

Effect of interaction of bioinsecticides and a carbamate insecticide on the larvae of the cotton leafworm, *Spodoptera littoralis* (boisd.), by successive applications

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ABSTRACT

Four bioinsecticides and a carbamate insecticide were bioassayed against the cotton leaf worm, *Spodoptera littoralis*, larvae by separate and/or successive applications. The obtained results reveal that Protecto was the most potent bioinsecticide compared with Viruset, Profect, and Bioranza. The obtained data also prove that the chemical insecticide, Kuik, was very toxic to the tested larvae. Successive applications with the bioinsecticides at different concentrations (LC₂₅, LC₅₀, and LC₉₀) then LC₂₅ of Kuik showed very promising results manifested by an outstanding increase in second and fourth larval instar mortality. Treatment of 2nd and 4th instar larvae with Protecto, Viruset, and Profect prolonged larval duration while treatment with Bioranza reduced larval duration. It was also observed that egg number and hatchability were affected by bioinsecticidal treatment.

Key word: Bioinsecticides, Carbamate . Cotton leafworm.

INTRODUCTION

In Egypt, many field crops as well as various vegetables are attacked by numerous insect pests. Lepidopterous insects in general and the cotton leafworm, *Spodoptera littoralis* (Boisd.), in particular, are the most dangerous pests in this respect. In fact, the cotton leaf-worm is a major limiting factor affecting crop and vegetable production, not only in Egypt, but also in many other countries. *S. littoralis* is similarly one of the most destructive agricultural lepidopterous pests within its subtropical and tropical range (Hosny *et al.* 1986). It can attack numerous economically important crops all year round. Of these crops are cotton, leguminous crops, and various vegetables. Chemical control of *S. littoralis* has been extensively used especially on cotton. In Egypt, *S. littoralis* was controlled by methyl-parathion, but resistance to this compound developed. Since then, numerous other organophosphorus, synthetic pyrethroids, insect growth regulators and other nonconventional insecticides have been used, with many reports of resistance and cross resistance development in many cases (Issa *et al.*, 1984a; Issa *et al.*, 1984b; and Abo-El-Ghar *et al.*, 1986). However, limitations of the application of synthetic pyrethroids to one application per year on cotton in Egypt has stopped the appearance of new resistance (Sawicki, 1986). The unwise use of synthetic insecticides gives rise to high resistance to many chemical pesticides, resurgence, and residues of chemical pesticides in environment (Forgash, 1984 and Georghiou, 1986). More attention should be paid to the use of bioinsecticides such as compounds based on bacteria, fungi, and viruses (Rao *et al.*, 1990). These groups have unique modes of action (Asher, 1993 and Thompson *et al.*, 1999) and their properties may differ considerably from the conventional agents with which growers are familiar. For the purpose of

shortening the latent period of most bioagents, meanwhile, minimizing the use of synthetic organic insecticides; this study was planned to evaluate the effect of four commercial bioinsecticides (Protecto® (*Bacillus thuringiensis kurstaki*), Viruset® (*Spodoptera littoralis* NPV), Profect® (*Btk* & *Sli*NPV), and Bioranza® (*Metarhizium anisopliae*)), and the synthetic carbamate (KUIK®), an acetylcholinesterase inhibitor, on the cotton leaf worm. These compounds will be applied either separately or successively. Data obtained from this study might be helpful to recommend the most efficient combination of conventional insecticides and bioinsecticides to suppress pest populations of this insect in the field.

MATERIALS AND METHODS

1- Insects used:

A laboratory susceptible strain of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), reared for more than 10 generations 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 $27 \pm 2^\circ \text{C}$, of $65 \pm 10\%$ R. H., and 8:16 L: D photoperiod at the Plant Protection Research Institute, Dokki-Giza, Egypt.

2-Chemicals used:

Four commercial bioinsecticides; Protecto® (*Bacillus thuringiensis kurstaki*), Viruset® (*Spodoptera littoralis* NPV), Profect® (mixture of *Btk* & *SL*NPV), and Bioranza® (*Metarhizium anisopliae*) as wettable powders, and the organic insecticide (KUIK®) as soluble powder were evaluated for their toxicity on the cotton leaf worm, *Spodoptera littoralis*. All tested bioagents were obtained from Plant Protection Research Institute Biopesticide Unit Production, while the organic insecticide, KUIK, was obtained from ROTAM Agrochemical Company- Egypt. Serial dilutions of Protecto, Viruset, Profect, Bioranza, and KUIK were prepared.

Serial dilutions from Protecto, Viruset, and Profect were prepared using 1gm of the wettable powder of each and dissolved in 100 ml of water. In case of Bioranza and Kuik several concentrations by weight were also dissolved in 100 ml of water.

3- Bioassay tests:

3-1. Application of tested compounds separately:

Two sets of five replicates each contain 10 newly molted 2nd and 4th instar larvae for each concentration of each tested product (Protecto, Viruset, Profect, Bioranza, and Kuik) were used. Treatment of larvae was conducted by the leaf dipping technique. Fresh and clean castor leaves, *Ricinus communis* L., were immersed for 10sec. 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 2nd and 4th 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 was used for control 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. Mortality rates were corrected according to Abbott's formula (Abbott, 1925) and plotted against concentrations as log/probit regression lines. LC₂₅, LC₅₀, and LC₉₀ values as well as the slope of the lines were calculated (Finney, 1971) using "LdPLine®" software [<http://embakr.tripod.com/ldpline/ldpline.htm>].

3.2 Successive application of tested compounds:

LC₂₅, LC₅₀, and LC₉₀ of the tested bioagents (Protecto, Viruset, Profect, and Bioranza) were used in this experiment by leaf dipping technique. Larvae were offered castor leaves treated with the bioagents for 48-h then mortalities were recorded. Larvae were then fed on castor leaves treated with LC₂₅ of KUIK for 24 hours and mortalities were recorded again.

3.3 Effect of bioagents on some biological aspects:

Larvae that survived treatment with LC₅₀ 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 life span of adult moths.

RESULTS AND DISCUSSION

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

Table (1) shows larval mortality rates due to treatment of the 2nd and 4th larval instars of *Spodoptera littoralis* with different concentrations of the tested compounds (Protecto, Viruset, Profect, Bioranza, and Kuik). LC₂₅, LC₅₀, and LC₉₀ values for both instars were determined. Protecto exhibited the highest effectiveness among the tested bioagents for both instars, followed by Viruset, Profect, and Bioranza. Kuik (carbamate insecticide) was extremely toxic to the tested larvae evidenced by the very low LC₂₅, LC₅₀ and LC₉₀ values.

Table (1): Susceptibility of the 2nd and 4th larval instars of the cotton leaf worm, *Spodoptera 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	2 nd	8.7×10 ⁻⁸	1.7×10 ⁻⁵	0.418	0.29 ±0.0297
	4 th	1.4×10 ⁻⁸	2×10 ⁻⁵	1.98	0.21±0.0225
Viruset	2 nd	4.8×10 ⁻⁹	1.1×10 ⁻⁵	2.34	0.20 ±0.0252
	4 th	4.7×410 ⁻⁷	1×10 ⁻⁴	3.134	0.29 ±0.0315
Profect	2 nd	5.0056×10 ⁻⁸	1.5×10 ⁻⁵	0.787	0.27 ±0.0318
	4 th	2.86×10 ⁻⁷	9.4×10 ⁻⁵	5.732	0.27 ±0.0310
Bioranza	2 ⁿ	0.059	0.230	3.093	1.35 ±0.118
	4 th	0.255	0.745	5.715	1.45 ±0.2001
KUIK	2 nd	0.0024	0.0054	0.025	1.92 ±0.2931
	4 th	0.0030	0.0062	0.025	2.12 ±0.2705

The tested bioagents did not result in instant mortalities 48hrs post treatment, however, mortality rates 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 Mabrouk, 2001; Mabrouk and El-Abbas, 2002, Hanafy *et al.*, 2005; Abdel-Aziz, 2007; and Abd El-Kareem, 2007.

2- Successive application of tested compounds:

Effects of successive application of tested bioagents (Protecto, Viruset, Profect, and Bioranza) then the tested carbamate (Kuik) represented in figure (1). Again, the tested bioagents did not result in instant mortalities 48hrs post treatment. The highest mortality was obtained when the 4th instar larvae were treated with LC₉₀ of Protecto (43.3% 48-h post treatment). The lowest mortality was obtained when the 4th instar larvae were treated with LC₂₅ of Profect (3.3% 48-h post treatment). In addition, when the 2nd instar larvae were treated with LC₂₅, LC₅₀, and LC₉₀ of

Bioranza, there was no killing effect on treated larvae. The same result was obtained when the 4th instar larvae treated with LC₂₅ of Bioranza. The mortality percentages of 2nd and 4th instar larvae treated with the LC₂₅ of Kuik successively were increased after 24-h of treatment. Full mortality was reached when 2nd instar larvae were treated with LC₉₀ of Bioranza then LC₂₅ of Kuik. In case of treatment of 2nd instar larvae with LC₂₅ of Protecto, the percentage of dead larvae was high but still lower than the other bioagents (i.e. 66.7%).

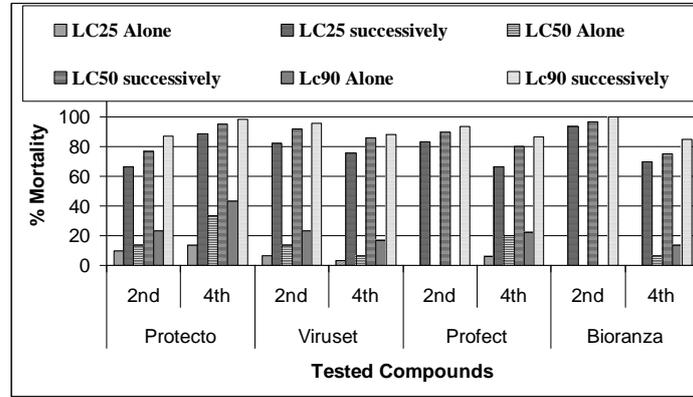


Fig. (1): Successive application of tested bioagents followed by Kuik

Generally speaking, application of bioagents for 48-h followed by the application of carbamate insecticide sharply increased the mortality of 2nd and 4th instars larvae. The obtained results show that there was a delay in the killing effect due to latent period of the tested bioagents (Protecto, Viruset, Protect, and Bioranza) even when high concentrations were used. This might be due to the fact that some microorganisms need an incubation period so that their toxic effect can appear. The treated larvae were weakened as a result of the action of the entomopathogens used. Treatment of these larvae with LC₂₅ of Kuik, has thus sharply increased mortality rates. High and instant mortality rates obtained through successive application, using the lowest concentrations of conventional insecticides can be very promising for the conservation of the environment. Environmental pollution with these insecticides can be minimized and the latent period during which larvae continue feeding on the crop can also be shortened. El-Ferjany *et al.* (2008) evaluated the consecutive and rotation applications of Chlorfenapyr, Aadirachtin, and Sodium dioctyl sulfosuccinate, against the two spotted spider mite, *Tetranychus urticae*. They stated that both types of applications have led to population suppression of the tested species.

1- Effect of bioagents on some biological aspects:

Treatment of the 2nd and 4th instar larvae with Protecto, Viruset, and Protect prolonged the duration of the larval stage (Table 2), while treatment of the 2nd and 4th instar larvae with Bioranza reduced mean larval duration.

Table (2): Effect of larval treatment with LC₅₀ of Protecto, Viruset, Protect and Bioranza on larval duration, pupation rate, and pupal duration of *Spodoptera littoralis*

Tested compounds	Mean larval duration (days) ± S. E.		%Pupation		Mean pupal duration (days) ± S. E.	
	2 nd	4 th	2 nd	4 th	2 nd	4 th
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 ^{***}
Protect	15.0±0.4 ^{ns}	9.3±0.58 [*]	52.5	57.0	12.6±0.6 ^{**}	11.0±1.6 ^{**}
Bioranza	13.5±0.3 ^{**}	10.0±0.3 [*]	49.2	50.0	12.2±0.1 [*]	13.3±0.1 [*]
Control	15.0±0.2	10.3±1.1	100	100	13.6±0.5	14.0±1.7

ns: not significant. *: Significant at P<0.05 **: highly significant a P<0.01 ***: Very highly significant a 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.

Reproductive potential of *S. littoralis* moths treated as 2nd or 4th instar larvae with LC₅₀ 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 researches 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 El-Khateeb and El-Sabagh, 2008).

Table (3): Effect of larval treatment with LC₅₀ of Protecto, Viruset, Profect and Bioranza on adult emergence and longevity of *Spodoptera littoralis*

Tested compounds	% Adult emergence		Mean adult longevity (days) ± S. E.			
	2 nd	4 th	2 nd		4 th	
			♂	♀	♂	♀
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***
Profect	81.25	94.10	16.3±0.58**	15.7±1.15*	14.0±1.0**	14.0±1.7*
Bioranza	93.00	95.00	12.3±0.4**	11±0.28*	13.7±0.28**	11.3±0.1**
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.

Table (4): Effect of larval treatment with LC₅₀ of Protecto, Viruset, Profect and Bioranza on fecundity and fertility of *Spodoptera littoralis*

Tested compounds	Mean no. of eggs/female ± S.E.		Mean no. of hatched eggs/female	
	2 nd	4 th	2 nd	4 th
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***
Profect	809±10.2***	882±15.7***	199±4.9***	668±6.11***
Bioranza	576± 4.1***	712±1.38***	415±3.6***	530±21.3***
Control	2135±60.6	1875±15.1	2103±4.04	1857±12.11

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

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ARABIC SUMMARY

تأثير التداخل بين المبيدات الحيوية ومركب كاربامات واحد ضد دودة ورق القطن بالتطبيق المتتالي

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1 معهد بحوث وقاية النباتات - الدقى - الجيزة

2 كلية العلوم - جامعة عين شمس - العباسية - القاهرة

تم تقييم تأثير أربعة مركبات حيوية ومبيد كويك من مجموعة الكارباميت على دودة ورق القطن عن طريق تطبيقها منفردة و /أو متعاقبة. من النتائج التي تم الحصول عليها وجد أن مركب البروتكتو كان أقوى المركبات الحيوية مقارنة بالفيروسست، البروفكت، والبيورانزا. كما أوضحت النتائج التي تم الحصول عليها السمية العالية جدا لمركب كويك على يرقات دودة ورق القطن. و مع التطبيق المتعاقب للتركيز القاتل للربع (LC₂₅) من مركب كويك ثم التركيزات المختلفة من المركبات الحيوية المختبرة (LC₂₅, LC₅₀, LC₉₀) أظهرت نتائج واعدة جداً ظهرت بشكل واضح في الزيادة الكبيرة في معدل موت يرقات كلا العمرين الثانى والرابع و أيضا النقص الحاد في فترة حضانة المرض (بين الأصابة بالمرض و الموت). أدت معاملة كلا العمرين الثانى والرابع بكل من البروتكتو والفيروسست والبروفكت إلى زيادة في مدة العمر اليرقى بينما أدت المعاملة بالبيورونزا إلى انخفاض في مدة العمر اليرقى. كما لوحظ أيضاً أن عدد البيض الموضع ونسبة الفقس قد تأثرتا بشكل واضح نتيجة المعاملة بالمركبات الحيوية محل الدراسة.