

## The Interaction of Some Bioinsecticides and Nicotinoids on The Toxicity and Biochemical Effects on *Spodoptera littoralis* Larvae

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### ABSTRACT

Toxicity effect of Dinotefuran; Acetampirid; Thiacloprid and Imidacloprid, and three Bioinsecticides: Spinosad; Diple-2X and Biosact, were determined against 2<sup>nd</sup> larval instar of *Spodoptera littoralis*. The results showed that Thiacloprid and Dinotefuran were the most potent toxicity followed by Imidacloprid; Acetampirid, and three Bioinsecticides: Diple-2X; Spinosad and Biosact. The effect of LC<sub>50</sub> of the tested Nicotinoids on the *in vivo* inhibition of AChE from *Spodoptera littoralis* was assayed. The interaction of Nicotinoids with Bioinsecticides on the *in vivo* inhibition of AChE was investigated. Results proved that pretreated of Spinosad; Diple-2X and Biosact with Thiacloprid increased the percentage inhibition which found that to be 75.25; 70.4% and 66.1% for Lab strain respectively, while the percentage inhibition found by pretreated Spinosad; Diple-2X and Biosact, with Dinotefuran were 71.4%; 66.1% and 63.3% respectively. Results proved that pretreated of Bioinsecticides with Nicotinoids caused more toxicity effect than single treatment. The results emphasized that I<sub>50</sub> and Ki values decreased when Bioinsecticides with Nicotinoids, so there were significant difference among the chemical combinations, which caused more reduction effect than single treatment. Generally, Bioinsecticides pretreated with Nicotinoids will produce a new trend so as increase toxicity of the Nicotinoids, enhance the role of beneficial insects. The results of the present study may add some forward steps to use Nicotinoids as alternative to conventional insecticides especially against this insect. So, the tested compounds can be involved in important steps necessary for successful IPM programs applied against *S. littoralis*

**Keywords:** Bioinsecticides, Nicotinoids, Toxicity, Cotton leaf worm

### INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* is one of the most important polyphagous pests (Smaghe and Degheele, 1997 & Quero *et al.*, 2002). It has quickly developed resistance to chemical pesticides (Chung and Cote, 1992 & Amin, *et al.*, 2001). Therefore, the cotton leafworm in Egypt exhibits multiple resistance to nearly all insecticides used (Keddis, *et al.*, 1988 & Ishaaya and Klein, 1990). The insecticide market has been dominated by the Organophosphate, Carbamate, and Pyrethroid classes of insecticides (Argentine *et al.*, 2002). There is a continuing need for new, safe, effective and economical insecticides for crop protection and public health (Casida and Quistad, 2005). The search for new insecticides is an ongoing process dependent on many factors including the ability of insect to develop resistance to conventional materials. High level of resistance to the synthetic insecticides had increased due to the intensive application of such insecticides for controlling *S. littoralis* larvae (Ishaaya and Klein, 1990; Martin *et al.*, 2000 & El-Aw *et al.*, 2002). If this trend continues, new compounds will be required to replace these insecticides. Recently, a number of new insecticides classes have been

discovered and commercialized (Argentine *et al.*, 2002).

Avermectins, a group of chemicals produced by soil-inhibiting streptomycete bacteria have demonstrated high toxicities to a number of insects (Putter *et al.*, 1981). Spinosyns are among the newest classes of insecticides, represented by Spinosad it has stomach activity against Lepidopteran larvae with long residual activity (Thompson *et al.*, 1999 & Dow AgroSciences Co., 2001).

The purpose of this investigation is to study the efficiency of some Nicotinoids of (Dinotefuran; Acetampirid; Thiacloprid and Imidacloprid) either alone or in their combination with some Bioinsecticides (Spinosad; Diple-2X and Biosact) on *Spodoptera* larvae. The study was directed to throw the light on the effect of these tested insecticides Nicotinoids as well as other environmentally-friendly compounds, for their possible use inhibition of AChE activity. Looking for a new aspect can be used in IPM programs for such deleterious subject pest. It is of great importance to take the suitable decisions to overcome this problem to avoid the outbreak of this serious pest.

## MATERIALS AND METHODS

### Insect:

Laboratory strain of cotton leafworm, *Spodoptera littoralis* was chosen for bioassays and biochemical assessment. This strain start as field strain reared under laboratory condition for several years in central lab. of pesticides, Agricultural Research Center (ARC) Cairo, Egypt the 2<sup>nd</sup> larval instar used for assessments.

### Chemical:

The one Organophosphorus-insecticide, Dursban (Chlorpyrifos 48% EC) was obtained from Dow Chemical Co. Four Nicotinoids where used in this study namely: Thiaclopride 48% SC, was obtained from MyTrade Co., Dinotefuran 20% SG, and Imidacloprid 35% SC, were obtained from NM Agro Egypt., and Acetamprid 20%Sp, was obtained from AL-Esraa Co.

Bioinsecticides *Bacillus thuringiensis* subsp. *Kurstaki* Diple-2X 6.4% (WP) (32,000 International Units/mg). The product was produced by Abbott Laboratories. Chemical and Agricultural Products Division North Chicago, USA, and Provided by Bayer Company. Spinosad (tracer 24% SC) it is a metabolite of the Actinomycete, *saccharopolyspora spinosa* Martz & Yao. It is a naturally occurring mixture of two active products (Spinsoyn A & D). It is a trademark of Dow AngroSciences Co., (Dow England). *Beansira bassiana*; Biosact (WP) ( $32 \times 10^{12}$  cells/kg), was obtained from Origanl Bio Technology Co.

### Bioassay tests:

#### 1- Toxicity of The Tested Bioinsecticides Against *S. littoralis*:

Second instar larvae were starved for 6hrs before exposed test the selected larvae were bioassayed against Bioinsecticides (Spinosad; Diple-2X and Biosact) using three replicates for each concentration with ten larvae in each replicate.

Disc dipping technique was used since it has been proved to be the most common procedure for assessing toxicity to Bioinsecticides (Tabashnik and Chushing, 1987). Each castor leaves disc (2Cm<sup>2</sup>) was dipped into the suspension of tested formulation for 10s. Tested concentration were prepared in glass distilled water (GDW) (Toni and Fred, 1996) disc were held vertically to allow excess solution to drip off and places on a rack to dry for at last 2hr. Treated discs were offered to starved larvae (one disc per cup) and left under constant conditions (27 ± 2 °C). The survivors were transferred with fresh castor oil plant leaves to clean cups and kept under the same conditions. Control larvae were allowed to fed on castor oil leave discs treated with distilled water. Mortality was percentage calculated for each concentration daily for 24; 48, and 72hrs and corrected according to Abbott (1925) and subjected to probit analysis using the computer program (Finney, 1971).

#### 2-Toxicity of The Tested Nicotinoids Against *S. littoralis*:

Dinotefuran; Acetamprid; Thiacloprid and Imidacloprid, were bioassayed against the 2<sup>nd</sup> larval instar of *S. littoralis*. The castor leaves were dipped in different concentrations of the tested Nicotinoids. All insecticides concentrations were prepared in distilled water. The treated leaves were placed in clean glass container at the laboratory conditions of (27±2 °C) and 65-70%RH. Ten larvae (Lab strain) were used for each test with three replicate. Mortality was recorded after 24; 48, and 72hr and subjected to probit analysis.

#### 3-Toxicity of Tested Nicotinoids in Presence of Bioinsecticides:

*S. littoralis* 2<sup>nd</sup> instar (Lab strain) was treated with solution of Dinotefuran; Acetamprid; Thiacloprid and Imidacloprid, at LC<sub>50</sub> values concentrations before 24; 48 and 72hr of feeding on discs of castor oil leaves discs treated with LC<sub>50</sub> of Spinosad; Diple-2X and Biosact, joint action experiments have tow controls. larvae of the first control were allowed to fed castor oil leaf discs treated with concentration equivalent LC<sub>50</sub> of Nicotinoids alone, while larvae of the second control were fed with untreated discs. Mortality counted and recorded daily for 3days. Percentage of mortality were calculated according to Abbott (1925) and subjected to probit analysis (Finney, 1971).

#### Enzyme Preparation and Activity Assay:

AChE was prepared from *Spodoptera littoralis* 2<sup>nd</sup> instar larvae was homogenized in Tris-HCl buffer (pH 7.4) at larvae/30ml buffer, with polytron mixer (at 50% power for 50sec.), then subjected to low speed centrifuged at 15,000rpm for 20min at 4 °C. The supernatant centrifuged at 25,000rpm for 1hr at 4 °C. Pellets were resuspended in 1ml of Tris-HCl buffer (pH 7.4) and stored at (-20 °C) for used as enzyme source.

The AChE activity measurements were done according to method reported by Ellman *et al.*, (1961). This method is based on the hydrolysis of acetylthiocholine iodide (ATChI) as substrate by enzyme to produce thiocholine and acetic acid. Thiocholine reacts with 5,5-dithio bis-(2-nitrobenzoic acid), "DTNB" to produce the yellow anion of 5-thio-2-nitrobenzoic acid. The rate of color production as a function of enzyme activity is measured spectrophotometrically at λ412nm. Enzyme specific activity was computed as mg protein/hr.

The protein content in prepared homogenates of *S. littoralis* was assayed pectrophotometrically by the method of Lowery *et al.*, (1951) at λ750nm using Bovine Serum Albumin (BSA) as a standard protein.

**Inhibition of AChE Activity:**

The inhibition of AChE Activity was determined in 2<sup>nd</sup> instar larvae using the values of each of the tested insecticides. The method of Dixon and Webb (1964) was adopted to draw the Dixon-plots by plotting 1/V versus concentrations of the inhibitor at two concentrations of the substrate. Acetylcholine iodide (the substrate of AChE) was used at concentrations of 5 and 10mM. Estimation of I<sub>50</sub> value (the concentration of the inhibitor which inhibits 50% of the enzyme activity) was carried out by pre incubating the enzyme with the inhibitor for 30min. Using the following concentrations 0.1; 1; 5; 10 and 50µM. Ki (the inhibition constant) values for each inhibitor were estimated from Dixon-plot.

**RESULTS AND DISCUSSION**

**Toxicity of Nicotinoids and Bioinsecticides:**

The results of the toxicity of the Nicotinoids and Bioinsecticides in terms of LC<sub>50</sub> are given in Table (1) for larvae of *S. littoralis*. LC<sub>50</sub> values after 24hr were 0.25; 0.40; 0.63; 0.81; 5.33; 7.25 and 9.21ppm for Thiacloprid; Dinotefuran; Imidacloprid; Acetamprid; Spinosad; Diple-2X and Biosact, respectively against *Spodoptera* Lab strain. While LC<sub>50</sub> values after 48hr were 0.057; 0.75; 0.087; 0.094; 2.13; 4.00 and 5.11ppm respectively. LC<sub>50</sub> values after 72hr were 0.041; 0.053; 0.068; 0.076; 0.093; 0.063 and 0.84ppm respectively. Present results demonstrated that the LC<sub>50</sub> values of both Bioinsecticides were decreased in general, by increasing the post treatment period of times. According to the LC<sub>50</sub> values, it is quite clear that the *Spodoptera* larvae is more susceptible to Spinosad in comparison to the Dipel-2X and Biosact. The report of (Dow AgroSciences, 2001) revealed that Spinosad has been found to be highly active on most Lepidoptera, Spinosyns are among the newest classes of insecticides, Represented by Spinosad. Spinosad is a fermentation metabolite of the Actinomycete, *Saccharopolyspora spinosad*, a soil-inhibition microorganism. It has both contact and stomach activity against lepidopteran larvae, with long residual activity (Entwistle, *et al.*, 1993; Abdel-Halim 1997; Rizk, *et al.*, 1999; Thompson *et al.*, 1999; Liburd *et al.*, 2000; Ali, 2001; Dow AgroSciences Co., 2001; Mona, *et al.*, 2004). Also Nicotinoids acts as effect on the larval through

reduction in its acetylcholine receptors (nAChRs) agonists (Millor and Denholm, 2007). These results are in agreement with many investigators, salahgado, 1998; samson *et al.*, 1988; Gupta, *et al.*, 1996; Dow AgroSciences Co., 2001; El-Aw, 2003; Pineda, *et al.*, 2004; Goettel, *et al.*, 2005 & El-Aw, 2006.

**Toxicity of Nicotinoids alone or pretreated with the LC<sub>50</sub> values of Bioinsecticides against *S. littoralis* larvae:**

The LC<sub>50</sub> values of Thiacloprid and Dinotefuran after 24hr were 0.25 and 0.40ppm against Lab *Spodoptera* strain respectively. The LC<sub>50</sub> values after for 48hr were 0.057 and 0.075ppm respectively Table (2). The interaction of Nicotinoids (Thiacloprid and Dinotefuran) with three Bioinsecticides against Lab strain of *Spodoptera* larvae were studied. Larvae were allowed to feed on castor oil leave discs treated with LC<sub>50</sub> of the different Bioinsecticides.

The LC<sub>50</sub> values of Thiacloprid and Dinotefuran pretreated with the LC<sub>50</sub> values of Spinosad; Diple-2X and Biosact on Lab strain of *Spodoptera* larvae are presented in Table (2). The LC<sub>50</sub> values of Nicotinoids when pretreated with Bioinsecticides was lower than LC<sub>50</sub> of Nicotinoids alone. The enhancement of toxicity is calculated as a Potentiation factor (P.f.) Table (2). P.f values for Thiacloprid and Dinotefuran are 2.08 and 1.54 respectively, when pretreated with Spinosad after 24hr treatment, while the P.f. values are 1.9 and 1.50 respectively, when pretreated with Spinosad after 48hr treatment. Also when pretreated with Diple-2X the P.f. values are 1.47 and 1.21 for Thiacloprid and Dinotefuran respectively, after 24hr, while the P.f. values are 1.16 and 1.17 respectively, after 48hr treatment. In case pretreated with Biosact the P.f. values are 1.14 and 1.08 for Thiacloprid and Dinotefuran respectively, after 24hr, while the P.f. are 1.08 and 1.06 respectively, after 48hr treatment. It is clear that the LC<sub>50</sub> values concentrations of Bioinsecticides enhancement the toxicity of the Nicotinoids on *S. littoralis* larvae. The mixture with Spinosad were the most toxic treatments than mixtures with Diple-2X and Biosact respectively.

**Table 1: LC<sub>50</sub> values of Nicotinoids and three Bioinsecticides to 2<sup>nd</sup> instar *S. littoralis* larvae.**

Compounds	LC <sub>50</sub> (ppm)		
	24hr	48hr	72hr
Thiacloprid	0.25	0.057	0.041
Dinotefuran	0.40	0.075	0.053
Imidacloprid	0.63	0.087	0.068
Acetamprid	0.81	0.094	0.076
Spinosad	5.33	2.13	0.093
Diple-2X	7.25	4.00	0.63
Biosact	9.21	5.11	0.84

Table 2: Comparative toxicities of Nicotinoids alone or pretreated with three Bioinsecticides on *Spodoptera* larvae

Compounds	LC <sub>50</sub> (ppm)			
	24hr	48hr		
		P.f.	P.f.	P.f.
Thiacloprid	0.25		0.057	
Thiacloprid+Spinosad	0.12	2.08	0.030	1.9
Thiacloprid+Diple-2X	0.17	1.47	0.049	1.16
Thiacloprid+Biosact	0.22	1.14	0.053	1.08
Dinotefuran	0.40		0.075	
Dinotefuran+Spinosad	0.26	1.54	0.050	1.50
Dinotefuran+Diple-2X	0.33	1.21	0.064	1.17
Dinotefuran+Biosact	0.37	1.08	0.071	1.06

\*Potentiation factor (P.f.) = LC<sub>50</sub> Nicotinoids alone / LC<sub>50</sub> Bioinsecticides + Nicotinoids.

In general the susceptibility of *Spodoptera* larvae to Nicotinoids increases when treatment after Bioinsecticides. The Nicotinoids+Bioinsecticides caused more toxic than effect single treatment with Nicotinoids. It could be concluded that Nicotinoids enhanced the toxicity effect of Bioinsecticides. Based on P.f. values, the Lab strain of *Spodoptera* larvae is more susceptible to Spinosad in comparison to the Diple-2X and Biosact. Generally, efficacy of Bioinsecticides have a very good additive toxicity for Nicotinoids either Lab *Spodoptera* strain.

(Chung & Cote 1992; Liburd, *et al.*, 2000 & Mona, *et al.*, 2004) whom found that when certain pairs of drugs or insecticides are administered together, the effects may be greater or less than might be expected from the sum of activities of the components when administered separately. The phenomena involved, included under the term "synergism" "potentiation" and "antagonism" are becoming increasingly important in, for example, practical insect control and mammalian toxicology.

The observation that Nicotinoids had the lowest effect when applied alone but it was the best when mixed with Bioinsecticides. These findings may be resulted insect cuticle easily penetration which caused by Bioinsecticides in the mixture, and these results show that Bioinsecticides are act in similar manner in reducing chitin incorporation in cuticle of *S. littoralis*. So may be these effect of Bioinsecticides in mixture and these are a good control of Lepidopterous larvae.

Generally, it could be concluded that the use of Nicotinoids and their mixtures with biological insecticides (Spinosad; Diple-2X and Biosact) instead of conventional hazardous insecticides; and these may reduce the environmental pollution and hazard management programs especially when mixed with Bioinsecticides. Using Bioinsecticides pretreatment with Nicotinoids looking forward in an intergrated pest management to overcome pest problems.

#### *In vivo* inhibition of brain *S. littoralis* AChE activity:

The *in vivo* inhibitory effect of the LC<sub>50</sub> values of four Nicotinoids against to the *Spedoptera* 2<sup>nd</sup> instar Lab strain larval AChE are shown in the data given in Table (3). The data declared that Thiacloprid and Dinotefuran exhibited the highest percentages of reduction of AChE activity as values were 63.4 and 60.2% respectively, while values were 54.1 and 51.2% respectively for Imidacloprid and Acetampirid, while Spinosad; Diple-2X and Biosact not active as inhibitor on AChE activity. Chlorpyrifos we used as a specifically inhibits the AChE.

Data in Table (3) summarize the interaction of Spinosad; Diple-2X and Biosact on the inhibitory effect of Thiacloprid and Dinotefuran on AChE activity. The resultes proves that pretreated of Spinosad; Diple-2X and Biosact with Thiacloprid and Dinotefuran induce increase the inhibition of enzyme activity. The inhibition of AChE activity by Thiacloprid and Dinotefuran were 63.4 and 60.2% while the increased to be 75.2; 70.4 and 66.1% for Spinosad; Diple-2X and Biosact respectively when three Bioinsecticides pretreated with Thiacloprid. Moreover the inhibition of enzyme activity increased to be 71.4; 66.1 and 63.3% for tested three Bioinsecticides respectively when three Bioinsecticides pretreated with Dinotefuran.

Result indicated that Bioinsecticides may be make activation of AChE activity these effect may be increased the Nicotinoids effects on AChE activity. It is quite clear that the Nicotinoids at LC<sub>50</sub> concentration acts as potential inhibitors for *Spodoptera* larvae AChE activity when pretreated with Bioinsecticides.

#### The *in vitro* inhibition of *S. Littoralis* AChE activity:

Table (4) show the *in vitro* interaction of Nicotinoids and three Bioinsecticides on AChE activity of *S.littoralis* 2<sup>nd</sup> instar brain. The I<sub>50</sub> values of Thiacloprid and Dinotefuran for Lab strain larval AChE are 0.57 and 0.64μM respectively. We have

**Table 3: *In vivo* inhibition of *Spodoptera* larvae 2<sup>nd</sup> instar AChE activity some compounds (LC<sub>50</sub>).**

Compounds	% Inhibition
Chlorpyrifos	84.1
Thiacloprid	63.4
Dinotefuran	60.2
Imidacloprid	54.1
Acetamprid	51.2
Spinosad	24.4
Diple-2X	19.2
Biosact	14.1
Chlorpyrifos+ Spinosad	90.5
Chlorpyrifos+ Diple-2X	88.2
Chlorpyrifos+ Biosact	86.3
Thiacloprid+ Spinosad	75.2
Thiacloprid+ Diple-2X	70.4
Thiacloprid+ Biosact	66.1
Dinotefuran+ Spinosad	71.4
Dinotefuran+ Diple-2X	66.1
Dinotefuran+ Biosact	63.3

**Table 4: *In vitro* inhibition of *Spodoptera* larvae AChE activity by certain compounds.**

Compounds	I <sub>50</sub> (μM)	Ki (μM)
Chlorpyrifos	0.41	22
Thiacloprid	0.57	42
Dinotefuran	0.64	56
Chlorpyrifos+Spinosad	0.24	12
Chlorpyrifos+Dipel-2X	0.30	17
Chlorpyrifos+Biosact	0.36	20
Thiacloprid+ Spinosad	0.38	28
Thiacloprid+ Dipel-2X	0.46	33
Thiacloprid+ Biosact	0.50	37
Dinotefuran+ Spinosad	0.50	40
Dinotefuran+ Dipel-2X	0.57	46
Dinotefuran+ Biosact	0.60	50

shown that efficacy of three Bioinsecticides has a very good additive toxicity for Thiacloprid and Dinotefuran in Lab *Spodoptera* strain (Table 2). Because for the enhancement toxicity of the three Bioinsecticides we study the *in vitro* biochemical interaction of them with the AChE activity and compare with the Thiacloprid and Dinotefuran *in vitro* effects. The I<sub>50</sub> values for Thiacloprid were 0.60; 0.72 and 0.81 μM respectively against Lab strain enzyme, respectively. While the I<sub>50</sub> values for Dinotefuran were 0.60; 0.72 and 0.81 μM respectively.

To characterize more details about the *in vitro* inhibition of AChE by the inhibitor, the I<sub>50</sub> and ki values of each inhibitor were estimated from the graphical method of Dixon and Webb, (1964) Table (4). The obtained data proved that compounds competitive inhibition of AChE activity and the ki values were 42 and 56 respectively.

It is concluded from the present results that the tested Nicotinoids are potentially potent for control of *S. littoralis* however, with new compounds, such as Organophosphate and Carbamate insecticides

currently in use, *S. littoralis* could be successfully included in the management programs.

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## الملخص العربي

## تدخل بعض المركبات الحيوية على سمية الفعل البيوكيماوى للمبيدات الحشرية على دودة ورق القطن

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الهدف من البحث هو تقييم التأثير الأبدى لأربعة من المبيدات الحشرية (دينوتيفوران، أسيتاميريد، ثياكلوبرايد وايداكلوبرايد) مع ثلاثة من المبيدات الحيوية هي سبينوساد، دايبيل-2 أكس وبيوساكت بهدف تلاشى تأثير المبيدات التقليدية الضار على البيئة. وقد تم تسجيل قيم التركيزات النصف مميتة ( $LC_{50}$ ) لكلا من المبيدات الحيوية والمبيدات الحشرية تحت الدراسة بصورة فردية. ثم تم معاملة اليرقات العمر الثانى لدودة ورق القطن بتركيزات مختلفة ( $LC_{50}$ ) من المبيدات الحيوية تحت الدراسة ثم معاملة هذه اليرقات بتركيز ( $LC_{50}$ ) من المبيدات الحشرية بعد 24، 48 و 72 ساعة من المعاملة بالمبيدات الحيوية تحت الدراسة، فأوضحت النتائج أن قيم  $LC_{50}$  بعد المعاملة انخفضت بدرجة ملحوظة ويتضح ذلك من قيم معامل التنشيط ( $P.f$ ) الذى تم حسابها. وكذلك تم دراسة المقدرة التنشيطية للمبيدات الحشرية المختبرة على النشاط الأنزيمى لأنزيم هام وحيوى بالنسبة للحشرة وهو أنزيم الأسيتايل كولين أستريز ولقد أوضحت النتائج أن فى حالة ثياكلوبرايد بعد المعاملة بالسبينوساد، الدايبيل-2 أكس والبيوساكت كانت النسبة المئوية للتنشيط هي 75.2، 70.4 و 66.1% للسلسلة المعملية على الترتيب، بينما فى حالة الدينوتيفوران بعد المعاملة بالسبينوساد، الدايبيل-2 أكس والبيوساكت كانت النسبة المئوية للتنشيط هي 71.4، 66.1 و 63.3% على التوالى. وكذلك دراسة تأثير المبيدات المختبرة بعد المعاملة بالمبيدات الحيوية تحت الدراسة على قيم  $I_{50}$  أوضحت النتائج حدوث انخفاض فى تلك القيم وكانت أعلى نسبة للانخفاض عند معاملة السبينوساد، الدايبيل-2 أكس والبيوساكت مع ثياكلوبرايد والدينوتيفوران على الترتيب وقد وجد أن هذه المركبات أظهرت تثبيط تنافسى على نشاط أنزيم الأسيتايل كولين أستريز. ومن هذه النتائج نجد أن الخلائط أعطت تأثير أكبر من المبيدات الحشرية والمبيدات الحيوية عند تطبيقهم بصورة فردية وهذا يوضح أن المبيدات الحيوية تنشط عمل المبيدات الحشرية ولذلك تعتبر هذه الدراسة خطوة فى اتجاه استخدام هذه المخاليط كأحد عناصر المكافحة المتكاملة لدودة ورق القطن حيث أنها أكثر أمانا للإنسان والبيئة.