Toxicity and Biochemical Impacts of Some New Insecticide Mixtures on Cotton Leafworm *Spodoptera littoralis* (Boisd.)

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Abstract

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The susceptibility to five new insecticide mixtures: chlorosan, feroban, cygron, engeo, and kingbo was studied in the 2nd and 4th instar larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.). The efficiency and residual effects of these compounds against *S. littoralis* under field conditions were also investigated. Obtained results revealed that feroban was the most effective compared with the other toxicants, while engeo was the least toxic insecticide in both instars after 2 and 5 days from treatment. Data also indicated that feroban had the longest half-life (Lt_{50}) while engeo recorded the shortest one. Biochemical analysis showed that the tested compounds caused pronounced changes in acetyl cholinesterase and phenol oxidase.

Keywords: insecticide mixtures; toxicity; enzymes; *Spodoptera littoralis*

Insecticide mixtures are usually applied in the field to enhance the spectrum of the control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of the control of a single pest to delay the development of insecticide resistance or to combat current resistance in a pest species. Using mixtures as a countermeasure for resistance management in insect pests has been advocated by several researchers (Ishaaya *et al.* 1985; Ascher *et al.* 1986; Mushtaq 2004). Insecticide resistance has become a major obstacle to successful chemical control with conventional insecticides. The evolution of resistance to insecticides is governed by a complex of events and factors; mainly, intense and repeated applications of insecticides which are often from the same chemical group or which employ the same mode of action. To prevent the resistance phenomenon, there is a need for different compounds having different modes of action (Aydin & Gürkan 2006). Mixtures are available as pre-mixes from pesticide companies or they are tank-mixed by farmers. Ideally, the insecticides having different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pest. When two compounds are mixed, they can be either potentiating or additive or antagonistic in an insect species. These effects can be varied on different insect species or strains depending upon their physiology and the mechanism(s) of resistance developed. If a mixture is potentiating, it is a useful tool in enhancing control efficacy and combating insecticide resistance. In this case, there may be a potential for reducing the application rate of one or both components of the mixture. If a mixture is antagonistic, it should not be used, because it will reduce the efficiency of pest control and aggravate the resistance problem (Swelam & Sayed 2006). Synergism between pyrethroids and organophosphates (OPs) or carbamates has already been demonstrated in the control of agricultural pests (Ozaki *et al.* 1984; Bynum *et al.* 1997; Martin *et al.* 2003). The mechanisms of resistance (specific biochemical changes that occur) and the speed with

which resistance develops vary according to the specific insect (e.g. the frequency of alleles for resistance inherent in the population), and the class and application rate of the insecticide. The occurrence of insect resistance to an insecticide is mainly due to the action of enzymes which are either insensitive to the insecticide or able to degrade it to nontoxic metabolites. Because of their dissimilar modes of action, pyrethroids and organophosphates (OPs) have commonly been mixed since the mid-1980s to manage the pest complex of cotton and other crops (Mushtaq 2004). Insect Growth Regulators (IGRs) have a much slower mode of action than synthetic chemical insecticides. IGRs include juvenile hormone (JH) mimics and chitin synthesis inhibitors (CSIs). CSIs, such as lufenuron, inhibit the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize a new cuticle, and therefore unable to successfully moult into the next stage. The efficiency of insecticides and their mixture with the IGRs against the cotton leafworm attracted several investigators (Ravi & Verma 1997; El-Aswad 2007).

Recently, due to the important role of insecticide mixtures in reducing insect resistance many mixtures of compounds have appeared in the market in Egypt, and so it is convenient to study the toxicity and enzymes that play a role in the change of response to some new insecticide mixtures on the cotton leafworm, *Spodoptera littoralis* (Boisd.), which has its importance as one of the most destructive phytophagous lepidopterous pests in Egypt because it causes various ravages not only for cotton plants but also for other field crops and vegetables.

MATERIAL AND METHODS

Toxicological studies. The present study was conducted to investigate the susceptibility of a laboratory strain of the 2nd and 4th instar larvae of the cotton leafworm *S. littoralis* (Boisd.) to five new insecticide mixtures (Table 1). A laboratory strain of the cotton leafworm *S. littoralis* (Boisd.) was maintained under constant conditions of 25 ± 1°C and 70 ± 5% RH and kept off any contamination by chemicals till the time of study in order to obtain a susceptible and homogenous strain as described by El-Defrawi *et al*. (1964).

Toxicity tests. A series of concentrations (in water) for each insecticide was prepared on the active ingredient (a.i.) based on ppm by diluting the commercial formulation. Castor-bean leaves were dipped in each concentration for 30 s and then left to dry for one hour. The 2nd and 4th instar larvae were confined with treated leaves in glass jars covered with muslin for 48 hours. Test also included a nontreated control in which leaves were dipped in water (as a check). Treated leaves were then removed and fresh untreated leaves were provided for three days. Four replications (each of 10 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded twice,

Table 1. Active ingredient and applied rate of tested insecticide mixtures

Tread name	Active ingredient and formulation	Active ingredient (mode of action)	Applied rate (ml/l)
Chlorosan[a]	29% EC	chloropyrifos 24% (cholinesterase inhibitor) + cypermethrin 5% (sodium channel modulator)	1.875
Feroban[b]	50% EC	chloropyrifos 47.5% (cholinesterase inhibitor) + lufenuron 2.5% (chitin synthesis inhibitor)	2.500
Cygron[b]	10% EC	flufenoxuron 3% (chitin synthesis inhibitor) + alpha-cypermethrin7% (sodium channel modulator)	0.625
Engeo[c]	24.7% SC	thiamethoxam 14.1% (acetylcholine agonist, mimic) + lambda-cyhalothrin 10.6% (sodium channel modulator)	0.400
Kingbo[d]	0.6% SL	oxymatrine 0.2% + prosuler 0.4% (combination of these two actives cause gastrointestinal disorder)	0.500

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for the first time after 48 h from treatment and for the second time at the end of the fifth day from treatment. The average of mortality percentage was corrected using Abbott's formula (1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971), from which the corresponding concentration probit lines (LC-p lines) were estimated in addition to determining 50% and 90% mortalities; slope values of the tested compounds were also estimated. In addition, the efficiency of different compounds was measured by comparing the tested compounds with the most effective compound using the following equation: Toxicity index = LC_{50} of the most effective compound/LC_{50} of the tested compound × 100 (Sun 1950).

Evaluation of residual effect. The purpose was to evaluate the efficiency and residual effects of tested insecticide mixtures against the 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) under field conditions of Aga district, Dakahlia Governorate, in a cotton (variety Giza 86) field during the period from 21st to 30th July, 2009. The field was divided into six treatments, five of them were treated with tested compounds, while the 6th treatment served as a control. Each treatment contained four replications (42 m^2 each) per plot. A knapsack sprayer provided with one nozzle delivering 200 l water/feddan was used. Samples of leaves were collected at random from both treated and untreated plants. Samples were taken immediately after one hour from spraying (zero time) and then after 3, 6, and 9 days from application. The collected leaves were instantly transferred to the laboratory and introduced to each group of larvae for each starved 2nd instar larvae in glass jars covered with muslin cloth, each jar contained ten larvae and four replications. Mortality percentages were assessed twice, for the first time following the feeding period for 48 h on treated leaves and for the second time after a feeding period for the next three days on untreated leaves for each time interval tested and corrected by the same technique as mentioned above.

Biochemical studies. This part of study was conducted in order to determine some enzymes activities in the 4th instar larvae of a laboratory strain of *S. littoralis* after treatment with tested insecticides. Castor-bean leaves were dipped in an aqueous solution of each of the tested compounds at the LC_{50} level for 30 s, then left to dry at room temperature for 1 h before being offered to the 4th instar larvae of a laboratory strain. Larvae were fed on the treated leaves for 48 h, and then transferred to fresh untreated leaves for three days. The haemolymph was obtained from approximately fifty larvae by removing one of the prolegs with forceps, applying gentle pressure on the larvae with the fingers and taking the haemolymph with syringe. The haemolymph was collected in cold tubes and stored in a refrigerator until the enzyme activities were determined (Sooker *et al*. 1999; Abd El-Mageed 2002; Abd El-Mageed *et al.* 2008).

Determination of enzyme activities. Invertase and amylase were determined based on the digestion of trehalose and starch by trehalase and amylase, respectively, according to the method described by Ishaaya & Swiriski (1976). Acid phosphatase (AC-P) and alkaline phosphatase (ALK-P) activities were determined according to the method described by Powell & Smith (1954). The activity of acetylcholine esterase (AchE) was measured according to the method described by Simpson *et al.* (1964). The activity of phenoloxidase was based on the method described by Ishaaya and Casida (1974). Chitinase was assayed using a 3,5-dinitrosalicylic acid reagent to determine the free aldehyde groups of hexoaminase liberated on chitin digestion according to the method described by Ishaaya & Casida (1974). Total proteins were determined by the method of Bradford (1976).

Statistical analysis. Statistical significance was assessed by Duncan's and Tukey's test at $P < 0.05$ (Snedecor & Cochran 1980).

RESULTS

Toxicological studies

Data in Table 2 show that feroban was proved to be the most effective insecticide against the 2nd instar larvae of *S. littoralis* after a feeding period for 48 h on treated leaves, followed by chlorosan, kingbo, cygron and engeo, showing the medium lethal concentration (LC_{50}) values of 0.079, 0.444, 1.523, 70.71 and 888.137 ppm, respectively.

The data also show that the order of efficiency of the tested insecticides was changed at the end of the fifth day from treatment (feeding period for 48 h on treated leaves and three days on untreated leaves). Cygron was more toxic than the other tested insecticides, its LC_{50} value was 0.019 ppm followed by feroban (0.030 ppm), while kingbo and chlorosan

Table 2. Susceptibility of 2nd and 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested insecticide mixtures

Tested compounds	After 2 days from treatment				After 5 days from treatment			
	LC_{50}(ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	slope	toxicity index (%)	LC_{50} (ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	slope	toxicity index (%)
2nd instar larvae								
Chlorosan 29% EC	0.444 0.287 0.686	4.089 2.645 6.322	1.329 ± 0.207	17.79	0.181 0.118 0.279	2.291 1.488 3.527	1.163 ± 0.158	10.50
Feroban 50% EC	0.079 0.053 0.117	0.583 0.393 0.862	1.475 ± 0.193	100.00	0.030 0.017 0.053	2.565 1.442 4.563	0.663 ± 0.114	63.33
Cygron 10% EC	70.710 27.900 151.700	995.960 561.170 6119.130	1.116 ± 0.125	0.112	0.019 0.003 0.104	9406.710 1726.419 51254.159	0.225 ± 0.099	100.00
Engeo 24.7% SC	888.137 501.022 2316.975	19721.069 5707.717 238953.765	0.952 ± 0.170	0.009	832.823 444.062 1561.929	20530.852 10947.086 38504.849	0.921 ± 0.165	0.002
Kingbo 0.6% SL	1.523 0.698 8.980	320.390 31.060 202664.810	0.552 ± 0.134	5.187	0.153 0.094 0.249	6.716 4.130 10.920	0.780 ± 0.134	12.42
4th instar larvae								
Chlorosan 29% EC	0.530 0.386 0.976	2.029 1.063 12.412	2.199 ± 0.556	100.00	0.437 0.317 0.601	1.646 1.196 2.263	2.224 ± 0.524	21.97
Feroban 50% EC	0.556 0.173 27.129	239.813 9.146 54701777.38	0.486 ± 0.146	95.32	0.096 0.051 0.257	8.223 1.565 466.087	0.663 ± 0.146	100.00
Cygron 10% EC	81.965 34.685 180.268	4103.194 1201.829 53070.92	0.754 ± 0.150	0.647	8.124 1.579 100.296	8979.003 444.804 7346183.19	0.421 ± 0.088	1.182
Engeo 24.7% SC	921.664 381.294 3189.370	168387.561 26276.381 7241039.40	0.567 ± 0.104	0.058	696.360 280.418 2340.11	168809.418 26041.599 6494385.695	0.538 ± 0.095	0.014
Kingbo 0.6% SL	2.324 1.218 7.795	78.915 17.788 1782.310	0.837 ± 0.155	22.81	0.270 0.174 0.430	9.586 3.659 57.717	0.827 ± 0.135	35.56

Toxicity index = LC_{50} of the most effective compound/LC_{50} of the tested compound × 100

were considered to be less toxic insecticides, their LC_{50} values were 0.153 and 0.181 ppm, respectively, but engeo was the least toxic insecticide, its LC_{50} value was 832.823 ppm.

Concerning the efficiency of the tested insecticides against the 4th instar larvae of *S. littoralis* after a feeding period for 48 hrs on treated leaves (Table 2), chlorosan was the most effective compound, its LC_{50} value was 0.530 ppm followed by feroban (0.556 ppm), kingbo showed an intermediate toxicity, its LC_{50} value was 2.324 ppm. Cygron and engeo were the least toxic insecticides with the LC_{50} values of 81.965 and 921.664 ppm, respectively.

The presented data indicate that feroban recorded the highest effect at the end of the fifth day from treatment (feeding period for 48 h on treated leaves and three days on untreated leaves) when its LC_{50} value was 0.096 ppm. Kingbo and chlorosan showed an intermediate toxicity, their LC_{50} were 0.270 and 0.437 ppm, respectively. Cygron and engeo were the least toxic insecticides with the LC_{50} values of 8.124 and 696.360 ppm, respectively.

It is worth to note that feroban seemed to be more potent compared with the other toxicants, while engeo was found to be the least toxic insecticide in both instars at two feeding periods.

Evaluation of residual effect

Residual effects of the tested insecticides at the recommended rates of application on the 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) intervals days are documented in Table 3. Summarised results show that the initial efficacy in the control of the 2nd instar larvae as determined one hour after spraying with feroban and kingbo was 94.74% and 81.58% after a feeding period for 48 h on collected leaves and the efficacy reached 100.00% after a feeding period for the next three days on untreated leaves for the above-mentioned insecticides. The latent toxicity effect was decreased to reach 24.32 and 78.79, 13.51 and21.21, 2.70 and 6.06, 5.41 and 3.03 and 0.00 and 3.03% in 9 days after the application of feroban, cygron, chlorosan, kingbo and engeo in the two recorded periods, respectively. Data indicate that feroban had the longest half-life (Lt_{50}) while engeo recorded the lowest value.

Reviewing the obtained results, it can be concluded that the efficiency of different tested insecticide mixtures against the 2nd instar larvae of the cotton leafworm *S. littoralis* (Boisd.) varied tremendously according to the type of component of the tested insecticide mixtures.

Biochemical impacts

Determination of phosphatase activities. Data in Table 4 indicate that engeo produced a significantly higher increase in acid phosphatase (AC-P) activity than in the control, it was 102.08%, while the lowest increase in AC-P activity was induced by chlorosan and cygron, by 13.43% and 4.13% higher than in the control, respectively. But a decrease in this enzyme activity was observed in the case of

Table 3. The residual effect of tested insecticide mixtures to the 2nd instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) at time intervals in days

Treatments	Corrected % mortality after treatment (days)								General mean of % reduction		Lt_{50} (days) its limits at 95%	
	IK		3		6		9					
	2nd day	5th day	2nd day	5th day	2nd day	5th day	2nd day	5th day	2nd day	5th day	2nd day	5th day
Chlorosan 29% EC	26.32	52.94	5.00	2.78	0.00	5.26	2.70	6.06	8.51	16.76	0.003 0.0002 0.012	0.05 0.02 0.09
Feroban 50% EC	94.74	100.00	77.50	97.22	87.50	100.00	24.32	78.79	71.02	94.00	13.92 6.50 29.82	19.62 15.36 25.06
Cygron 10% EC	13.16	41.18	5.00	27.78	10.00	26.32	13.51	21.21	10.42	29.12	1.36E-22 5.4E-28 3.4E-17	0.004 2.2E-7 0.052
Engeo 24.7% SC	2.63	17.65	0.00	5.56	0.00	2.63	0.00	3.03	0.66	7.22	–	2.40E-4 4.1E-10 0.003
Kingbo 0.6% SL	81.58	100.00	7.50	41.67	5.00	2.63	5.41	3.03	24.87	36.83	0.24 0.16 0.35	2.61 2.12 2.98

IK – initial kill after one hour from application

Table 4. Phosphatase activity in haemolymph of the 4th instar larvae *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each tested insecticide mixtures

Tested compounds	Acid phosphatase		Alkaline phosphatase	
	µg phenol/ml/min	% of control	µg phenol/ml/min	% of control
Chlorosan 29% EC	3.049[b]	13.43	1.786[d]	–23.31
Feroban 50% EC	2.217[c]	–17.52	2.252[c]	–3.31
Cygron 10% EC	2.799[b]	4.13	5.163[a]	121.68
Engeo 24.7% SC	5.432[a]	102.08	3.494[b]	50.02
Kingbo 0.6% SL	1.580[d]	–41.22	0.641[e]	–72.48
Control	2.688[bc]		2.329[c]	
LSD = 5%	0.518		0.403	

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test

kingbo and feroban (–41.22% and –17.52% lower than in the control, respectively).

The obtained data in Table 4 reveal that there was a significant increase in the activity of alkaline phosphatase (Alk-P) in larvae treated with both cygron and engeo with the values higher by 121.68% and 50.02% than in the control, respectively. While kingbo and chlorosan produced a significant decrease in the Alk-P activity, it was by –72.48% and –23.31% lower than in the control, respectively. But a negligible decrease in Alk-P activity was observed in the case of feroban.

Determination of activities of carbohydrate hydrolyzing enzymes. Our results (Table 5) document that all tested compounds caused a reduction in amylase activity ranging between –1.05% and –25.26% compared to the control except for feroban, which increased the enzyme activity (12.63% higher than in the control).

Obtained data in Table 5 show that the significantly highest activity of trehalase enzyme was noticed in cygron treatment (14.33%) followed by engeo, chlorosan and feroban, 10.42%, 1.950% and 1.30% above the control level, respectively. While kingbo recorded a significant decrease in the enzyme activity with the value –26.38% lower than in the control.

At the same respect, all tested insecticide mixtures caused a significant increase in invertase activity ranging between 3.42% and 16.44% more than in the control, except for kingbo, which decreased the enzyme activity (–4.45 %).

Determination of acetyl cholinesterase enzyme activity. Our results indicated that all tested

Table 5. Carbohydrate hydrolyzing enzymes activity in haemolymph of the 4th instar larvae of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each tested insecticide mixtures

Tested compounds	Amylase		Trehalase		Invertase	
	µg glucose/ml/min	% of control	µg glucose/ml/min	% of control	µg glucose/ml/min	% of control
Chlorosan 29%EC	32.430[bc]	–1.05	35.995[b]	1.95	34.730[bc]	3.42
Feroban 50% EC	36.915[a]	12.63	35.765[b]	1.30	39.100[a]	16.44
Cygron 10% EC	30.015[bc]	–8.42	40.365[a]	14.33	35.190[b]	4.79
Engeo 24.7 % SC	28.750[c]	–12.28	38.985[a]	10.42	35.420[b]	5.48
Kingbo 0.6%SL	24.495[d]	–25.26	25.990[c]	–26.38	32.085[d]	–4.45
Control	32.775[b]		35.305[b]		33.580[c]	
LSD = 5%	4.016		1.754		1.296	

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test

Table 6. Acetyl cholinesterase and chitinase activity in haemolymph of the 4th instar larvae *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each tested insecticide mixtures

Tested compounds	Acetyl cholinesterase		Chitinase	
	µg AchBr/ml/min	% of control	N.acetyl glucose amine/min/ml	% of control
Chlorosan 29% EC	32.235[a]	857.10	6.307[bc]	17.14
Feroban 50% EC	19.245[c]	471.41	8.256[a]	53.34
Cygron 10% EC	12.990[d]	285.69	7.538[ab]	40.01
Engeo 24.7% SC	25.981[b]	671.41	7.025[abc]	30.48
Kingbo 0.6% SL	17.321[c]	414.28	2.769[d]	–48.57
Control	3.368[e]		5.384[c]	
LSD = 5%	2.841		1.533	

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test

compounds caused a significant increase in acetyl cholinesterase activity (Table 6), the enzyme activity reached its maximum in chlorosan treated larvae (857.10% higher than in the control), while the lowest increase in the enzyme activity was observed in cygron (285.69%).

Determination of chitinase enzyme activity. From the results in Table 6 it can be seen that all tested compounds caused an increase in chitinase activity ranging between 17.14% and 53.34% more than in the control, except for kingbo, which caused a significant decrease in enzyme activity, it was –48.57 % lower than in the control.

Determination of phenol oxidase enzyme activity. In this study, we noticed that there was an elevation in phenol oxidase activity (Table 7). Kingbo was the most effective insecticide, which caused a significant increase in this enzyme activity (2858.33% higher than in the control) followed by engeo (1191.67%) and chlorosan (291.67%). Feroban and cygron caused an increase in the enzyme activity but these inductions were not statistically significant (156.25% and 62.50%, respectively).

Determination of total proteins. Data in Table 7 show that no significant differences among all tested compounds were observed as compared with the control except for kingbo, which caused a significant increase in total proteins, it was by 583.00% more than in the control.

DISCUSSION

The efficiency of different tested compounds in the control of the 2nd and 4th instar larvae of the cotton leafworm *S. littoralis* (Boisd.) varied

Table 7. Total proteins and phenol oxidase activity in haemolymph of the 4th instar larvae *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of each tested insecticide mixtures

Tested compounds	Total proteins		Phenol oxidase	
	mg/ml haemolymph	% of control	extinction units (E)/g at 3 min	% of control
Chlorosan 29% EC	0.824[b]	–18.58	0.188[c]	291.67
Feroban 50% EC	0.991[b]	–2.08	0.123[cd]	156.25
Cygron 10% EC	0.984[b]	–2.77	0.078[d]	62.50
Engeo 24.7% SC	0.936[b]	–7.51	0.620[b]	1191.67
Kingbo 0.6% SL	6.912[a]	583.00	1.420[a]	2858.33
Control	1.012[b]		0.048[d]	
LSD = 5%	0.672		0.079	

% of control = (Test – Control)/Control × 100; Letters mean the significant differences between treatments according to Duncan's test

tremendously according to the instar of larvae and the chemical structure of the tested mixtures. Generally, the 2nd instar larvae were more sensitive to the five tested compounds than the 4th instar larvae of *S. littoralis*, moreover the effect at the end of the fifth day from treatment (feeding period for 48 h on treated leaves and three days on untreated leaves) was remarkably higher compared with the effect on the second day from treatment (feeding period for 48 h on treated leaves).

The reasons for employing mixtures of insecticides of various chemical types in agriculture are as follows: a mixture may give the best control of a complex of pests with varying susceptibilities to the different components of the mixture; insects that are resistant to one or more insecticides may be susceptible to a combination of toxicants; or synergism may be exhibited by the combinants (All *et al.* 1977). Feroban was proved to be the most effective (initial kill) of the tested mixtures because it contains a double concentration of chloropyrifos (47.5%) compared with chlorosan (it contains 24% of chloropyrifos). Also, feroban contains lufenuron (2.5%), which has a much slower mode of action (residual toxicity) and inhibits the production of chitin, therefore the larvae are unable to successfully moult into the next stage. Kassem *et al.* (1986) found out that the mixture of diflubenzuron with chloropyrifos was more effective (initial kill) than diflubenzuron alone. Pyrethroids are always used in combination with organophosphates (OPs) to control the broad mite, the sucking pests *Aphis gossypii* Glover and *Bemisia tabaci* Gennadius, and the leafworm (Martin *et al.* 2003). Among the insect growth inhibitors tested, lufenuron required a shorter time at a lower concentration as compared with the other insecticides of this group tested. Although a high level of resistance was observed against lufenuron (Sudhakaran 2002), yet it was proved as an effective insecticide against *S. littoralis* (Boisd.). Kingbo is extracted, refined and produced from several Chinese wild medicinal plants, such as *Sophora japonica*, *Sophora flavescens* and *Veratrum nigrum*. Also, this insecticide is used in inorganic farming according to Anonymous (1991). El-Aswad (2007) reported that oxymatrine/prosuler (kingbo) had a low residual effect on *S. littoralis*.

In general, it was documented by the aforementioned results that the treatment of *S. littoralis* larvae with the five compounds caused pronounced changes in acetyl cholinesterase and phenol oxidase. The data also revealed that the other tested enzymes may be playing an important role in insecticidal poisoning according to the type of component of the tested insecticide mixtures.

Motoyama and Dauterman (1974) reported that phosphatases can hydrolyse organophosphorus insecticides by clearing off the leaving groups and result in nontoxic dialkyl phosphorothioic acid or phosphoric acid. Ishaaya *et al.* (1983) reported that the susceptibility of *S. littoralis* larvae to pyrethroids appeared to be limited by pyrethroid esterases in the gut. Organophosphorus compounds inhibiting these detoxifying enzymes serve as synergists.

Some authors have shown that synergism between pyrethroids and OPs is caused by the inhibition of either esterases (Gunning *et al.* 1999) or oxidases (Kulkrani & Hodgson 1980) by OPs, thereby preventing the degradation of pyrethroids. In such cases, pyrethroid and OP mixtures provide a level of synergism by competitive substrate inhibition. The mechanism of this synergism is as follows: pyrethroids are detoxified in insects by esterases and oxidases, as demonstrated by Casida and Ruzo (1980). About the same results were reported by Radwan *et al.* (1984), who measured the activity of amylase, invertase and trehalase in the haemolymph of *S. littoralis* with insecticide-growth regulator mixtures, and found that the treatment with diflubenzuron-chlorpyrifos alone or in a sequential system with other chemicals led to a reduction in the activity of all three enzymes. Diflubenzuron reduced amylase activity *in vivo* in *S. littoralis*, the reduction in activity being positively correlated with concentration, but invertase, trehalase and protease (proteinase) were not affected. In the 6th instar larvae, diflubenzuron probably inhibits amylase indirectly by acting on the physiological system affecting amylase activity or secretion (El-Saidy *et al.* 1990). Abdel-Hafez *et al.* (1988) found out that there was a reduction in the level of proteins and free amino acids in laboratory and resistant strains of *S. littoralis* as a result of IGR (diflubenzuron and triflumuron) treatments. Abdel-Hafez *et al.* (1993) stated that the IGR/insecticide mixtures or their residues induced a variable decrease in the activity of alkaline phosphatase, much lower than in the control, while acid phosphatase enzyme showed a higher increase in its activity in the field strain larvae of *S. littoralis*. As a general trend, acid phosphatase activity appeared to be lower in field strains of *S. littoralis*

than in a susceptible strain with IGRs and binary mixtures. Salem (1998) revealed that there was no pronounced effect of cypermethrin or profenofos on carboxyesterase activity in *S. littoralis* larvae, carboxyesterase might increase if these pesticides were used sequentially and the insect was exposed to them for several generations.

This demonstration agrees with the finding of Corbel *et al*. (2003, 2006). They found out that repetitive firing of nerves induced by pyrethroids stimulated an acetylcholine release at cholinergic nerve terminals. Then, the application of pyrethroid and carbamate insecticides may contribute to an increase in acetylcholine concentration to a critical level leading to the block of cholinergic synaptic transmission.

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