Effect of Recombinant Baculovirus on the Mulberry Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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ABSTRACT

A system of the baculovirus *Bombyx mori* nucleopolyhedrovirus (BmNPV) and silkworm larvae, *B. mori*, was applied to analyze the potential use of the insect-specific toxin for improving the efficacy of recombinant baculoviruses for pest control. Recombinant baculoviruses carrying a gene encoding an insect specific natural toxin, scorpion toxin, AaIT and LqhIT2 have been constructed. Larvae were infected with BmNPV(T3), Bm AaIT and BmLqhIT2 by injection.

The present resul. , suggest that LqhIT2 is more efficient than AaIT for improving the insecticidal activity of baculoviruses. The reduction in the development rate in silkworm larvae averaged 0.290gm in the treatment with LqhIT2. BmLqhIT2 was more effective than AaIT in reducing the development rate of *B. mori* larvae. The 4th larval instar was more susceptible than 5th instar. The body weight in 4th instar was 0.145gm and 0.665gm in 5th instar larvae.

Keywords: scorpion insect-specific toxins, LqhIT2, Bombyx mori nucleopolyhedrovirus.

INTRODUCTION

The combination of molecular biology and biotechnology has put the recombinant baculovirus far ahead of other genetically engineered bioagents. Baculovirus is a natural insect virus that infects insects and kills their larvae. Baculoviruses are invertebrate-specific viruses characterized by circular double-stranded DNA genomes and enveloped rod-shaped virions. The family Baculoviridae is composed of three subgroups, nuclear polyhedrosis viruses (NPVs), granulosis viruses and nonoccluded baculoviruses NPVs have been extensively used as vectors for the expression of foreign genes in insect cells and larvae (Maeda, 1994). Although NPVs have been used as agents for insect pest control since the 1930s, their use in agriculture has been limited (Maeda, 1995; Miller, 1995). The baculovirus expression vector system has been used extensively to produce numerous proteins originating from both prokaryotic and eukaryotic sources. In addition to easy cloning techniques and abundant viral propagation, the system's insect cell environment provides eukaryotic post-translational modification machinery. The baculovirus displaying vector system provides a number of advantages over prokaryotic systems (Anna *et al.*, 2007). Recombinant baculoviruses carrying various toxin genes have been constructed to increase their insecticidal effects.

Among the foreign genes that have been considered for the improvement of viral insecticides, several genes have already been expressed and analyzed. Diuretic hormone expressed in silkworm (*Bombyx mori*) larvae by a recombinant baculoviruses was the first to show an induction of insecticidal activity, by reducing the volume of hemolymph (Maeda, 1989b). A number of different scorpion venom-derived peptide toxins which target sodium channels have been isolated and characterized (Zlotkin *et al.* 1991).

The insecticidal effects of a recombinant AaIT have been studied in insects by injection of recombinant baculoviruses carrying the AaIT gene (Maeda *et al.*, 1991; McCutchen *et al.*, 1991) and the insecticidal effect of a recombinant LqhIT2 have been studied (Somaia, 1997; Noriko *et al.* 2000). AaIT and LqhIT2 have shown synergy when injected into *Heliothis virescens* (Herrmann *et al.*, 1995).

Aim of the present investigation is to determine the effect of recombinant baculoviruses carrying scorpion toxin genes on the developmental rate of 4th and 5th larval instars of mulberry silkworm *B. mori*.

MATERIALS AND METHODS

The mother colony of the mulberry silkworm *Bombyx mori* was maintained under laboratory conditions of 27±2°C and 60±5% R.H. The newly hatched larvae and all instars were reared in carton boxes on mulberry leaves. The newly moulted 4th and 5th instar larvae were used in the present work.

Recombinant BmNPV expressing AaIT and recombinant BmNPV expressing LqhIT2 were examined in this work.

The two scorpion toxin genes, were *Androctonus australis* Insect Toxin (AaIT) and *Leiurus quinquestriatus hector* Insect Toxin (LqhIT2). AaIT consists of 70 amino acids translated from 210 nucleotides coding sequence while LqhIT2 consists of 61 amino acids translated from 183 nucleotides coding sequence. AaIT was created by Maeda *et al.* (1991) as follows:

SacI

GACCTCATGAAGATACTCCTTGCTATTGCATTAATGTTGTCAACAGTAATGTGGGTGTCAACAAAAAAAAAACGGCTACGCTGTTGACTCTTCTGC

M K I L L A I A L M L S T V M W V S T K K N C Y A V D S S C

<.....Bombyxin Signal Sequence .....>II<.....

AAAGCTCCGGAATGCCTGCTGTCTAACTACTGCAACAACCAGTGCACTAAAGTTCATTACGCTGACAAAGGCTACTGCTGCCTGCTGTCTTGCTC

K A P E C L L S N Y C N N Q C T K V H Y A D K C Y C C L L S C Y

..... Scorpion Neurotoxin Sequence .....

XbaI

TGCTTCGGCCTGAACGACGACAAAAAAGTTCTGGAAATCTCTGACACTCGTAAATCTTACTGCGACACTACTATCATCAACTAGGATCCTCTAGA

C F C L N D D K K V L E I S D T R K S Y C D T T I I N*

.....>I

The LqhIT2 gene was modified from the original gene LqhIT. The synthetic coding sequence of the scorpion toxin gene LqhIT2 was chemically synthesized by a DNA synthesizer, (Somaia 1997), as shown.

HindIII BglII

AAGCTTAGATCTATGAAAATCCTTCTCGCCACCGCTCTAATGCTGAGCACTGTGATGTGGGTTTCGACAGACGGCTACATTAAGCGTCGCGATGGTTG

M K I L L A T A L M L S T V M W V S T D G Y I K R R D G C

<.....Bombyxin Signal Sequence.....> II <.....

CAAAGTCGCATGTTTAATAGGAAACGAAGGGTGTGACAAGGAGTGCAAAGCGTATGGCGGATCCTACGGATACTGCTGGACCTGGGGCTTAGCCTGT

K V A C L I G N E G C D K E C K A Y G G S Y G Y C W T W G L A C

..... Scorpion Neurotoxin Sequence .....

TGGTGTGAAGGACTTCCCGATGACAAGACGTGGAAATCTGAGACGAATACCTGTGGATAAGAATTC.

W C E G L P D D K T W K S E T N T C G *

.....> EcoR1

A sequence specifying the bombyxin signal peptide was fused to the sequence encoding the LqhIT2. The complete gene was cloned between HindIII and EcoRI sites in a pUC19-based plasmid. According to Maeda, (1989a), the recombinant plasmid was cotransfected to the baculovirus, *Bombyx mori* nucleopolyhedrovirus (BmNPV).

T3 virus (BmNPV), BmAaIT and BmLqhIT2 (University of California, Davis, CA, USA, personal communications- shipped in liquid nitrogen).

The effect of AaIT and LqhIT2 on the development rate of the mulberry silkworm was tested by injection in the intersegmental membrane of the thoracic segments of *B. mori* larvae at a dose of 30 µl/larva after moulting using microsyring (Somaia, 1997). Three replicates each of 15 larvae were treated. Two control groups of larvae were used, one of them was injected with T3 virus and the second control reared normally. The treated larvae were reared in petri dishes. The *B. mori* larvae from each replicate, were weighted and recorded daily, zero, 24, 48, 72 and 96 hrs.

The data were statistically analyzed to check the significance of difference between treatments using F test and L.S.D. test, according to Snedcor and Cochran, 1980.

RESULTS AND DISCUSSION

The effect of BmAaIT and BmLqhIT2 on the development rate measured at the body weights of 4th and 5th instar larvae at zero h., 24, 48, 72, 96 hrs. Table (1) illustrated the analysis of variance for larval body weight as affected by viruses (A), instars (B),time after injection and their interactions. Significant responses to virus type, instar of larvae and time after injection ($p \geq 0.05$) were recorded. First order (viruses × time) and (instar × time) and second order (viruses × instar×time) interactions had not reached the level of significance. Insignificant first order interactions between viruses and time mean that the studied viruses behaved the same at various periods in treated larvae. Also, the insignificant instar × time interaction refer to stable response of instars to time after injection. Meanwhile, the significant viruses × instar interaction, indicate that, instars responded differently to the studied viruses. Similary, the insignificant second order interaction among viruses × instar×time refere to stable body weight response with variable viruses, instars and time after injection. Figure (1) showed the relation between virus type and larval weight over times and instars. LqhIT2 was significantly the most effective in reducing body weight (about 32.24% relative to control) Meanwhile, AaIT was insignificantly similar to control.

Table1: Analysis of variance for mulberry silkworm larvae body for weight development as affected by viruses, instars and time after injection.

Source of variation	df	Sum of squares	Mean squares	F value	
				estim.	Table
Viruses (A)	3	2.862	0.954**	19.733	2.61
Instars (B)	1	40.584	40.584**	839.478	3.85
A × B	3	2.348	0.783**	16.1914	2.61
Time (C)	4	0.344	0.086 NS	1.781	2.83
A × C	12	0.940	0.078 NS	1.620	1.84
B × C	4	0.268	0.067 NS	1.385	2.83
A × B × C	12	0.713	0.059	1.229	1.84
Error	480	23.206	0.048		

** highly significant at 0.01 level of probability
NS; Not significantly different.

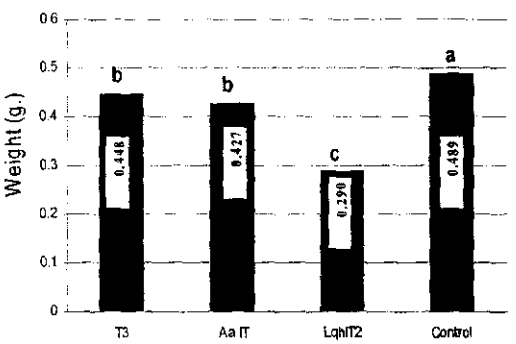

Fig. 1: The relation between virus types and larval weight over times and instars.

Figure (2) illustrated the variable response of larval, weight development over the studied viruses and times. It was valuable to notice that, the fourth instar larvae were significantly suppressed by viruses over times. The recorded value of larval body weight development at 4th instar reached about 22% of the respective recoded value for 5th instar., the average of the body weight in 4th instar was 0.145gm comparing with 5th instar (0.665gm). This results agree with those of (Somaia 1997); the treated larvae with LqhIT2 stopped moving, the lack of movement was probably due to LqhIT2 induced paralysis resulting from LqhIT2 expression by BmLqhIT2.

The significant first order interaction between the studied viruses and larval instar are presented in Figure (3). The most significant suppressive effect was expressed by LqhIT2 at fourth larval instar. The second rank of larval body weight suppression was expressed by AaIT at 4th instar. The third rank of suppression was represented by the effect of T3

virus. In the mean times, larval body weight of 5th instar larvae was significantly reduced by LqhIT2. This results agree with larvae injected with LqhIT2 that started to die at 96hrs compared with BmAaIT larvae which died between 97 and 105h and BmNPV(T3) larvae died between 128and 144 h post injection (Maeda *et al.*, 1991; Ohkawa *et al.*, 1994).

Values of larval body weight at various times after injection with viruses, over viruses and instars are presented at Figure (4). Although, the rate of development in larval weight had not reached the level of significance, it was obvious that a reasonable reduction in weight was expressed after 24h after injection and then started reducing weight after72 hrs. Commonly; from this work we concluded that BmLquIT2 is more effective in silkworm than T3and BmAaIT in reducing weight, and the 4th instar more susceptible than 5th instar larvae.

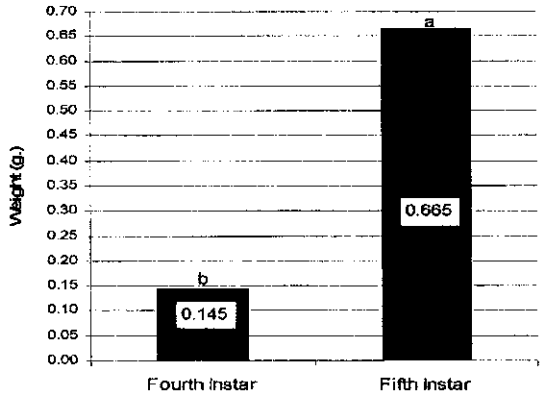

Fig. 2: Larval body weight for fourth and fifth instars larvae of *B. mori* affected by viruses and time.

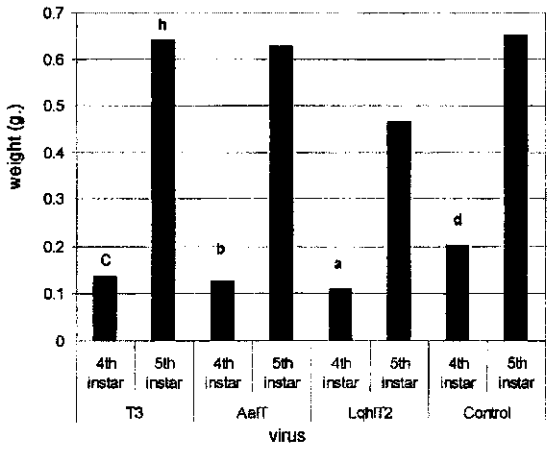

Fig. 3: The interaction between viruses and larval instars as body weight over time of injection in *B. mori*.

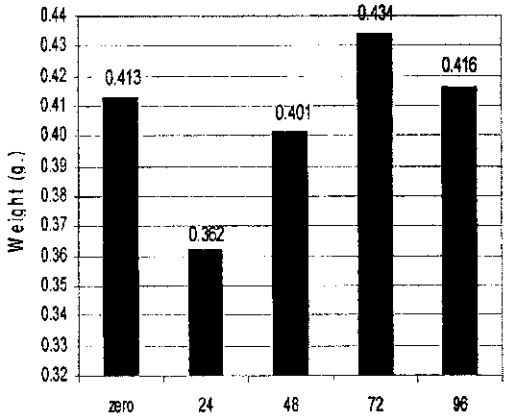

Fig. 4: Effect of time after injecting larvae on body weight over viruses and times in *B. mori*.

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الملخص العربى

تأثير فيروسات الباكولوفيروس المهندس وراثيا على يرقات دودة حرير القز

سمية السيد على السيد

قسم علم الحشرات التطبيقى ــ كلية الزراعة ــ جامعة الإسكندرية

تناولت هذه الدراسة تأثير بعض السموم الطبيعية من أصل حيوانى (سموم العقرب المتخصصة ضد الحشرات وذلك ضد دودة حرير القز). حيث تم فى هذا البحث استخدام اثنين من سموم العقرب المهندسة وراثيا لدراسة تأثيرها على معدل نمو يرقات دودة حرير القز وتمت هذه الدراسة على يرقات العمر الرابع والخامس. وذلك عن طريق الحقن فى غشاء ما بين الحلقات الصدرية بجرعة ٣٠ ميكروليتر لكل يرقة. قد اظهرت النتائج أن فيروسات الباكولوفيروس المهندسة وراثيا والمحتوية على الجينات LqhIT2, AaIT فروقاً عالية المعنوية بين الفيروسات بحوالى ٠,٩٥٤ حيث تم الحصول على وزن ٠,٢٩٠جرام بالمعاملة بـ LqhIT2 . كما اظهرت النتائج معنوية عالية بين الأعمار حيث أظهر العمر الرابع حساسية عالية عن العمر الخامس عند الحقن بـ LqhIT2 حيث أظهرت أنخفاض كبير فى أوزان يرقات العمر الرابع.