Minufiya J. Agric. Res. Vol. 34 No.2: 755 - 762 (2009) "http://www.mujar.net"

EFFICACY OF THE IMPACT OF SUBLEATHAL DOSAGES OF NUCLEAR POLYHEDROSIS VIRUS AGAINST SPODOPTERA LITTORALIS (BOISD) LARVAE

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(Received: Feb., 24, 2009)

ABSTRACT: *The relative susceptibility of 2^{nd} and 4^{th} instar of Spodoptera littoralis (Boisd) larvae to nuclear polyhedrosis virus was studied by bioassay in the laboratory .The obtained results showed that larval mortality increased with increased dosage , whereas the dosage incubation relationship was reversed . Larval age inversely affected mortality and incubation. The number PIBs / larva required to reduce 50 % mortality in 2^{nd} and 4^{th} larval instars were 48 and 124 polyhedral inclusion bodies (PIB'$_S$ / Larval) .The median lethal doses calculated as number of PIB'$_S$ / larva body weight showed that 2^{nd} and 4^{th} instar larvae were only three more susceptibility to virus fourths . The LT_{50} values for 3 x 10^7 , 3 x 10^6 , 3 x 10^5 , 3 x 10^4 and 3 x 10^3 PIB'$_S$ / Larval were 2.9, 3.4, 4.5, 4.9 and 5.2 days respectively in 2^{nd} instar assay .Compared with the LT_{50} values for 3 x 10^7 , 3 x 10^6 , 3 x 10^5 , 3 x 10^4 and 3 x 10^3 PIB'$_S$ / Larva were 6.2, 7.5, 4.8, 9.3 and 10.2 days respectively in 4^{th} instar assay.*

Key word : *Spodoptera littoralis (Boisd) , nuclear polyhedrosis virus (NPV)*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd) has been and still is a serious pest of cotton and other crops in Egypt, this pest seems to be more difficult to control with various insecticides. The insecticidal value of entomogenous microorganisms depends on their infectivity and pathogenicity to target pests and disease vectors. Much effort has gone into investigations on the qualitative and quantitative values of such pathogens as agents for microbial control, in both the field and in the laboratory (Lomer *et al*. (1999), Huber (2003), Miller (1990), Vail *et al.* (2005) and Podgywaite, 1999). Lack of information on the quantity of test pathogen consumed by test insects makes comparison of the infectivity of different pathogens, or even the same pathogen tested at different times. This in turn would lead to difficulty in the standardization of insect pathogenic microorganisms and hence also in the evaluation of their potential as agents for microbial control. This factor has attracted considerable attention in recent times.

The present investigations were intended to determine whether a nuclear polyhedrosis virus (NPV) can be effectively used to control *Spodoptera littoralis in* Egypt. The precision of the assay estimates hingedmainly on the used larvae that had completely consumed a known quantity of infective material.

MATERIALS AND METHODS

All experiment were conducted under controlled conditions of temperature 26ºC ± 2ºC and relative humidity 65-70%. This work has been conducted under controlled conditions in the Cotton Leafworm Research Division Plant Protec. Res. Inst., Agric. Res. Center, Dokki, Giza, Egypt.

One hundred of the 2^{nd} and 4^{th} instar *Spodoptera littoralis* (Boisd) larvae was maintained for each replicate. Caster oil leaves were used for feeding purposes of larvae reared under the different concentration.

Preparation of polyhedral inclusion bodies :-

Cadavers of *Spodoptera littoralis* larvae dead from NPV infection were triturated in 20 ml of cold sterilized water. The aqueous (pH 7.4) semipurified PIB suspension was brought to the top of cellulose nitrate tubes (15 ml) containing four-layered sucrose gradient 35- 65% (w/w) at 10% intervals, and purified further according to the method of Grzywacz, *et al.* (1997). The clean PIBs were then suspended in sterile distilled ice water and centrifuged at 8500g for 30 min. The pellet of polyhedra collected at the bottom of the tube and was finally suspended in sterile distilled water, pH 7.4. The suspension (concentration 1.3×10^9 PIBs / ml) was back diluted to give five levels of PIB concentration. Freshly molted second and fourth instar larvae. To estimate the pathogenicity of the virus six decimal dilutions of the stock suspension were each tested against 100 larvae. Each treatment was replicated four times on 2^{nd} larvae and twice on 4^{th} instar .

RESULTS AND DISCUSSION

Dose mortality data for both 2^{nd} and 4^{th} instar *Spodoptera littoralis* (Boisd) are summarized in Table (1) . Mortality increased directly with dosage , but susceptibility to virus was inversely affected by the age of the larvae . Dosages of $3x10^6$ and $3x10^7$ PIBs / larva) only caused over 90% and 82% mortatlity when applied during the 2^{nd} and 4^{th} instar ,respectively . None of the control larvae died from disease. The pathogenicity of the NPV for 2^{nd} ana 4^{th} instar larvae was compared by plotting the larval mortality as probits (Finney, 1971) This result was in agreement with (Lynn *et al.* 1990. Butani *et al*. 1997 and Belisle *et al.* 1990) . May have caused some deviation of laraval responses from the straight line . Since variation of mortality

responses were not excessive and the values for (g) were less than unity, the normal deviate for the 5% level of probability was used as multiplier of the standard error of (y) to obtain the limits of probit mortality. These limits for the expected 10,30,50,70,and 90% lethal doses were tabulated as PIBs / larva or PIBs / mg body weight (Table 2) .A comparison based on the median lethal doses (LT_{50}) showed that 2^{nd} instar larvae were about two times more susceptible to virus than 4^{th} instar larvae .However , only a twofold difference between the same two stages was obtained when the LT_{50} doses were calculated as number of inclusion bodies / mg of body weight . Further, the dosage –mortality lines indicated that 928 and 4.982 PIBs / larva or 48 and 124 PIBs/ mg body weight were sufficient to induce lethal infections in a few 2^{nd} and 4^{th} instar larvae, respectively .This results agree with Jones et al (1993) .

Table (1) Number PIBs / Larva required to produce 10, 30, 50, 70 and 90 % mortality in 2^{nd} and 4^{th} instar of *Spodoptera littoralis* (Boisd).

% mortality	2^{nd} Instar Larvae			4^{th} Instar Larvae		
	PIBs / larva	95% fiducial limits		PIBs / larva	95% fiducial limits	
		Lower	Upper		Lower	Upper
10	85	59	154	387	184	582
30	315	146	537	1.542	824	1.254
50	928	752	1.894	4.982	2.498	9.245
70	2.482	1.845	4.587	9.542	2.985	17.254
90	15.249	7.246	28.749	47.254	13.592	245.321

The time of mortality at different concentrations of the virus fed to 2^{nd} and 4^{th} instar larvae was studied (Table 3) . Mortality of 2^{nd} and 4^{th} instar larvae feeding on dosages of $3x10^7$, $3x10^6$, $3x10^5$, $3x10^4$ and $3x10^3$ PIBs / larva first occurred on the 2.9 days. 4^{th} instar larvae treated with the same concentrations began to die after 6.2 and 10.2 days, respectively. An incubation time of 6 and 9 days was required for initial mortality ,respectively. In 2^{nd} and 4^{th} instar larvae exposed to $3x10^5$ PIBs / larva. The LT_{50} values, estimated according to (Jons 1999). In general the time of mortality at a given dosage was longer in the older larvae with a fourfold increase in body weight of the larvae the median lethal time increased 5.2 and 10.2 days, respectively , at all dosages of $3x10^7$, $3x10^6$, $3x10^5$, $3x10^4$ and $3x10^3$ PIBs/larva. The increasing slope difference at the given decreasing concentrations provided evidence that the course of the disease was significantly altered by the age of the larvae at the lower dosage .With concentrations producing less than 50% mortality, the relation between larval age and period of lethal infection was reversed .(Cherry *et al*. 1999). It should be noted that the interval between any two LT_{50} times was statistically significant .

Table (2) Number PIBs / body wight required to produce 10, 30, 50, 70 and 90 % mortality in 2nd and 4th instar of *Spodoptera littoralis* (Boisd).

% mortality	2nd Instar Larvae			4th Instar Larvae		
	PIBs/Body wight	95% fiducial limits		PIBs/Body wight	95% fiducial limits	
		Lower	Upper		Lower	Upper
10	9	5	14	17	9	19
30	15	11	23	29	18	35
50	48	32	59	124	98	169
70	164	101	235	397	248	421
90	958	358	1.265	2.457	1.987	3.105

From the above mentioned results it may be concluded that the *Spodoptera littoralis* (Boisd) is highly susceptible to the NPV larval mortality susceptible to the NPV larval mortality varied with dosage. However a twofold resistance to virus infection was developed by the larvae with a fourfold increase in body weight . The dosage incubation relationship was reversed. The dosage –incubation relationship was reversed. At a given dosage of virus inoculum, the period of lethal infection .The larvae surviving a second molt after virus ingestion developed and emerged normally comparable to the controls this results agree with Eilenberg (2000) , Granados *et al* (2001) , Keller et al (1996) , Wood and Granados (1991) and Wu *et al* (1999).

Table (3) The LT_{50} values and 95% fiducial limits for 2nd and 4th *Spodoptera littoralis* (Boisd). Larval instar treated with nuclear polyhedrosis virus .

No. PIBs / larva	2nd Instar Larvae			4th Instar Larvae		
	LT_{50} / days	95% fiducial limits		LT_{50} / days	95% fiducial limits	
		Lower (days)	Upper (days)		Lower (days)	Upper (days)
3×10^7	2.9	2.8	3.8	6.2	5.4	8.1
3×10^6	3.4	3.1	4.2	7.5	6.7	9.1
3×10^5	4.5	4.2	5.1	8.4	7.1	9.2
3×10^4	4.9	4.8	5.9	9.3	8.4	9.9
3×10^3	5.2	5.1	6.4	10.2	9.4	11.4

According to the result given in (Table 4) all larval stages ranging from very susceptible to apparently resistant were included in these tests. As the larvae matured an inverse relationship between mortality and larvae age (weight) became apparent. This decrease in susceptibility can be measured either as a decrease in mortality. This apparent increase in resistance could be partially due to the normal increase in a body weight which may serve to dilute the constant virus dose . Another factor of importance to consider when evaluating resistance based on maturation is that the incubation period is never less than two days and increases up to five days with increasing larval age , The apparent total resistance of older larvae could be due to the fact that pupation takes place before the virus can exert its influence on the larval this would especially apply when final-instar larvae are infected .

Table (4) Mortalities , LT_{50} value and *b* -value of *Spodoptera littoralis* (Boisd) larvae of different ages treated with $1.4x10^5$ Polyhedral inclusion bodies

Larvae age (days)	Larvae (mg)	No. larvae treatment	Average Mortality %	LT_{50} (days)	*b*-Value
Newly hatched	_	25	98.2	3	-
4	0.00785	100	97.5	4	1.72
5	0.02985	100	90.8	5	1.31
6	0.05435	100	86.2	8	0.34
7	0.12318	100	53.8	12	0.22
8	0.15089	100	48.2	13	0.21
9	0.16243	100	33.2	14	0.20
	0.38512	100	11.0	0	0.20

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تقييم التاثير للجرعة النصفية غير المميتة لفيروس NPV ضد يرقات دودة ورق القطن الكبرى

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الملخص العربى

يهدف البحث الى دراسة حساسية كل من يرقات العمر الثانى و الرابع لدودة ورق القطن للجرعة النصفية غير المميتة لفيرس النبوكيلير بوليهيدروزيس فيرس NPV و قد أوضحت النتائج الاتى :

أدت زيادة الجرعة إلى زيادة نسبة الموت لكل من يرقات العمر الثانى و الرابع لدودة ورق القطن بزيادة الجرعة .

وجدت علاقة طردية بين عمر اليرقات و الجرعة و الوقت اللازم لحدوث موت اليرقات.

عدد وحدات PIB اللازم لقتل ٥٠% من يرقات العمر الثانى و الرابع لدودة ورق القطن كان ٤٨ و ١٢٤ PIB لكل يرقة على التوالى .

أظهرت يرقات العمر الثانى حساسية للجرعة النصفية المميتة تعادل ثلاث مرات عن مثيلتها من يرقات العمر الرابع لدودة ورق القطن

وجد أن الوقت اللازم لقتل ٥٠% من يرقات العمر الثانى للجرعات المختلفة كان كالاتى : ٢.٩ و ٣.٤ و ٤.٥ و ٤.٩ و ٥.٢ للجرعات - 3X106- 3X105 -3X104 -3X103 3X107على التوالى

كان الوقت اللازم لقتل ٥٠% من يرقات العمر الرابع للجرعات المختلفة كان كالاتى : ١٠.٢ و ٩.٣ و ٨.٤ و ٧.٥ و ٦.٢ للجرعات - 3X106- 3X105 -3X104 -3X103 3X107على التوالى .