from selected larvae with nomolt toxicity was achieved 82.o9% in the parent generation (F0), while the lowest percentage (37.71%) was attained at F3. The recorded emergence percentages of developed resistance in adult moths were 70.33, 55.06, 41.18 and 43.65% in F1, F6, F8 and F10, respectively compared with 90.38 % in control**.** The reduction percentage in adult emergence was ranged between 8.9 and 58.1% during the selected generations.

The prolongation in both larval and pupal durations and the reduction percentages in pupation adult emergence of *S. littoralis* due to treatment with

nomolt action against the pest were studied by several authors on the same insect species treated with other chitin synthesis inhibitors. In this field of study Sabry (2007) showed that a resistance strain of *Pectinophora gossypiella* to both atabron and cascade having a distinct latent effects on the larval and pupal durations. The reduced number of larvae entering pupation or moth emergence could be a result of accumulation of toxic material in the insect's body.

Menka and Gupta (2013) found that the effect of novaluron on prolongation of *Euproctis icilia* the life span was less than buprofezin which exhibited greater increase in the larval longevity.

Table 2. Latent effect of nomolt on the larval and pupal durations, pupation and adult emergence percentages in *Spodoptera littoralis* pretreated as 4thinstar larvae during developed resistance in the pest to the insecticidal action

No. Generations	Mean larval duration (days ± S.E.)	Mean pupal duration (days ± S.E.)	Pupation (%) + % reduction	Adult emergence (%) + % reduction
Control	10.0^{e}±0.32	10.5^{b}±0.3	97.0	90.08
Treated parent (F0)	11.25^{d}±0.25 (12.5)	10.9^{b}±0.3 (3.81)	56.7 (42.3)	82.09 (8.9)
F1	12.01^{cd}±0.29 (20.1)	12.5^{a}±0.29 (19.05)	55.0 (43.3)	70.33 (21.9)
F3	11.75^{d}±0.25 (17.5)	11.5^{ab}±0.61 (9.52)	43.85 (54.8)	37.71 (58.1)
F6	13.5^{b}±0.29 (35.0)	11.0^{b}±0.41 (4.76)	27.35 (71.8)	55.06 (38.9)
F8	14.5^{a}±0.62 (45.0)	10.5^{b}±0.29 (0.0)	24.66 (74.6)	41.18 (54.3)
F10	12.8^{bc}±0.41 (20.8)	12.7^{a}±0.29 (20.95)	33.56 (65.4)	43.65 (51.5)
F. Value	23.81***	5.2333**		
L.S.D.	0.9047	1.2024		

Numbers between brackets presented % prolongation in case of larval and pupal durations and % reduction in case of pupation and adult emergence percentages.
Numbers of the same letters have no significant difference

3. Biochemical studies:

Results illustrated in Tables 3 showed the changes in alpha and beta non-specific esterases, chitinase and protease activities, in six days after treatment of the 4th instar larvae of *S. littoralis* during selection with nomolt. The data expressed as percentages either increase or decrease in the enzymatic activities.

During selection with nomolt, the activities of α-esterase showed significant difference in the selected generations comparing with control. At the beginning, in the

treated parent (F0), the activity of α esterase were increased significantly from 529.97 µg α-naphthyl acetate/ min/ mg in the normal larvae to 632.93 then this activity increased to reach 983.45 and 992.58 µg α-naphthyl acetate/ min/ mg in F3 and F6 generations, respectively. Again the activities were decreased to reach 509.60µg α-naphthyl acetate/ min/ mg in F10 generation more than the control**.**

Assummarized in Table (3), β-esterase activity were significantly decreased in the treated parent (247.31 µg β-naphthyl acetate/ min/ mg) compared with the control which recorded 507.21 µg β-naphthyl acetate/ min/ mg then this activity was significantly increased to be 612.79 µg β-naphthyl acetate/ min/ mg in F3. In F6 and F10, the level of activity decreased to reach 479.21 and 497.89µg β-naphthyl acetate/ min/ mg, respectively as compared with the control.

It could be concluded that nomolt caused a significant decrease or increase alternation in the activities of α- and β-esterases, in the treated larval instar. The changes in esterases activities during the larval life was coincide with the titer of JH (Riddiford and truman, 1978).

The variation in the measused esterases activity by nomolt treatment indicated that, these enzymes may play an important role in resistance in the cotton leafworm *S. littoralis* to chitin synthesis inhibitors toxicity. In this respect, Farag (2005) suggested that the most of tested *S. littoralis* field strains tissue showed a high activity of esterases than the laboratory strain with exception in haemolymph. It was concluded that the increase in esterase activities could play an important role in cotton leafworm resistance development to nomolt action. Bakr *et al*. (2010) evaluated the effect of sublethal doses LC_{25}, LC_{50} and LC_{90} of Cascade (Flufenoxuron) on enzymatic activities against 2^{nd} and 4^{th} larval instars of *S. littoralis*. The treated larvae showed a significant decrease in enzyme activities of acid phosphatase as well as α, β-esterases non- specific esterases, at different intervals times post treatments.

The activity of chitinase enzyme of untreated *S. littoralis* larvae was 20.94 µg N-acetylglucosamine (NAGA)/min/g. The results showed high significant increase in the treated larvae with nomolt in F3, F6 and F10 which generation being 33.35, 28.70 and 28.77 µg NAGA/min/g, respectively. On the other hand, significant decrease was recorded in the treated parent (F0) which recorded 15.88 µg NAGA/min/g comparing with that in the untreated larvae.

The previous results are going in line with those published by Al-Shannaf *et al*. (2012). They declared that atabron and admeral gave highly significant increase in the activity of chitinase enzyme. On contrast, Sabry (2007) revealed that, atabron, cascade and Xentari reduced the chitinase activity of *P. gossypiella* resistant strain to especially in cascade -resistant field strain.

As shown in Table 3, the activity of protease enzyme in the treated parent was significantly decreased from 293.60 µg casein/min/ mg in the control to 156.25

μg casein/min/ mg in the treated parent then this activity was increased to reach 280.21 μg casein/min/ mg in F3. This activity significantly increased to 422.57 μg casein/min/ mg in F6 and then it was decreased to 305.02 μg casein/min/ mg in the generation.

It is interest to note that protease enzyme could play an important role in the resistance development throughout protein digestion by releasing amino acids from the peptides. The results are accordance with those published Sabry (2007). They stated found that the activity of protease was increased in Xentari resistant strain and chitin synthesis inhibitors resistant strain in the pink bollworm *P. gossypiella*.

Table 3. enzymes activities of *S. littoralis* larvae after six days of treatment as 4th instar larvae with at LC_{50}values of nomolt.

Treated Generations No.	α-Esterase μg α- naphthyl acetate/ min/ mg	β-Esterase μg β-naphthyl acetate/ min/ mg	Chitinase μg NAGA/min/g	Protease μg casein/min/ mg
Control	529.97^{c}± 18.47	507.21^{b}± 7.56	20.94^{c}±0.556	293.60^{b}± 8.987
Treated parent(F0)	632.93^{b}± 17.01	247.31^{d}± 3.30	15.88^{d}± 0.441	156.25^{c}± 4.701
F3	983.45^{a}± 36.91	612.79^{a} ± 9.65	33.35^{a}± 0.978	280.21^{b}± 8.621
F6	992.58^{a}± 35.29	479.21^{c}± 8.13	28.70^{b}± 0.825	422.57^{a}± 13.098
F10	509.60^{c}± 17.28	497.89^{bc}± 8.45	28.77^{b}± 0.824	305.02^{b}± 9.306
F. Value	81.906^{***}	302.933^{***}	87.0346^{***}	102.989^{***}
L.S.D.	83.7076	24.3268	2.3637	29.3688

Numbers of the same letters have no significant difference.
NAGA: N-acetylglucosamine

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تطور الحساسية فى يرقات دودة ورق القطن المعاملة بمركب النومولت وعلاقتها بالتغيرات البيولوجية والكيميائية فى جسم الحشرة

الشيماء نجيب إبراهيم[1]– عزة كمال إمام[2]– قدري وشاحى محمود[2]– عزيزة السيد عبد العال[1]

1. *معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي– جيزة – مصر.*
2. *قسم وقاية النبات– كلية الزراعة – جامعة عين شمس– شبرا الخيمة – القاهرة– مصر.*

أجري هذا البحث لدراسة حساسية يرقات العمر الرابع لحشرة دودة ورق القطن (رتبة حرشفية الأجنحة) لمركب نومولت، حيث تمت تغذية اليرقات على أوراق الخروع المعامله بتركيزات مختلفة من المركب لمدة 48 ساعة ثم قدم لهم أوراق غير معامله وتم حساب التركيزات نصف المميته فى اليوم السابع بعد المعامله. وأجريت المعامله في كل جيل حتى الجيل العاشر مع حساب التركيزات نصف المميتة في كل جيل. وقد سجلت النتائج زيادة نسبة المقاومة إلى 1,2709 ضعف بعد الجيل الثالث ثم إلى 1,2894 و 2,3034 و 3,0117 ضعف فى الجيل السادس و الثامن والعاشر على التوالى. كما زاد العمر اليرقي بالمقارنة باليرقات غير المعاملة أدت المعاملة إلى إنخفاض نسبة التعذر ومعدل خروج الفراشات خلال الأجيال تحت الدراسة حيث كانت النسبه المئوية للتعذر 56,7، 55,0، 43,85، 27,35، 24,66 و 33,56 وكان النسب المئوية لخروج الفراشات 82,09، 70,33، 37,71، 55,05، 41,18 و 43,65 لجيل الآباء، الجيل الأول، الجيل الثالث، الجيل الثامن و الجيل العاشر على التوالى.

أظهرت النتائج ارتفاعا فى نشاط الإنزيمات المختبرة بعد 6 أيام من المعاملة ليرقات العمر الرابع إرتفاعا وإنخفاضا خلال الأجيال العشره المختبره.