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THE VIRULENCE OF TWO BIOINSECTICIDES (PROTECTO AND VIRUSET) AND THEIR EFFICACY ON SOME BIOLOGICAL ASPECTS OF *Spodoptera littoralis* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Two commercial bioinsecticides; Protecto (*Bacillus thuringiensis* var. *kurstaki*) and Viruset (*Spodoptera littoralis*/NPV*)* were bioassayed against the cotton leaf worm (CLW), *Spodoptera littoralis* (Boisd.) (2^{nd} and 4^{th} instar larvae). The obtained results reveal that Protecto was the most potent bioinsecticide compared with Viruset. Treatment of 2^{nd} and 4^{th} instar larvae with LC_{50}'s of Protecto and Viruset affected estimated biological aspects. The larval, pupal, and adult longevity durations were prolonged due to treatment. It was also observed that egg number and hatchability were affected by treatment.

INTRODUCTION

The cotton leafworm (CLW), *Spodoptera littoralis* is a polyphagous pest in Egypt. Without a hibernation period, cotton leafworm is active all year round, attacking cotton as well as more than 29 hosts from other crops and vegetables (Temerak, 2006). The rate of cotton leafworm infestation can reach up to 50,000 egg-masses/acres, causing severe damage to leaves, buds, flowers, and bolls (Temerak, 2006).

Development of an effective control method against the cotton leaf worm, *S. littoralis* is urgently needed since it does serious damage to many important agricultural crops in Egypt. There is a serious interest in the use of microbial insecticides for biological control of the cotton leafworm, as alternatives to chemical control, since they neither leave toxic chemical residues in the environment nor do they develop resistance in their insect hosts (Ahmed and El-Katatny, 2007). And hence, the public awareness and concern for environmental quality, has led to more focused attention on research aiming at developing biological agents. A promising strategy with good potential to control insect pests and, at the same time, to minimize the adverse effects of chemical insecticides is the use of microbial agents (Ahmed and El-Katatny, 2007).

These groups have unique modes of action and their properties may differ considerably from the conventional agents with whom growers are familiar (Asher, 1993 and Thompson *et al.*, 1999).

The virulence of two bioinsecticides; Protecto® (*Bacillus thuringiensis* var. *kurstaki*) and Viruset® (*Spodoptera littoralis* NPV [*Spli*NPV]) on 2^{nd} and 4^{th} instar larvae of *S. littoralis* was evaluated. In addition, the efficacy of tested agents on some biological aspects of both larval instars was evaluated.

MATERIALS AND METHODS

Used insects:

A laboratory susceptible strain of the cotton leaf worm, *Spodoptera littoralis*, was reared for more than 10 generations. It was obtained from the Research Division of the cotton leaf worm, Plant Protection Research Institute. Insects were reared under controlled conditions in an incubator at 27 ± 2ºC, of 65 ± 10% R. H., and 8:16 L: D photoperiod at the Plant Protection Research Institute, Dokki-Giza, Egypt.

Chemical used:

Two commercial bioinsecticides; Protecto® (*Bacillus thuringiensis kurstaki*) and Viruset® (*Spodoptera littoralis* NPV) as wettable powders were evaluated for their toxicity on the CLW, *S. littoralis*. The tested bioagents were obtained from Plant Protection Research Institute Biopesticide Unit Production. Serial dilutions of Protecto and Viruset (9.4%) were prepared using 1g the wettable powder of each and dissolved in 100 ml of water.

Bioassay tests:

Two sets of five replicates each contain 10 newly molted 2^{nd} and 4^{th} instar larvae for each concentration of each tested product were used. Treatment of larvae was conducted by the leaf dipping technique. Fresh and clean castor leaves, *Ricinus communis* L., were immersed for 10 sec. in the prepared suspensions of the tested compounds. The treated leaves were then left to dry at room temperature before being offered to the newly molted 2^{nd} and 4^{th} instars of *S. littoralis* larvae. Larvae were left to feed on treated leaves for 48-h. They were then offered fresh clean leaves. The same numbers of larvae were used for check experiments in which larvae were offered fresh clean castor leaves dipped in water. Mortality was recorded daily and cumulative mortalities were recorded up till pupation.

Biological studies:

Larvae which survived treatment with LC_{50} of the tested bioagents were observed for the following biological aspects: the duration of the rest of larval stage, pupation rate, duration of the pupal stage, adult emergence, reproductive potential of moths (fecundity per female and fertility per egg mass), and longevity of adult moths.

Statistical analysis:

Mortality rates were corrected according to Abbott's formula (Abbott, 1925) and plotted against concentrations as log/Probit regression lines. LC_{25}, LC_{50}, and LC_{90} values as well as the slope of the lines were calculated (Finney, 1971) using "LdPLine®" software [http://embakr.tripod.com/ldpline/ldp line.htm]. Means were tested for significance by the one way analysis of variance (ANOVA) using SPSS statistics 17.0 release 17.0.0 software.

RESULTS AND DISCUSSION

1-Virulence of tested compounds on 2nd and 4th instar larvae of *S. littoralis*:

Table (1) shows larval mortality rates due to treatment of the 2nd and 4th larval instars of *S. littoralis* with different concentrations of the tested compounds. LC_{25}, LC_{50}, and LC_{90} values for both instars were determined. Protecto exhibited higher effectiveness than Viruset for both instar larvae.

Table (1). Susceptibility of the 2nd and 4th larval instars of the cotton leaf worm, *S. littoralis*, to the tested compounds

Tested compounds	Larval instar	LC_{25} (gm/ml)	LC_{50} (gm/ml)	LC_{90} (gm/ml)	Slope ± S.E.
Protecto	2nd	8.7×10^{-8}	1.7×10^{-5}	0.418	0.29± 0.0297
	4th	1.4×10^{-8}	2×10^{-5}	1.98	0.21± 0.0225
Viruset	2nd	4.8×10^{-9}	1.1×10^{-5}	2.34	0.20± 0.0252
	4th	4.7×10^{-7}	1×10^{-4}	3.134	0.29± 0.0315

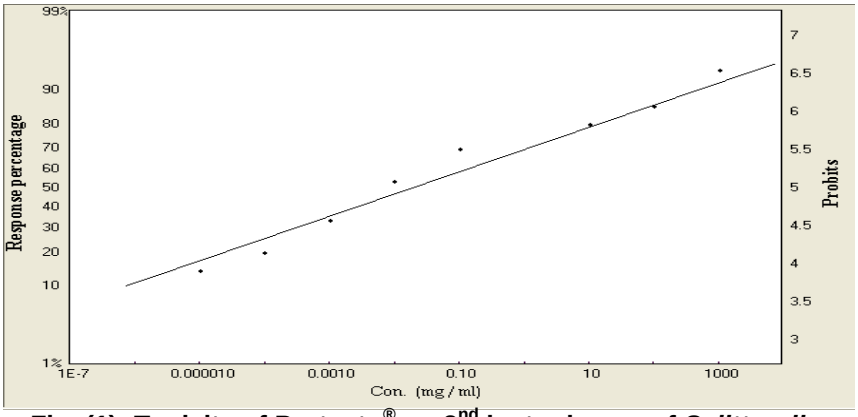

Fig. (1): Toxicity of Protecto® on 2nd instar larvae of *S. littoralis*.

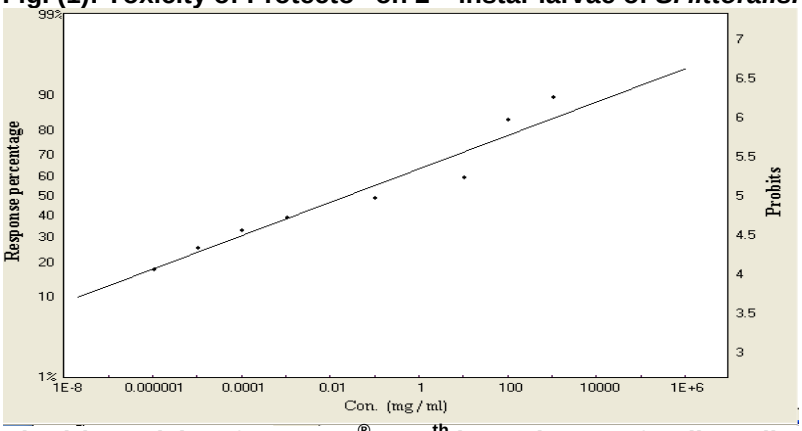

Fig. (2): Toxicity of Protecto® on 4th instar larvae of *S. littoralis*.

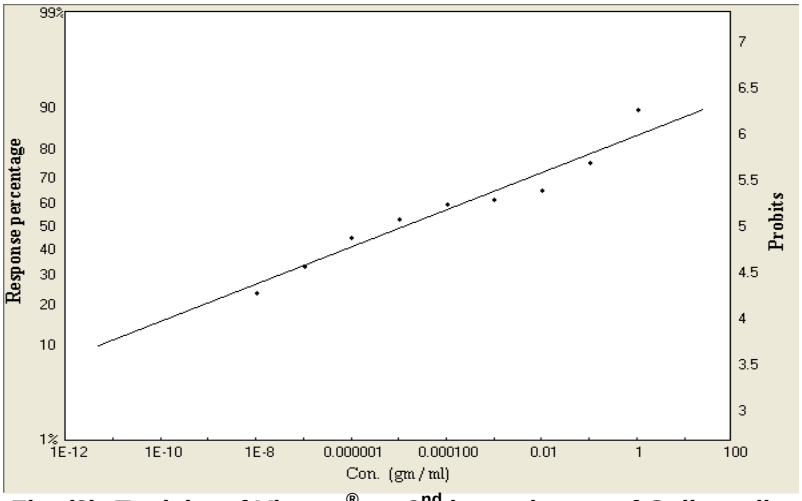

Fig. (3): Toxicity of Viruset® on 2nd instar larvae of *S. littoralis*.

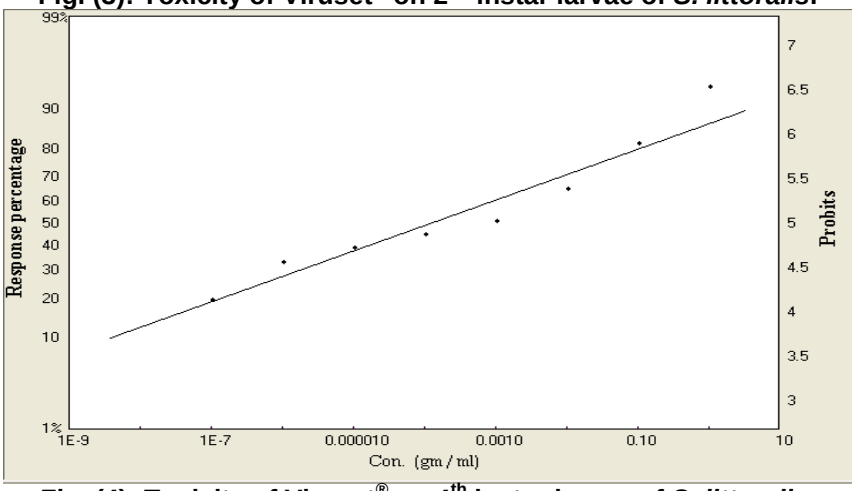

Fig. (4): Toxicity of Viruset® on 4th instar larvae of *S. littoralis*.

The tested bioagents did not result in instant mortalities 48 hrs. post treatment. However, mortality rates were increased at the termination of the larval stage. Second larval instar showed higher susceptibility to all the tested compounds than the 4th larval instar. This might be due to differences in sizes and defense mechanisms between instars. It is well documented that older instars of the cotton leaf worm are able to tolerate the toxic effect of these bioagents. Similar observations were reported by Hanafy *et al*. (2005); Abdel-Aziz (2007); and Abd El-Kareem (2007).

2- Effect of tested compounds on some biological aspects:

Treatment of the 2nd and 4th instar larvae with Protecto and Viruset prolonged the mean duration of the larval stage (Table 2).

Table (2). Effect of larval treatment with LC_{50} of Protecto and Viruset on larval duration, pupation rate, and pupal duration of *S. littoralis*

Compounds	Mean larval duration (days) ± S. E.		%Pupation		Mean pupal duration (days) ± S. E.	
	2nd	4th	2nd	4th	2nd	4th
Protecto	14.5±0.1*	12.0±1.7**	67.5	47.0	11.3±1.5***	9.3±1.3***
Viruset	16.0±0.5*	11.6±0.6*	52.5	56.6	11.3±1.5***	10.0±1.7***
Check	15.0±0.2	10.3±1.1	100	100	13.6±0.5	14.0±1.7

***: Significant at P> 0.05 **: highly significant at P> 0.01 ***: Very highly significant at P> 0.001**

The mean pupal duration was reduced as a result of treatment with the tested compounds. In addition, there was a decrease in adult emergence and mean adult longevity (Table 3). Mohamed (2006) reported a similar delay of ecdysis in larvae treated with NPV. Atwa *et al*. (1984) showed that latent effects of *Bt* on treated insects manifested as decrease of pupation and adult emergence. Abd El-Halim (1993); Abd El-Latif (2001); Dutton *et al*. (2003); Gamil (2004) and Mohamed (2006) also found that the development time of larvae and pupae were extended as well as adult emergence after treatment with bacterial or viral agents.

Table (3). Effect of larval treatment with LC_{50} of Protecto and Viruset on adult emergence and longevity of *S. littoralis*

Tested compounds	% Adult emergence		Mean adult longevity (days) ± S. E.			
	2nd	4th	2nd		4th	
			♂	♀	♂	♀
Protecto	94.40	94.20	8.3±1.15***	9.3±0.57***	14.3±1.2**	12.0±1.7***
Viruset	100.00	94.10	9.0±1.0***	10.3±0.57**	14.0±1.1**	13.0±1.5***
Control	100.00	100.00	13.6±1.15	14.6±0.58	17.0±1.0	15.3±0.57

***: Significant at P> 0.05 **: highly significant at P> 0.01 ***: Very highly significant at P> 0.001.**

Reproductive potential of *S. littoralis* moths treated as 2nd or 4th instar larvae with LC_{50} of the tested bioagents was reduced (Table 4). It is likely that the used compounds interfered with egg formation or development and consequently, led to reduction in the number of laid eggs. Results obtained could be explained by those reported by Santiago-Alvarez and Osuna (1988) and Aldebis *et al*. (1993). They found that males of *S. littoralis* infected with NPV and allowed to mate with untreated females produced normal number of eggs but showed a significant reduction in egg hatchability. In addition, the observed reduction in the percentage of egg hatch may be attributed to impairment of either eggs and/or sperms as a result of treatment. Furthermore, it may be due to inability of the sperms to be transferred to the females during copulation, as suggested by Ismail (1980) and Aldebis *et al*. (1993). Many researchers reported a low reproductive capacity in the cotton

leafworm moths treated with bioagents (Hassan, 2004; Mohamed *et al*., 2005; Hatem, 2006; Abdel-Aziz, 2007; and Abd El-Kareem *et al*., 2010).

Table (4): Effect of larval treatment with LC_{50} of Protecto and Viruset on fecundity and fertility of *S. littoralis*

Tested compounds	Mean no. of eggs/female ± S.E.		Mean no. hatched eggs/female ± S.E.	
	2nd	4th	2nd	4th
Protecto	422±12.5***	698±14.2***	282±4.7***	646±15.3***
Viruset	847±16.8	671±18.4	528±8.5	622±4.04
Control	2135±60.6	1875±15.1	2103±4.04	1857±12.11

*****: Very highly significant at P> 0.001.**

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فاعليـة مركبـان حيويـان (بروتكتـو وفيروسـت) وتأثيرهمـا علـى بعـض المظـاهر البيولوجية لدودة ورق القطن الكبرى

عادل حسن عبد السلام[١]، آمال محمد مبروك[٢] و نيفين محمد فايز[٢]
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تم تقييم تأثير مركبان حيويان هما بروتكتو (*Bacillus thuringiensis* var. *kurstaki*) and وفيروست (*Spodoptera littoralis*/NPV) على يرقات العمرين الثانى والرابع لدودة ورق القطن. من النتائج التى تم الحصول عليها وُجد أن مركب بروتكتو كان أكثر المركبات فاعلية مقارنة بمركب فيروست. أشارت الدراسة إلى أن تأثير المعاملة بكل من مركبى بروتكتو وفيروست العمرين الثانى والرابع بالتركيز القاتل للنصف (LC_{50}) أطال فترات العمر اليرقى، وعمر العذارى، وعمر الحشرات الكاملة نتيجة المعاملة. كما وُجد أيضاً أن عدد البيض الموضوع ونسبة الفقس قد تأثرتا بشكل واضح نتيجة المعاملة.

قام بتحكيم البحث

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