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EFFICACY OF SOME BIOINSECTICIDES AGAINST THE IMMATURE STAGES OF THE COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The toxicity of chlorpyrifos, spinosad, phenothrin and emamectin compounds were evaluated against the immature stages of the cotton leafworm, *Spodoptera littoralis* (Boisd.) field strain. The results showed that chlorpyrifos was the most potent tested compound followed by spinosad, phenothrin and the least one was emamectin. Tested compounds, chlorpyrifos at 15.0 ppm achieved 48.0% mortality of treated egg masses, while spinosad, emamectin and phenothrin at the same concentration caused 17.6 , 14.4 and 30.4 % mortality, respectively. The toxicity of tested compounds were evaluated against the newly hatched larvae at 24 , 48 and 72 hr., post treatment. The results showed that spinosad and emamectin were the most potent than phenothrin and chlorpyrifos. Spinosad, emamectin, chlorpyrifos and phenothrin indicated no toxic action against pupae of *S. littoralis*. The interactions of phenothrin and chlorpyrifos with bioinsecticides *in vivo* on the inhibition of AChE and Na^+, K^+-ATPase were investigated. Results proved that pretreated of spinosad and emamectin with chlorpyrifos increased the percentage inhibition for AChE to 82.2 and 81.4% respectively, while the percentage inhibition for Na^+, K^+-ATPase by pretreated spinosad and emamectin with phenothrin were 93.8 and 89.7% successively. Results proved that pretreated of bioinsecticides with phenothrin caused more toxic effect than single treatment. The present results proved that the two bioinsecticides are potentially potent for control of cotton leafworm. Generally, bioinsecticides will produce a new trend to increase toxicity of the newly hatched larvae of this insect pest and enhance the role of beneficial insects. The results of the present study may add some forward steps to use bioinsecticides as an alternative to conventional insecticides especially against this insect. So, the bioinsecticides can be involved in important steps necessary for successful IPM programs applied against *S. littoralis*.

Key words: *Spodoptera littoralis*, immature stages, cholinesterase, Na^+,K^+-ATP-ase, bioinsecticides, chlorpyrifos, spinosad, phenothrin, emamectin.

INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) is a common pest, found in different areas in the world. This caterpillar is very polyphagous causing important economic losses in both greenhouses and open fields on a broad range of ornamental, industrial and vegetable crops in Egypt. This insect pest attacks all parts of plants of more than 70 cultivated crops. It has acquired resistance towards most insecticides groups commonly used on various crops in Egypt (Abo-Elghar *et al.*, 2005). Nowadays, the scientists of pest control and environmental protection oriented their activities to limit the environmental pollution. If this trend continues, new compounds will be required to replace these insecticides. Recently, 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

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demonstrated high toxicities to a number of insects. 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; Anonnomys, 2001). 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).

The purpose of this investigation is to study the ovicidal activity of two bioinsecticides, spinosad and emamectin against the egg stage and to evaluate their toxicity to the newly hatched larvae as well as pupae of *S. littoralis* (Thompson *et al.*, 1999; El-Aw, 2006). Also, the study was directed to throw the light on the effect of these tested bioinsecticides as well as other environmentally-friendly compounds for their possible use inhibition of Na^+, K^+-ATPase and AChE activity.

MATERIALS AND METHODS

Insect

Field strain of the cotton leafworm, *S. littoralis* egg masses were collected from cotton fields at Abeis area, Alex. Province, Egypt. Experiments were carried out using egg masses (24 hrs-old) and newly hatched larvae, which were chosen for bioassays as well the 2nd larval instar were chosen for biochemical assessment. Pupae (48 hrs-old) were also investigated.

Insecticides and Chemicals

Dursban (chlorpyrifos 48% EC) was obtained from Dow Chemical Co. Sumithrin (phenothrin) provided as technical grade pyrethroids insecticide from USA, Environmental Protection Agency (EPA). Ouabain is a cardiac glycoside which specifically inhibits the Na^+, K^+-ATPase. A pure sample was obtained from Sigma Chem. Co. ST. Loius.

Bionisecticides, emamectin 5% SG (emamectin benzoate) which is like abamectin supplied by Syngenta Co. 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 AgroSciences Co., (Dow England). *Beauveria bassiana*, Biosact (WP) ($32x10^{12}$ cells/kg), was obtained from Origanl Bio Technology Co.

Bioassay Tests

The determination of the ovicidal activity

Ovicidal activity of the phenothrin, chlorpyrifos, spinosad, and emamectin against the field strain of *S. littoralis* egg masses (0-24 hrs old) was investigated. They were removed gently with a fine hair brush. The lower layer in each egg mass was counted by the binocular. The counted egg samples were dipped (5 sec.) in different concentrations of the tested compounds, while control was dipped in water according to Dittrich (1967). Each treatment was replicated three times. Treatment and control were held in a clean plastic cup (9x4 cm) at 27 ± 2 °C, 65-75% RH and observed until hatching. The number of unhatched eggs, dead neonates and alive larvae were counted and the mortality percentages were calculated.

Toxicity of the tested bionisecticides against larvae

Newly hatched larvae of *S. littoralis* were starved for 6 hrs before exposure test. The selected larvae were bioassayed against bionisecticides (spinosad and emamectin) 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 leaf disc (2 cm^2) was dipped into the suspension of tested formulation for 10 sec. Tested concentrations were prepared in glass distilled water (GDW) (Toni and Fred, 1996). Discs were held vertically to allow excess solution to drip off and placed on a rack to dry for at last 2 hrs. Treated discs were offered to starved larvae (one disc per cup) and left under constant conditions (27 ± 2 °C and 65-70% RH). Thereafter, survivors were transferred with fresh castor oil plant leaves to clean cups and kept under the same conditions. Control larvae were allowed to feed on castor oil leaf discs treated with distilled water. Mortality percentage was calculated for each concentration daily for 24, 48 and 72 hrs and corrected according to Abbott's equation (Abbott, 1925) and subjected

to probit analysis using the computer program (Finney, 1971).

Toxicity of the tested insecticides against larvae

Phenothrin and cholorpyrifos were bioassayed against the newly hatched larvae of *S. littoralis*. The castor leaves were dipped in different concentrations of the tested insecticides. Phenothrin concentrations were prepared in pure acetone while chlorpyrifos concentrations were prepared in distilled water. Treated and control leaves were air-dried for 3 hrs and 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 replicates at least. Number of alive and dead larvae per replicate was counted 24, 48, and 72 hrs after treatment. Concentrations–mortality percentages were calculated and corrected for natural mortality according to Abbott's equation (Abbott, 1925). LC_{50} values were calculated by using probit-analysis method of Finney (1971).

Toxicity of the tested compounds on pupae

Using the residual film method to determine the LC_{50} values of 48 hrs-old *S. littoralis*, pupae with different concentrations of tested compounds. The experiment was left for 24, 48 and 72 hrs in pre-treated petri-dishes. Concentrations-mortality percentages were calculated and corrected for natural mortality according to Abbott's equation (Abbott, 1925). LC_{50} values were calculated by using probit-analysis method of Finney (1971).

AChE Preparation and Activity Assay

AChE was prepared from *Spodoptera littoralis* 2nd instar larvae and homogenized in Tris-HCl buffer (pH 7.4) at 30 larvae/30ml buffer, with polytron mixer (at 50% power for 50 sec.), then subjected to low speed centrifuge at 5000 rpm for 15 min at 4 °C. The resulting supernatant was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was centrifuged at 25,000 rpm for 1hr at 4 °C. Pellets were resuspended in 1ml of Tris-HCl buffer (pH 7.4) and stored at -20 °C for using 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 a 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 colour production as a function of enzyme activity is measured spectrophotometrically at λ 412 nm. 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 and homogenized in a solution of 0.32 M sucrose, 1mM EDTA and 40 mM Tris-HCl buffer (pH 7.4). The homogenate was filtered through two layers of cheesecloth. Mitochondrial ATPase was prepared according to the method reported by Koch *et al.* (1969), by differential centrifugation of the homogenate at 8000 Xg for 10 min. The supernatant was then centrifuged at 20,000 Xg for 30 min. The formed pellets were then suspended in the same buffer and stored at -20 °C for use.

ATPase activity was measured according to the method reported by Koch *et al.* (1969) with slight modification by Morshedy (1980) using Tris-HCl buffer instead of imidazole buffer. Absorbancy of inorganic phosphate (Pi) was measured at λ750 nm (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 (700 µl) containing 100 mM Na^+; 20 mM K^+; 5 mM Mg^{2+} chlorides; 40 mM Tris-HCl buffer (pH 7.4) and 5 mM ATP. The volume was completed to 850 µl with buffer. The mixture was incubated for 15 min in a shaking water bath at 37 °C. The reaction was stopped by adding 150 µl 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 λ750 nm using Bovine Serum Albumin (BSA) as a standard protein.

In vivo Inhibition of AChE and Na^+, K^+-ATPase Activity

The inhibition of AChE and Na^+, K^+-ATPase activity were determined in the 2nd instar larvae using the values of each of the tested compounds. In the inhibition studies of AChE and Na^+, K^+-ATPase activity 10 µl of the enzyme preparation was incubated with the inhibitor for 30 min, the enzyme-inhibitor mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:

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

Where:

(V) is the specific activity without inhibitor.

(Vi) is the specific activity in presence inhibitor

RESULTS AND DISCUSSION

Ovicidal Activity Against Egg Masses

Plant leaves containing egg masses were dipped in different concentrations of each tested compound. Data in Table 1 show the ovicidal activity of the tested compounds against 24 hrs-old eggs of *S. littoralis*. The results showed that, spinosad and emamectin have low ovicidal activity compared with chlorpyrifos and phenothrin.

Percentages of unhatched eggs at 0.5 ppm were 9.1, 6.4, 20.0 and 16.4 for spinosad, emamectin, phenothrin and chlorpyrifos, respectively against eggs of *S. littoralis* field strain. Percentages of unhatched eggs at 1.0 ppm were 9.3, 8.3, 22.3 and 39.7; whereas at 5.0 ppm they were 11.9, 10.3, 25.7 and 43.1, successively. On the other hand, mortality percentages of unhatched eggs at 10.0 ppm were 16.9, 11.1, 28.8 and 47.2%, as well as at 15.0 ppm they were 17.6, 14.4, 30.4 and 48.0% respectively. However, significant differences in percentages of unhatched eggs were found between all tested concentrations of the tested compounds and control eggs. The present results demonstrated that the *S. littoralis* eggs were more susceptible to chlorpyrifos in comparison to spinosad and emamectin. In general the susceptibility of eggs to the tested bioinsecticides indicated low ovicidal activity.

These results are in agreement with some investigators such as Ascher *et al.* (1987) who recorded that the tested bioinsecticides have a low ovicidal activity compared with methomyl and chlorpyrifos. Bueno and Freitas (2004) reported that abamectin has no effect on the *Chrysoperla externa* egg viability. Also, these results are in accordance with those obtained by many investigators (Mitri and Kamel, 1970; El-Gayar *et al.*, 1979; El-Guindy *et al.*, 1983; Does *et al.*, 1985; Bret *et al.*, 1997; Canela *et al.*, 2000; Raslan, 2002; Bueno and Freitas, 2004; Saleem *et al.*, 2008a).

Toxicity on Newly Hatched Larvae

Table 2 shows that the LC_{50} values of tested compounds were decreased. In general the two bioinsecticides decreased LC_{50} values by increasing the post treatment period. The toxicity results of the tested compounds in terms of LC_{50} are given in Table 2 for the larvae of *S. littoralis*. LC_{50} values after 24 hrs were 1.89, 3.66, 0.75 and 0.86 ppm for spinosad, emamectin, phenothrin and chlopyrifos against the field strain of *S. littoralis*, respectively. While LC_{50} values after 48 hrs were 0.25, 0.40, 0.079 and 0.094 ppm, successively. LC_{50} values after 72 hrs were 0.041, 0.053, 0.061 and 0.085 ppm, respectively.

The interactions of phenothrin and chlorpyrifos with two bioinescticides against filed strain of *S. littoralis* larvae were studied. Larvae were allowed to feed on castor oil leaf discs treated with LC_{50} of the different bioinsecticides. The LC_{50} values of phenothrin and chlorpyifos pretreated with the LC_{50} values of spinosad and emamectin on filed strain of *S. littoralis* larvae are presented in Table 3. The LC_{50} values of tested insecticides when pretreated with two bioinsecticides were lower than LC_{50} of tested insecticides alone. The enhancement of toxicity was calculated as a potentiation factor (P.f.). P.f values for phenothrin and chlorpyrifos were 2.34 and 1.13

Table 1. Ovicidal activity of tested insecticides against 48 hrs-old eggs of the cotton leafworm, *S. littoralis*

Conc. (ppm)	Spinosad		Emamectin		Phenothrin		Chlorpyrifos	
	H*%	UH*%	H%	UH%	H%	UH%	H%	UH%
Control	91.8	8.2	93.8	6.2	94.7	5.30	86.6	13.4
0.5	90.9	9.1	93.6	6.4	80.0	20.0	83.6	16.4
1	90.7	9.3	91.7	8.3	77.7	22.3	60.3	39.7
5	88.1	11.9	89.7	10.3	74.3	25.7	56.9	43.1
10	83.1	16.9	88.9	11.1	71.2	28.8	52.8	47.2
15	82.4	17.6	85.6	14.4	69.6	30.4	52.0	48.0

*H; Hatched eggs * UH; Unhatched eggs

Table 2. Toxicity of tested compounds against the newly hatched larvae of *S. littoralis*

Compounds	LC_{50} (ppm)		
	24 hrs	48 hrs	72 hrs
Spinosad	1.89	0.25	0.041
Emamectin	3.66	0.40	0.053
Phenothrin	0.75	0.079	0.061
Chlorpyrifos	0.86	0.094	0.085

Table 3. Comparative toxicities of tested insecticides alone or pretreated with two bioinsecticides on *S. littoralis* larvae

Compounds	LC_{50} (ppm)			
	24 hrs		48 hrs	
	LC_{50}	P.f.	LC_{50}	P.f.
Chlorpyrifos	0.86		0.094	
Spinosad+chlorpyrifos	0.76	1.13	0.077	1.22
Emamectin+chlorpyrifos	0.79	1.09	0.080	1.18
Phenothrin	0.75		0.079	
Spinosad+ phenothrin	0.32	2.34	0.024	3.29
Emamectin+phenothrin	0.44	1.71	0.033	2.39

*Potentiation factor (P.f.) = LC_{50} of insecticide alone / $LC_{50}$of bioinsecticide + insecticide.

respectively; when pretreated with spinosad after 24 hrs from treatment, while the P.f. values were 3.29 and 1.22, respectively when pretreated with spinosad after 48 hrs from treatment. Also, when pretreated with emamectin the P.f. values were 1.71 and 1.09 for phenothrin and chlorpyrifos respectively after 24 hrs, while the P.f. values were 2.39 and 1.18, respectively after 48 hrs from treatment. It is clear that the LC_{50} values concentrations of bioinsecticides enhanced the toxicity of the tested insecticides on *S. littoralis* larvae. The mixture with spinosad were the most toxic treatments than mixtures with emamectin.

These results are in agreement with those found by many authors (Ascher *et al.*, 1987; Canela *et al.*, 2000; Thompson *et al.*, 2000; Bueno and Freitas 2004; Pineda *et al.*, 2004; Pineda *et al.*, 2007; Saleem *et al.*, 2008b).

In general, the susceptibility of *S. littoralis* larvae to tested insecticides increased when treated after bioinsecticides, the observation that tested insecticides had the lowest effect when applied alone but it was the best when mixed with bioinsecticides.

The tested insecticides+bioinsecticides caused more toxic than the effect of single treatment

with tested insecticides. It could be concluded that tested insecticides enhanced the toxicity effect of bioinsecticides. Based on P.f. values, the field strain of *S. littoralis* larvae is more susceptible to spinosad in comparison to the emamectin. Generally, efficacy of bioinsecticides have a very good additive toxicity for tested insecticides with the field strain of *S. littorallis*.

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 (Liburd *et al.*, 2000; El-Mandarawy *et al.*, 2004). Generally, it could be concluded that the use of tested insecticides and their mixtures with biological insecticides (spinosad and emamectin) 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 tested insecticides is looking forward in an intergrated pest management to overcome pest problems.

Toxicity on Pupae

The toxic action of tested compounds against pupae of *S. littoralis* was also investigated. Data in Tables 4 and 5 show treatment of 48 hrs-old pupae of *S. littoralis* with different concentrations of tested compounds. The results demonstrated that no significant differences in the percentages

Table 4. Pupicidal activity of the two bioinsecticides to 48 hrs-old pupae of the cotton leafworm, *S. littoralis*

Time posttreatment (hrs)	% Accumulative mortality of pupae											
	Control		Concentrations (ppm)									
			0.5		1.0		5.0		10.0		15.0	
	S*	E*	S	E	S	E	S	E	S	E	S	E
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	1.1	1.0	2.2	2.2	3.2	2.3
72	0.0	0.0	0.0	0.0	3.3	2.2	4.3	2.4	4.9	2.8	5.0	3.5
% Died pupae	0.0	0.0	0.0	0.0	3.8	2.2	5.4	3.4	7.1	5.0	8.2	5.8
% Emerged adults	100	100	100	100	96.2	97.8	95.3	96.6	93.9	95.0	90.8	92.2
% Nonemerged adults	0.0	0.0	0.0	0.0	3.8	2.2	5.4	3.4	7.1	5.0	8.2	5.8

* S; spinosad and * E; emamectin*

Table 5. Pupicidal activity of the two insecticides to 48 hrs-old pupae of the cotton leafworm, *S. littoralis*

Time posttreatment (hrs)	% Accumulative mortality of pupae											
	Control		Concentrations (ppm)									
			0.5		1.0		5.0		10.0		15.0	
	C*	Ph*	C	Ph	C	Ph	C	Ph	C	Ph	C	Ph
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	2.2	1.1	1.1	2.3
72	0.0	0.0	1.4	1.0	2.0	2.4	2.6	2.0	4.9	2.5	3.0	3.5
% Died pupae	0.0	0.0	1.4	1.0	2.0	2.4	3.6	3.0	7.1	3.6	4.1	5.8
% Emerged adults	100	100	98.0	99.0	98.4	98.6	97.0	96.6	96.6	95.4	95.9	94.2
% Nonemerged adults	0.0	0.0	0.0	1.0	3.3	2.4	3.6	3.0	7.1	3.6	4.1	5.8

*C; chlorpyrifos and *Ph; phenothrin

of pupal mortality or deformed adults (2.2-8.2%) compared with untreated pupae for spinosad and emamectin, respectively. The percentages of pupal mortality or deformed adults about (1.0-7.1%) compared with untreated pupae for chlopyrifos and phenothrin, respectively. It was observed from the present results that the two tested bioinsecticides and two tested insecticides had the lowest effect on pupae of *S. littoralis*.

These results are in agreement with many investigators (Thompson *et al.*, 1999; Argentine *et al.*, 2002; Vergoulas and Jousseaume, 2002; El-Aw, 2006; Pineda *et al.*, 2004).

The *in Vivo* Inhibition of AChE and Na^+, K^+-ATPase Activity

The *in vivo* inhibitory effects of the LC_{50} values of tested compounds against the *S. littoralis* 2nd instar field strain larvale AChE and Na^+, K^+-ATPase are shown in Table 6. The data declared that phenothrin, spinosad and emamectin exhibited the highest percentages of reduction of Na^+, K^+-ATPase activity as values were 83.7, 60.6 and 54.7%, respectively; while the values of 27.4% for chlorpyrifos proving that it was not active as inhibitor on Na^+, K^+-ATPase activity. Chlorpyrifos was used as a specifically inhibits the AChE. The data declared that chlorpyrifos exhibited the highest percentage of reduction of AChE activity as the value was 80.9%; while values were 28.1, 26.2 and 21.4% for phenothrin, spinosad and emamectin, respectively. It was clear that the phenothrin and the two bioinsecticides tested were not active as inhibitor on AChE activity.

Data in Table 6 summarize the interaction of spinosad and emamectin on the inhibitory effect of phenothrin and chlorpyrifos on AChE and Na^+, K^+-ATPase activity. The results proved that the pretreatment of spinosad and emamectin with phenothrin and chlorpyrifos induce increase the inhibition of enzyme activity. The inhibition of AChE activity by chlorpyrifos was 80.9%, while it increased to be 82.2 and 81.4% for spinosad and Emamectin, respectively when the two bioinsecticides pretreated with chlorpyifos. Morover, the inhibition of Na^+, K^+-ATPase activity increased to be 93.8 and 89.7% for spinosad and emamectin, respectively when two bioinsecticides pretreated with phenothrin.

These results are in agreement with many investigators (Smagghe and Degheele, 1997; Salahgado, 1998; El-Aw, 2006; Saleem *et al.*, 2008a).

Results indicated that bioinsecticides may make activation of Na^+, K^+-ATPase activity and these may be increased the phenothrin effects on Na^+, K^+-ATPase activity. It is quite clear that the Pyrethroids at LC_{50} concentration acts as potential inhibitors for *S. littoralis* larvae Na^+, K^+-ATPase activity when pretreated with bioinsecticides. It was concluded from the present results that the tested pyrethroids were potentially potent for control of *S. littoralis* however, with new compounds, such as bioinsecticides currently in use, *S. littoralis* could be successfully included in the management programs.

Table 6. *In vivo* inhibition of *S. littoralis* 2nd instar larvae AChE and Na^+, K^+-ATPase activity by some compounds (LC_{50})

Compounds	(%) inhibition	
	AChE	Na^+,K^+-ATPase
Spinosad	26.2%	60.6%
Emamectin	21.4%	54.7%
Chlorpyrifos	80.9%	27.4%
Phenothrin	28.1%	83.7%
Spinosad+chlorpyrifos	82.2%	23.1%
Spinosad+ phenothrin	26.3%	93.8%
Emamectin+ chlorpyrifos	81.4%	20.4%
Emamectin+ phenothrin	22.6%	89.7%

REFERENCES

Abbott, W. S. (1925). A method for computing the effectiveness of insecticides. J. Econ. Entomol., 18: 265-267.

Abo-Elghar, G.E., Z.A. Elbermawy, A.G. Yousef and H.K. Abd-Elhady (2005). Monitoring and characterization of insecticides resistance in the cotton leafworm, *Spodoptera littoralis* (Biosd.) (Lepidoptera: Noctuidae). J. Asia-Pacific Entomol., 8: 397-410.

Anonnomys (2001). Spinosad. Technical Bulletin. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268-1054, Y 45-000-001 (01/01) CBK, ML, 6596150.

Argentine, J.A., R.K. Jansson, W.R. Halliday, D. Rugg and C.S. Jany (2002). Potency, spectrum and residual activity of four new insecticides under glasshouse conditions. Florida Entomologist, 85: 552-562.

Ascher, R.S., V. Melamed-Madjar, E.N. Nemny and S. Tam (1987). The effect of benzoyl-phenyl urea molting inhibitors on larvae and eggs of the European corn borer, *Ostrinia nubitalts* Hbn. (Lepidoptera, Pyralidae). J. Plant Disease Protection, 94: 584-589.

Bret, B.L., L.L. Larson, R.J. Schoonover, C.T. Sparks and D.G. Thompson (1997). Biological properties of spinosad. J. Econ. Entomol., 60: 13-15.

Bueno, A.F. and S. Freitas (2004). Effect of the insecticides abamectin and lufenuron on eggs and larvae of *Chrysoperla externa* under laboratory conditions. BioControl., 49: 277-283.

Canela, R., M. Balcells, L. Dalmau and J. Avilla (2000). Ovicidal, larvicidal and juvenilizing activity of a picolinephenoxanilide against *Cydia pomonella*. Pest Manag. Sci., 56: 586-590.

Dittrich, V. (1967). A formamidine acaricide as an ovicide for three insect species. J. Econ. Entomol., 60: 13-15.

Does, S.A., S. Saddik and A.M. Assem (1985). Efficiency of some insecticides in controlling the cotton leafworm, *Spodoptera littoralis* (Boisd.) on vegetable crops (Lepidoptera : Nocuidae). Bulletin of Entomological Society of Egypt, Econ. Series, 8: 215-220.

El-Aw, A.M. (2006). Efficacy of two biorational insecticides, emamectin benzoate and spinosad, against the immature stages of the cotton leafworm, *Spodoptera littoralis* (Biosd.) (Lepidoptera: Noctuidae). Alex. J. Agric. Res., 51: 55-68

El-Gayar, F.H., M. Abbossy, F. Khalil and W. Watson (1979). Efficiency of certain insecticides in controlling *Spodoptera littoralis* (Boisd.) and persistence of these insecticides under field conditions. Alex. J. Agric. Res., 27: 433-437.

El-Guindy, M.A., M.M. Abdel-Sattar and M.A. El-Refai (1983). The ovicidal action of insecticides and insect growth regulator/ insecticide mixtures on the egg of various ages of susceptible and diflubenzuron-resistant strains of *Spodoptera littoralis* Boisd. Pestic. Sci., 14: 253-260.

Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacy, 7: 88-95.

El-Mandarawy, M.B.R., M.A.Z. El-Naggar and S.A. Abdel-Samae (2004). Evaluation of feeding stimulant mixed with chemical and bioinsecticides on certain lepidopterous pests and their natural enemies in cotton and soybean fields. J. Appl. Sci. Environ. Mgt., 8: 77-83.

Finney, D.J. (1971). Probit analysis, 3rd ed. Cambridge Univ. Press, Cambridge, England.

Koch, R.B., L.K. Cutkomp and F.M. Do (1969). Chlorinated hydrocarbon insecticide inhibition of cockroach and honey bee ATPase. Life Sci., 8: 289-297.

Liburd, E.O., J.E. Funderburk and S.M. Olson (2000). Effect of biological and chemical insecticides on *Spodoptera littoralis* (Lep., Noctuidae) and marketable yield of tomatoes. J. Applied Entomol., 124: 19-25.

Lowery, H.O., N.J. Rosbrough, A.L. Farr and R.J. Ranball (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mitri, S. and A.A. Kamel (1970). The ovicidal effect of certain newer insecticides on *Spodoptera littoralis* egg masses. J. Econ. Entomol., 63: 676-678.

Morshedy, M. (1980). Comparative study on enzymes and metabolic inhibitors. Ph.D. Thesis, Fac. Agric. Alexandria Univ.

Pineda, S., F. Budia, M.I. Schneider, A. Gobbl, E. Vifiuela, J. Valle and P. Del Estal (2004). Effect of two biorational insecticides, spinosad and methoxyfenozide, on *Spodoptera littoralis* (Lepidoptera: Nocuidae) under laboratory conditions. J. Econ. Entomol., 97: 1906-1911.

Pineda, S., M.I. Schneider, G. Smagghe, A.M. Martinez, P. Del Estal, E. Vifiuela, J. Valle and F. Budia (2007). Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Nocuidae). J. Econ. Entomol., 100: 773-780.

Raslan, S.A.A. (2002). Preliminary report on initial and residual mortality of the natural product, spinosad for control of egg masses of cotton leafworm in cotton at Sharkia Governorate, Egypt, Proceeding of the 2nd Intern. Conf., Plant Pro. Res. Ins., Cairo, Egypt, 21-24.

Salahgado, V.L. (1998). Studies on the mode of action of spinosad: symptoms and physiological correlates. Pestic. Biochem. Physiol., 60: 91-102.

Saleem, H.S., A. Munir, A. Mushtaq, A. Muhammad and A.H. Sayyed (2008a). Resistance to selected organochlorine, organophosphate, carbamate and pyrethroid in *Spodoptera littoralis* (lepidoptera: Nocuidae) from Pakistan. J. Econ. Entomol., 101: 1667-1675.

Saleem, H.S., Z.A. Motagatly and U. Skatulla (2008b). On the mode of action of Dimilin as a moulting inhibitor in some lepidopterous insects. J. Appl. Entomol., 80: 396-407.

Smagghe, G. and D. Degheele (1997). Comparative toxicity and tolerance for the ecdysteroid mimic tebufenozide in a laboratory strain of cotton leafworm (Lepidoptera : Noctuidae). J. Econ. Entomol., 90: 278-282.

Sparks, T.C., G.D. Thompson, H.A. Kirst, L.L. Hertlein, T.V. Worden and S.T. Thibauult (1998). Biological activity of the spinosyns new fermentation derived insect control agents on tobacco budworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol., 91: 1277-1283.

Tabashink, B.F. and L.N. Chushing (1987). Leaf residue vs. topical bioassays for assessing insecticide resistance in the diamond back moth *Plutella ylostella.* FAO Plant Prot. Bull., 35: 11-14.

Taussky, H.H. and E. Shorr (1953). A microcolorimetric method for the determination of inorganic phosphorus. J. Biol. Chem., 202: 675-685.

Thompson, G.D., R. Dutton and C.T. Sparks (2000). Spinosad a case study: an example from a natural products discovery programme. Pest Management Sci., 56: 696-702.

Thompson, G.D., S.H. Hutchins and T.C. Sparks (1999). Development of spinosad and attributes of a new class of insect control products. Dow AgroSciences LLC, 9330 Zionsville Rd., Indianapolis, IN 46268, USA. Minnesota Univ.

Toni, M. and G. Fred (1996). Effect of surfactants, *Bacillus thuringiensis* formulations and plant damage on oviposition by diamond back moth (Lepidoptera: Plutellidae). J. Econ. Entomol., 89: 891-897.

Vergoulas, P. and C. Jousseaume (2002). Development of spinosad, a naturally derived insect control agent, in agriculture in the Mediterranean countries. Proceedings of the 2nd International Conference, Plant Prot. Res. Inst., Cairo, Egypt: 81-91.

Wang, W., J. Mo, J. Cheng, P. Zhuang and Z. Tang (2006). Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Pesticide Biochem. and Physiol., 84: 180-187.

كفاءة بعض المركبات الحيوية ضد الأطوار غير الكاملة لحشرة دودة ورق القطن

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الهدف من البحث هو تقييم التأثير الإبادى لإثنين من المبيدات الحيوية هما سبينوساد وبروكلام مع إثنين من المبيدات الحشرية فينوثرين وكلوربيريفوس ضد الأطوار غير الكاملة للسلالة الحقلية لحشرة دودة ورق القطن *Spodoptera littoralis* بهدف تلاشى تأثير المبيدات التقليدية الضار على البيئة. أوضحت النتائج أنه سبينوساد وبروكلام وفينوثرين لهم سمية ضعيفة على البيض مقارنة بمبيد كلوربيريفوس فلقد أوضحت النتائج أنه فى حالة الكلوربيريفوس كانت النسبة المئوية للبيض غير الفاقس هى ٤٨,٠% عند تركيز ١٥جزء فى المليون بينما السبينوساد والبروكلام والفينوثرين عند نفس التركيز كانت النسبة المئوية للبيض غير الفاقس هى ١٧,٦و١٤,٤ و ٣٠,٤% على الترتيب، وعلى الرغم من أن سبينوساد وبروكلام لهما سمية منخفضة على البيض إلا النشاط الإبادى للمتبقى على اليرقات حديثة الفقس كان كبيراً. وقد تم تسجيل قيم التركيزات النصف مميتة (LC_{50}) لكل من المبيدات الحيوية والمبيدات الحشرية تحت الدراسة بصورة فردية، وقد أظهرت قيم الـ LC_{50} أن كلاً من السبينوساد والبروكلام كانا أكثر سمية على اليرقات حديثة الفقس بالمقارنة بفينوثرين وكلوربيريفوس. ثم بعد ذلك تم معاملة يرقات العمر الثانى لدودة ورق القطن بتركيزات مختلفة (LC_{50}) من المبيدات الحيوية تحت الدراسة ثم معاملة هذه يرقات بتركيز (LC_{50}) من المبيدات الحشرية تحت الدراسة بعد ٢٤، ٤٨ و ٧٢ ساعة من المعاملة بالمبيدات الحيوية، فأوضحت النتائج أن قيم الـ LC_{50} بعد المعاملة انخفضت بدرجة ملحوظة ويتضح ذلك من قيم معامل التنشيط (P.f) الذى تم حسابها. ثم أيضاً تم تقييم الفعل السام لكلٍ من المبيدات المختبرة على طور العذراء وأظهرت النتائج أنه بعد ٢٤، ٤٨ و ٧٢ ساعة من المعاملة بأى من المبيدات المختبرة لم يلاحظ وجود فروق معنوية فى النسب المئوية للموت فى العذارى. وكذلك تم دراسة المقدرة التثبيطية للمبيدات المختبرة على النشاط الإنزيمى لإنزيمين من الإنزيمات الهامة والحيوية بالنسبة للحشرة وهم إنزيم الأسيتايل كولين أستريز وإنزيم الصوديوم-بوتاسيوم أدينوسين تراى الفوسفاتيز. ولقد أوضحت النتائج أنه فى حالة ألسبينوساد والبروكلام بعد المعاملة بالكلوربيرفوس كانت النسبة المئوية للتثبيط هى ٨٢,٢% و ٨١,٤% على الترتيب وذلك بالنسبة لأنزيم الأسيتايل كولين أستريز، بينما فى حالة السبينوساد والبروكلام بعد المعاملة بالفينوثرين كانت النسبة المئوية للتثبيط ٩٣,٨% و ٨٩,٧% على التولى لإنزيم الصوديوم-بوتاسيوم أدينوسين تراى الفوسفاتيز. ومن هذه النتائج إتضح أنه يمكن استخدام السبينوساد وخاصة مع المبيدات البيرثرويدية وتشير النتائج إلى أن هناك تأثير سام قوى للمبيدات الحيوية المختبرة وخاصة السبينوساد على الأطوار غير الكاملة لحشرة دودة ورق القطن، وبذلك يمكن استخدام هذه المبيدات الحيوية فى برامج المكافحة المتكاملة لدودة ورق القطن.

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