Evaluation of Some Compounds on *Spodoptera littoralis* Larvae.

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ABSTRACT

Toxicity effect of Spinosad and three insecticides from different groups: Chlorpyrifos; Phenothrin and Thiodicarb were determined against 2nd larval instar of *Spedoptera littoralis*. The results showed that Phenothrin was the most potent toxicity followed by Chlorpyrifos; Thiodicarb and Spinosad. The effect of LC_{50} of the tested compounds on the *in vivo* inhibition of AChE and Na^+,K^+-ATPase from *Spedoptera littoralis* was assayed. The interaction effect of Phenothrin with Spinosad the percentage inhibition which found that to be 92.6% for Na^+,K^+-ATPase, while the percentage inhibition found by pretreated the Chlorpyrifos with Spinosad and pretreated Thiodicarb with Spinosad showed very weak inhibitory effect (less than 50%) on the Na^+,K^+-ATPase activity, so results proved that Na^+,K^+-ATPase was sensitive to the Phenothrin with Spinosad. Generally of Pyrethroid (Phenothrin) pretreated with Bioinsecticides (Spinosad) will produce a new trend so as reduce the field does of Pyrethroid insecticides, enhance the role of beneficial insects and reduce the cost of pest control.

Keywords: Toxicity of *Spodoptera littoralis*, Inhibition of AChE, and Na^+,K^+-ATPase by different 4 insecticide.

INTRODUCTION

The Egyptian cotton leafworm, *Spedoptera littoralis* is the major pest attacking several crops and vegetables in Egypt, this pest cause the greatest part of cotton yield losses (Smagghe and Degheele, 1997; Amin, *et al.*, 2001, and Quero, *et al.*, 2002). Number of insecticides currently in widespread use: Organophosphates, Carbamates and Pyrethroides are usually used in Egypt (Devonshire and Moores, 1982 and Argentine, *et al.*, 2002) to suppress the *S. littoralis* resistance populations. The protection of crop plants from different pests is one of the most importants of profitable farming system. Pest-controlling by chemical pesticides has an important role in management insect pests attacking crops, which can easily be applied, give rapid control and have been successful against insects. Furtherment, the insecticides are the only tool for pest management that is reliable for emergency action threshold (Metcalf, 1982, and Aydin and Gurken, 2006). Although most of them does not give satisfactory results probably because of development of resistance (Ishaaya and Klein, 1990; Martin *et al.*, 2000, and El-Aw, *et al.*, 2002). Spinosad has strong insecticidal activity with low level of mammalian toxicity and relatively little toxicity to non-target insects (Sparks, *et al.*, 1998). Spinosad is highly toxic to insect especially Lepidoptera insect pests (Wang, *et al.*, 2006).

From this point the need for insect control is essential through chemical control (pesticides) (Casida and Quistad, 2005), so in present study which is concentrated on the combination of Spinosad with insecticides from different groups gives high reduction in cotton leafworm infestation.

MATERIALS AND METHODS

Insect:

Field strain of cotton leafworm, *Spedoptera littoralis* egg masses were collected from cotton fields at Abeis area, the 2nd larval instar chosen for bioassays and biochemical assessment.

Chemicals:

Phenothrin (Pyrethroids) provided as technical grade insecticides from U.S.A. Environmental Protection Agency (EPA). Thiodicarb (Carbamate) provided as technical grade insecticides from JinHung Fin Chem. Co. LTd. Koria. Chlopyrifose 48% EC (Organophosphorus) was obtained from Dow Chemical Co. Bionisecticides Spinosad (tracer 24% SC) it is a metabolite of the Actinomycete, *Saccharopolyspora spinosa* Martz and Yao., it is a naturally occurring mixture of two active products (Spinsoyn A and D). It is a trademark of Dow AngroSciences Co., (Dow England). Ouabain is a cardiac glycoside which specifically inhibits the Na^+,K^+-ATPase (McIlwain, 1963). A pure sample was obtained from Sigma Chem., Co. St. Loius.

Bioassay tests:

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

Second instar larvae were starved for 6hrs before exposed test the selected larvae were bioassayed against bionisecticide (Spinosad) 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 bionisecticide (Tabashink and Chushing, 1987). Each castor leaves disc ($2Cm^2$) 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 (on disc per cup) and left under constant conditions of 27 ± 2 °C and 65-70%RH. There after 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 Insecticides Against *S. littoralis*:

Phenothrin; Chlopyrifose and Thiodicarb, were bioassayed against the 2nd larval instar of *S. littoralis*. The castor leaves were dipped in different concentration of the tested insecticides. Thiodicarb and Phenothrin concentrations were prepared in pure acetone while Chlopyrifose concentration was 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 (Field strain) were used for each test with three replicate at least, number of alive and dead larvae per replicate was counted 24; 48, and 72hr after treatment. Concentration-mortality percentages were calculated and corrected for natural mortality according to Abbott equation (Abbott, 1925) LC_{50} values were calculated by using the probit-analysis method of Finney (1971).

3- Toxicity of Tested Spinosad in Presence of Tested Insecticides:

S. littoralis 2nd instar (Field strain) were treated with solution of Chlopyrifose; Thiodicarb, and Phenothrin at LC_{50} values concentrations before 24: 48, and 72h of feeding on discs of castor oil leaves discs treated with LC_{50} of Spinosad, joint action experiments have two controls. Larvae of the first control were allowed to fed castor oil leaf discs treated with concentration equivalent LC_{50} of Spinosad 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).

AChE Preparation and Activity Assay:

AChE was prepared from *Spodoptera littoralis* 2nd instar larvae was homogenized in Tris-HCl buffer (pH 7.4) at 30 larvae/30ml buffer, with polytron mixer (at 50% power for 50sec.), then subjected to low speed centrifuged at 5,000rpm for 15min at 4 °C. The resulting supernatant was 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.

Na^+,K^+-ATPase Preparation and Activity Assay:

Na^+,K^+-ATPase was prepared from *Spodoptera littoralis* 2nd instar larvae was homogenized in a solution of 0.32M sucrose, 1mMEDTA and 40mM Tris-HCl buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. Mitochondrial ATPase was prepared according to the method reported by Koch (1969), by differential centrifugation of the homogenate at 8000Xg for 10min. The supernatant was then centrifuged at 20000Xg for 30min. The formed pellets were then suspended in the buffer and stored at (-20 °C) for use.

The ATPase activity was measurements according to the method reported by Koch (1969) with slight modification by Morshedy (1980) using Tris-HCl buffer instead of imidazole buffer. Absorbancy of inorganic Phosphate (Pi) was measured at λ750nm (Taussky and Shorr, 1953). This method was based on the spectrophotometric determination of the inorganic Phosphate (Pi) liberated from the hydrolysis reaction of the ATP, mediated by the enzyme.

The ATPase activity was measured in total volume of 1ml. The mitochondrial preparation was mixed with a reaction mixture (700µl) containing 100mM Na^+; 20mM K^+; 5mM Mg^{2+} chlorides; 40mM Tris-HCl buffer (pH 7.4) and 5mM ATP. The volume was completed to 850µl with buffer. The mixture was incubated for 15min, in a shaking water bath at 37 °C. The reaction was stopped by adding 150µl trichloroacetic acid (TCA, 30%). Hydrolyzed Pi was determined according to the method, described by Taussky and Shorr, (1953). The activity of Mg^{2+}-ATPase was measured after

the addition of 1mM Ouabain, whereas the activity of Na^+,K^+-ATPase was calculated as the difference between the total ATPase and Mg^{2+}-ATPase activities.

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

***In Vivo* Inhibition of AChE and Na^+,K^+-ATPase Activity:**

In the inhibition studies, of AChE and Na^+,K^+-ATPase activity, 10µl of the enzyme preparation was incubated with of the inhibitor for 30min, the enzyme- inhibitor mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:-

$$\%\text{Inhibition} = \frac{V - Vi}{V} \times 100$$

Where:- (V) is the specific activity without inhibitor.

(Vi)is the specific activity in presence of inhibitor.

RESULTS AND DISCUSSION

The results of the toxicity of the tested compounds in terms of LC_{50} are given in Table (1) for 2nd instar larvae of *S. littoralis*. LC_{50} values after 24hr were 6.44; 0.64; 0.80, and 0.89 ppm for Spinosad; Phenothrin; Chlopyrifose and Thiodicarb respectively against *Spedoptera,* while LC_{50} values after 48hr were 5.36; 0.40; 0.73, and 0.80ppm for these tested compounds respectively. LC_{50} values after 72hr were 0.82; 0.23; 0.54, and 0.66ppm for these tested compounds respectively. Phenothrin was the most potent followed by Chlopyrifose; Thiodicarb and Spinosad

It is clear that Phenothrin was more toxic than Chlopyrifose; Thiodicarb and Spinosad in controlling of *S. littoralis*. These results are in agreement with many investigators, (Feshawi, *et al.*, 1991; Mohan and Katiyar 2000; Tadros, 2003; El-Aswad and Aly, 2007; El-Aswad and Abou-Taleb, 2008 and Aboel-Kassem, *et al.*, 2010).

Table 1: LC_{50} values of Spinosad and three tested insecticides to 2nd instar *S. littoralis* larvae.

Compounds	LC_{50} (ppm)		
	24hr	48hr	72hr
Spinosad	6.44	5.36	0.82
Phenothrin	0.64	0.40	0.23
Chlopyrifose	0.80	0.73	0.54
Thiodicarb	0.89	0.80	0.66

Toxicity of Spinosad Alone or Pretreated with the Tested Insecticides:

Data in Table (2) show the LC_{50} values of Spinosad are 6.44; 5.36 and 0.82 ppm after 24; 48 and 72hr against Field *Spedoptera* strain respectively. The interaction of Phenothrin; Chlopyrifose and Thiodicarb with Spinosad against Field strain of *Spedoptera* larvae were studied. Larvae were allowed to feed on castor oil discs treated with of the three tested insecticides.

The LC_{50} values of Spinosad pretreated with the Phenothrin; Chlopyrifose and Thiodicarb on Field strain of *Spedoptera* larvae are presented in Table (2). The enhancement of toxicity is calculated as a Potentiation factor (P.f.) Table (2). a Potentiation factor (P.f.) values for Phenothrin; Chlopyrifose and Thiodicarb are 11.93; 8.15 and 7.49 respectively, after 24hr treatment, the P.f. values of three tested insecticides are 16.24; 8.38 and 6.87 respectively, after 48hr treatment, while the P.f. values are 5.86; 2.05 and 1.28 for three tested insecticides respectively, after 72hr treatment. It is clear that the LC_{50} values concentrations of three tested insecticides enhancement the toxicity of the Spinosad on *S. littoralis* larvae. The mixtures of Phenothrin+Spinosad were the most toxic treatments than Chlopyrifose+Spinosad and Thiodicarb+Spinosad respectively.

In general, the susceptibility of *Spedoptera* larvae to Spinosad increases after Phenothrin. Phenothrin+Spinosad caused more toxic effect than single treatment, it could be concluded that Phenothrin enhanced the toxicity effect of Spinosad. Generally, efficacy of Spinosad has a very good additive toxicity for Phenothrin in Field *Spedoptera* strain. These results are agreement with finding (Entwistle, *et al.*, 1993; Abdel-Halim 1997; Rizk, *et al.*, 1999; Thompson, *et al.*, 1999; Liburd, *et al.*, 2000; Ali, 2001; Dow AgroSciences, 2001, and Mona, *et al.*, 2004), reported that Spinosad has been found to be highly active on most Lepidoptera.

***In Vivo* Inhibition of AChE and Na^+,K^+-ATPase Activity:**

The *in vivo* inhibition effect of the LC_{50} values of four compounds against to the *Spedoptera* 2nd instar field strain larval AChE and Na^+,K^+-ATPase are shown in the data given in Table (3). The data declared that Chlopyrifose and Thiodicarb exhibited the highest percentages of reduction of AChE activity while Phenothrin and Spinosad not active as inhibitor on AChE activity. On the other hand, Phenothrin exhibited the highest percentages of reduction of Na^+,K^+-ATPase activity followed by Spinosad while Chlopyrifose and Thiodicarb not active as inhibitor on Na^+,K^+-ATPase activity.

Data in Table (3) summarize the interaction of Spinosad and Three tested insecticides on the activity of AChE and Na^+,K^+-ATPase. The inhibition to be 24.2% and 92.6% for AChE and Na^+,K^+-ATPase respectively, when Spinosad pretreated with Phenothrin, while the inhibition of enzymes activity to be 75.7% and 17.4% for two enzymes respectively, when Spinosad pretreated with Chlopyrifose, morever the inhibition of enzymes activity to be 67.5% and 14.2% for two enzymes respectively, when Spinosad pretreated with Thiodicarb.

Table 2: Comparative toxicities of Spinosad alone or pretreated with three tested insecticides on *Spodoptera* larvae.

Compounds	LC_{50} (ppm)					
	24hr		48hr		72hr	
		P.f.		P.f.		P.f.
Spinosad	6.44		5.36		0.82	
Phenothrin +Spinosad	0.54	11.93	0.33	16.24	0.14	5.86
Chlopyrifose +Spinosad	0.79	8.15	0.64	8.38	0.40	2.05
Thiodicarb +Spinosad	0.86	7.49	0.78	6.87	0.64	1.28

Potentation factor (P.f.) = LC_{50} Spinosad alone / LC_{50} three tested insecticides+ Spinosad

Table 3: *In vivo* inhibition of *Spodoptera* larvae 2nd instar AChE and Na^+,K^+-ATPase activity by LC_{50} of some compounds.

Compounds	%Inhibition	
	AChE	Na^+,K^+-ATPase
Spinosad	18.1	56.2
Phenothrin	13.3	80.3
Chlopyrifose	75.7	11.4
Thiodicarb	70.2	10.3
Phenothrin+Spinosad	24.2	92.6
Chlopyrifose+Spinosad	75.7	17.4
Thiodicarb+Spinosad	67.5	14.2

It is quite clear that the *Spodoptera* larvae is more susceptible to Spinosad+ Phenothrin, it was observed that the high effective Bionisecticide compound and Pyrethroids compound. This result could be explained as the different modes of action of tested compounds allow these compounds to increase inhibition, also Duhoon and Banerjee (1984) who reported that synthetic pyrethroids gave good results followed by organophosphorus compounds these results are in agreement with (Gupta, 1990; Khidr, *et al.*, 1996, and Abou-Taleb, *et al.*, 2010).

In this work, suggest that the toxicity of Spinosad and three tested insecticides from different groups describe the sensitivity of AChE and Na^+,K^+-ATPase activity to tested compounds. It is concluded from the present results that the using bioinsecticide pretreated with synthetic insecticides and looking forward to an integrated pest management to overcome pest problems.

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الملخص العربى

تقييم بعض المركبات على يرقات دودة ورق القطن.

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الهدف من البحث هو تقيم التأثير الأبادى للمبيد الحيوى سبينوساد مع ثلاث مبيدات من مجاميع مختلفة هى فينوثرين وكلوربيرفوس وثيوديكارب ومخاليطهما وذلك على يرقات العمر الثانى لدودة ورق القطن للسلالة الحقلية بهدف تلاشى تأثير المبيدات التقليدية الضار على البيئة. وقد تم تسجيل قيم التركيزات النصف مميتة (LC_{50}) لكلاً من المبيد الحيوي (سبينوساد) والمبيدات الحشرية (فينوثرين والكلوربيرفوس وثيوديكارب) تحت الدراسة بصورة فردية. ثم تم معاملة يرقات العمر الثانى لدودة ورق القطن بتركيزات مختلفة (LC_{50}) من المبيدات الحشرية تحت الدراسة ثم معاملة هذه اليرقات بتركيز(LC_{50}) من السبينوساد بعد ٢٤ و٤٨ و٧٢ ساعة من المعاملة بالمبيدات الحشرية تحت الدراسة، فأوضحت النتائج أن قيم الـ LC_{50} بعد المعاملة أنخفضت بدرجة ملحوظة ويتضح ذلك من قيم معامل التنشيط (P.f.) الذى تم حسابها. وكذلك تم دراسة المقدرة التثبيطية للمركبات المختبرة على النشاط الأنزيمى لأنزيمين من الأنزيمات الهامة والحيوية بالنسبة للحشرة وهم أنزيم الأسيتايل كولين أستريز وأنزيم الصوديوم-بوتاسيوم أدينوسين تراى الفوسفاتيز. ولقد أوضحت النتائج أن فى حالة السبينوساد بعد المعاملة بالفينوثرين كانت النسبة المئوية للتثبيط هى ٩٢,٦% وذلك بالنسبة لأنزيم الصوديوم-بوتاسيوم أدينوسين تراى الفوسفاتيز، بينما فى حالة الكلوربيرفوس وثيوديكارب بعد المعاملة بالسبينوساد كانت النسبة المئوية للتثبيط أقل من ٥٠% لأنزيم الصوديوم-بوتاسيوم أدينوسين تراى الفوسفاتيز. ومن هذه النتائج أتضح أن يمكن أستخدام السبينوساد وخاصة مع المبيدات البيرثرويدية وبذلك يمكن أستخدامها فى برامج المكافحة المتكاملة لدودة ورق القطن.