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Efficacy of certain insecticides and their mixtures with the tested IGRs against a field strain of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) under laboratory conditions

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Abstract: Toxicity of thirteen insecticides of different classes was established under laboratory conditions against egg mass and 4th instare larvae of field strain of cotton leafworm, *Spodoptera littoralis* (Boisd.) obtained from Gharbia Governorate in Egypt in 2010. The field population showed high resistance to pyrethroids and moderate resistance to organophosphorus and carbamates; however it had low resistance to insect growth regulators (IGRs) against 4th instar larvae. However, there different levels of tolerance to 1-day-old eggs for all insecticides and IGRs tested. Mixtures of organophosphorus compounds with IGRs were assessed for their potentiation in resistant field population. The results of co-toxicity factor against 4th instar larvae showed that chlorpyrifos when mixed with hexaflumuron and triflumuron produced high synergism, and produced additive effect when mixed with chlorfluazuron, chromafenozide and tobufenozite. As for mixtures of profenofos with IGRs, profenofos combined with hexaflumuron caused high synergism, however the mixtures of profenofos with triflumuron, lufenuron, chromafenozide, flufenoxuron, tobufenozite or chlorfluazuron produced additive effect. On the other hand, the same mixtures were applied on the 1st - day old masses and the results were different, the combination of flufenoxuron, triflumuron or tobufenozite with chlorpyrifos produced high synergism. The same results were also observed with profenofos when combined with flufenoxuron or triflumuron. Use of mixtures is potentiating and useful strategy to breakdown insecticide resistance, the use of antagonistic mixtures exacerbates resistance problem. Resistance to mixtures and their components should therefore be monitored regularly. For season-long pest control, mixtures should be rotated with other insecticide classes.

Key words: Organophosphorus,resistance,IGRs Spodoptera littoralis (Boisd.)

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is known as one of the most destructive agricultural lepidopterous pests, attacking numerous economically important crops. Use of chemicals control of *S.littoralis* has been extensively reported in relation especially to cotton pests in Egypt. The overlapping of crops that serve as hosts of this insect throughout the annual cropping cycle encourages high population densities. As a result of this and the intensive use of chemical control measures to manage this pest in particular on cotton has resulted in the development of resistance to almost all classes of insecticides used (El-Guindy *et al*., 1989; Abdallah, 1991; Abo-Elghar *et al*., 2005).

Chemical control of multiple pests caused resistance to different classes of insecticides. Therefore, now it has become necessary to search for alternative means of pest control which can minimize the use of chemicals (El-Aswad, 2007), the growers resort to the use of insecticide mixtures in an effort to obtain acceptable control of pests. Availability of cheaper, generic insecticides has further popularized the application of pre-and tank-mixed mixtures (Ahmed 2009).

Moreover, some levels of resistance to insect growth regulators (IGRs), as unconventional insecticides, have been documented in this pest for methoprene (juvenile hormone analogue, (JHA) (El-Guindy *et al.*, 1989), and also for benzoylphenylureas (chitin synthesis inhibitors) (El-Guindy *et al*., 1983; Abo-Elghar *et al*., 1992). In Egypt, management strategy against this pest has been settled upon the last decade on cotton to preserve and extend insecticide efficacious based on rotating various insecticides including organophosphates, carbamates, insect growth regulators and pyrethroids every year (Sawicki and Denholm, 1987; Temerak, 2002).

The objectives of this study were to further describe the expression of resistance to different insecticides in both eggs and larvae of field population of the cotton leafworm collected from cotton fields and also determine the pattern of revconducted to evaluate the potentiation in the used binary mixtures of organophosphorus, chlorpyrifos and profenofos plus insect growth regulators (IGRs) as unconventional insecticides against a field strain of *S.littoralis* (Boisd.) under laboratory conditions.

MATERIALS AND METHODS

Insects: Field strain of *S. littoralis* were collected at the commercial cotton field which had been exposed to the chemical control program for controlling different cotton pests, where several insecticides belonging to

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different groups (pyrethroids, organophosphates, carbamates and insect growth regulators). Eggs of field strain were allowed to hatch and reared at laboratory conditions on fresh castor bean, *Ricinus communis,* and leaves as described by (Eldefrawi *et al*., 1964). A laboratory susceptible strain of *S. littoralis* was obtained from the Central Agricultural Pesticides Laboratory, Dokki, Egypt. It has been reared on fresh castor bean leaves for many years without exposure to insecticides. It served as reference strain for the comparisons with studied strain.

Chemicals: The commercial formulations of insecticides used in bioassays were:

I- Pyrethroids: Es-fenvalerate (Sumi-alpha 5% EC), Fenpropathrin (Meothrin 20% EC, Fenvalerate (Sumicidin 20% EC).

II-Organophosphorus: Chlorpyrifos (Dursban,48% EC), Profenofos (Selecron72% EC).

III-Carbamates: Methomyl (Lannete, 90% SP), thiodicarb (Larvin 80% DF).

VI- Insect growth regulators (IGRs): Lufenuron (Match 5% EC), Hexaflumuron (Consult 10% EC), Triflumuron (Alsystin 48% SC), Flufenoxuron (Cascade 10% DC), Chromafenozide (Virto 5% SC), Tobufenozite (Mumic 24% FL), Chlorfluazuron (Tobron 5% EC).

All insecticides were obtained from manufacturers. Organophosphorus plus growth regulators mixtures were prepared by mixing insecticides in desired ratios just before treatment.

Bioassays: In the case of eggs stage, one-day-old eggs masses were used to determine the ovicidal action of the tested insecticides. After removing the hair that covers the egg-masses with a brush, they were dipped in water dilution of each insecticide for 10 seconds and left for air dryness. Seven concentrations for each insecticide were used with four replicates . The treated egg-masses were transferred to glass tube covered with muslin cloth until hatching. The newly hatched larvae and the unhatched eggs were counted in all treatments. Abbott's formula (1925) was used to correct the obtained unhatability percentage using the natural unhatability in the control treatment.

In the case of larval stage, 4^{th} instar larvae were used to determine the toxicity action of the same insecticides which used in the test of eggs stage. $5cm^2$ of cotton leaf discs were cut and dipped into the water dilution of each insecticide for 20 seconds with gentle agitation, then left for air drayness. Ten larvae were released on to each leaf disc placed in a 7-cm diameter Petri dish. At least six concentrations and three replications were used to estimate each concentration-mortality line. The same number of leaf discs per treatment was dipped into tap water as an untreated check. Before and after treatment, larvae were maintained under laboratory conditions (constant temperature 25 (±2)^{0}C with photoperiod of 14:10(L: D). After 24 h of treatment the mortality was recorded and the data were corrected relatively to control mortality (Abbott, 1925). The data were subjected to statistical analysis by the method of (Busvine 1971). The software program used to calculate the LC_{50} and slop values.

The joint action of tests pesticide mixtures was studied by mixing concentrations equivalent to LC_{25} values at the ratio of 1:1. The combined action of the different mixtures was expressed at the Co-toxicity factor (CF), estimated according to the equation given by Mansour *et al.,* 1966. (CF) were determined by dividing the observed mortality percentage mince expected mortality by expected mortality percentage.

$$\text{C0-toxicity factor} = \frac{\text{Observed mortality \% - Expected mortality \% X 100}}{\text{Expected mortality \%}}$$

The Co-toxicity factor Differentiate the results as following:

A positive factor of 20 or more is considered as potentiation, a negative of 20 or more is considered as antagonism, while intermediate values (-20 & +20) indicated additive effect.

RESULTS AND DISCUSSION

The response of eggs of field strain to insecticides and IGRs is presented in Table (1). Among the insecticides tested, es-fenvalerate was the highest ovicidal action followed by chlorpyrifos, fenpropathrin and fenvalerate while thiodicarb was the least toxic insecticides followed by profenofos and methomyl. These results agree with those obtained by Ghoneim (1981) who reported that the pyrethroid cypermethrin was the most potent ovicide against egg of *S. littoralis* field strain followed by the chlorpyrifos, methomyl, profenofos and chlordimeform.

Within the IGR tested, hexaflumuron was the most ovicidal action followed by chlorfluazuron, lufenuron and chromafenozide, while tobufenozite was the least toxic action followed by triflumuron. On the other hand, insecticides were higher ovicidal activity than IGR, where LC_{50} values ranged between 23.7-182.0 ppm for insecticides and 197.0-1491.8 for IGR.

Table 1: Effect of tested insecticides against one-day-old egg mass of *Spodoptera littoralis* (Boisd.) field strain.

Insecticide	Lab. Strain		Field strain		TR*	TI**
	Slope	LC_{50} ppm	Slope	LC_{50} ppm		
Profenofos Selecron 72% EC	4.7 ± 1.19	33.3	2.52± 0.23	180.1 (158-205.1)	5.4	13
Chlorpyrifos Dursban, 48% EC	2.58 ± 0.23	16.7 (14.77-18.9)	3.95± 0.91	35.8	2.2	66.2
Methomyl Lannete, 90% SP	2.37± 0.22	28.3 (24.6-32.3)	3.71 ± 0.40	125.5 (113.2-139.6)	4.4	18.9
Thiodicarb Larvin 80%DF	2.76 ± 0.24	28.8 (25.6-32.5)	3.14 ± 0.30	181.9 (163.2-204.0)	6.3	13
Fenvalerate Sumicidin 20%.EC	3.21 ± 0.36	13.4 (11.9-15.4)	2.24 ± 0.24	65.9 (56.6-79.8)	4.9	35.9
Fenpropathrin Meothrin 20% EC	2.75± 0.73	15.4	3.10 ± 0.35	57.2 (50.8-64.9)	3.7	41.4
Es-fenvalerate Sumi-alpha 5% EC	2.21 ± 0.22	7.2 (6.2-8.7)	2.34 ± 0.23	23.7 (20.6-27.1)	3.3	100
lufenuron Match 5% EC	1.96± 0.16	272.2 (235.3-396.2)	2.51 ± 0.21	324 (285.6-396.2)	1.3	60.8
Chromafenozide Virto 5% SC	1.97± 0.21	207.4 (177.5-244.9)	3.27 ± 0.25	477.1 (426.0-536.4)	2.3	41.3
Flufenoxuron Cascade 10% DC	2.02 ± 0.17	171.1 (147.9-199.1)	2.80± 0.51	547.9 (265.5-1095.80)	3.2	36
Tobufenozite Mumic 24% FL	2.27 ± 0.23	869.8 (757.2-1011.1)	1.12 ± 0.14	1491.8 (1173.3-1993.5)	1.7	13.2
Hexaflumuron Consult10% EC	3.62 ± 0.38	124.9 (112.5-140.1)	2.10 ± 0.17	197 (171.4-226.4)	0.8	100
Triflumuron Alsystin 48% SC	1.94± 0.27	187.2 (116.3-281.4)	1.31± 0.15	741.4 (591.2-910.8)	3.9	26.6
Chlorfluazuron Tobron 5% EC	1.65 ± 0.30	109.3 (88.5-137.9)	2.44 ± 0.23	216.1 (187.5-246.4)	1.9	91.2

TR*(Tolerance ratio) = LC_{50} of Field strain /LC_{50} of Lab. Strain

TI** (Toxicity index) = (LC_{50} of the most effective insecticide /LC_{50} of the least effective insecticide) x 100

Concerning the resistance to insecticides in eggs of field strain, it is clear from Table (1) that egg masses showed low level of tolerance to chlorpyrifos (2.2-fold) while other insecticides tested exhibited moderate levels of tolerance, where TR ranged between (3.3-6.3- fold). The same results were also observed with IGR tolerance levels but were lower than that obtained with insecticides (TR ranged between 1.6-4.0-fold). The low or moderate levels of resistance of eggs to insecticides and IGRs may be discussed in the light of the findings concencerning egg selection with insecticides. Guirguis *et al* (1985) exposed egg masses of field strain of *S. littoralis* to the selection pressure of each of chlorpyrifos, permethrin and chlordimeform for 13 generations. A low level of tolerance was obtained (2.6, 3.2 and 1.2 fold) respectively. They suggested that the low level of resistance obtained after thertin selected generations with these insecticides might be regarded as an indication that the selection pressure of eggs led to lack of resistance potential towards selected agent

The response of 4th instar larvae of field strain to insecticides and IGRs is presented in Table (2).

Table 2: Effect of the tested insecticides against 4th instar larvae of *Spodoptera littoralis* (Boisd.) field strain

Insecticide	Lab. Strain		Field strain		TR*	TI**
	Slope	LC_{50} ppm	Slope	LC_{50} ppm		
Profenofos Selecron 72% EC	5.29 ± 0.15	10.6	1.24 ± 0.36	272.1 (198-374)	25.8	33
Chlorpyrifos Dursban, 48% EC	4.06 ± 0.24	6.3	2.34 ± 0.42	93.4 (66-121)	14.8	96.2
Methomyl Lannete, 90% SP	3.03 ± 0.16	11.4	2.80 ± 0.60	210 (160-270)	18.5	42.6
Thiodicarb Larvin 80%DF	3.62 ± 0.13	24.9	2.11± 0.44	238.9 (168-312)	9.6	37.6
Fenvalerate Sumicidin 20%.EC	1.31 ± 0.26	14.5	3.88 ± 0.72	386 (314-464)	26.7	23.3
Fenpropathrin Meothrin 20% EC	2.80 ± 0.11	1.2	2.98 ± 0.54	89.8 (71-115)	78.1	100
Es-fenvalerate Sumi-alpha 5% EC	1.99 ± 0.27	1.1	3.76 ± 0.68	181 (149-222)	168.1	49.5
lufenuron Match 5% EC	0.5 ± 0.25	2.6	0.66 ± 0.11	7.2 (3.6-15.1)	2.9	91.7
Chromafenozide Virto 5% SC	1.10 ± 0.19	2.9	1.04 ± 0.24	8.4 (2.8-16.8)	2.9	78.6
flufenoxuron Cascade 10% DC	0.6 ± 0.34	1.7	0.42 ± 0.10	9.3 (2.8-32.6)	5.5	71
Tobufenozite	0.70 ± 0.34	6.2	0.87 ± 0.15	14.5	2.3	45.5

Mumic 24% FL				(6.6-26.1)		
hexaflumuron Consult10% EC	0.60 ± 0.17	2.7	0.75 ± 0.14	8.6 (3.9-16.8)	3.2	76.7
Triflumuron Alsystin 48% SC	1.03 ± 0.28	9.6	0.55 ± 0.15	57.3 (20-306)	5.9	44.5
Chlorfluazuron Tobron 5% EC	0.56 ± 0.12	3.5	0.69 ± 0.15	6.6 (1.8-14.3)	1.9	

TR: Tolerance Ratio = LC_{50} of Field strain /LC_{50} of Lab. Strain

TI** (Toxicity index) = (LC_{50} of the most effective insecticide /LC_{50} of the least effective insecticide) x 100

As regarded with the toxic action of insecticides, the pyrethroid fenvalerate was the most toxic action followed by the OP chlorpyrifos, while the other insecticides tested recorded moderate toxic action followed by the IGRs lufenuron, chromafenozide, hexaflumuron and flufenoxuron, while the IGRs triflumuron and tobufenozite were the least toxic action.

Concerning the resistance to insecticides, 4th instar larvae of field strain showed very high levels of tolerance to the pyrethroids es-fenvalerate (168.1- fold), fenpropathrin (78.1-fold) and fenvalerate (26.7-fold) and the OP profenofos (25.8-fold), while the carbamates thiodicarb, methomyl and the OP chlorpyrifos showed slightly high levels of tolerance (18.5, 9.6 and 14.8- fold, respectively). As for IGRs, very low levels of tolerance were observed for all compounds (TR ranged between 1.9-3.2-fold) except with flufenoxuron and triflumuron which exhibited moderate tolerance (5.5 and 6.0 fold, respectively). On the other hand, IGRs was higher toxic action with lower resistance level than that obtained with insecticides. High levels of resistance to pyrethroids, OPs and carbamates had already been observed in cotton leafworm (El-Guindy *et al* 2002a and 2002b). Low resistance to IGRs in field strains of cotton leafworm was also found by Ghoneim (2002).

The joint action between LC_{25} of insecticide and LC_{25} of IGR against the egg-masses and the 4th instar larvae of Spodapetra littoralisis tabulated in Table (3). The LC_{25} of Triflumuron and Tobufenozite caused potentiated when used in admixture with the LC_{25} of chlorpyrifos. The toxicity was increased by a Co-toxicity factor ranging between +22 and +88.

Summarized results from Tables (1, 2 and 3) showed that all insecticides tested recorded low resistance on eggs, while larvae showed very high levels of resistance except for thiodicarb, chlorpyrifos which had toxic action on both eggs and larvae.

Table 3: The joint action between the (LC_{25} : LC_{25}) of the tested insecticides with the tested IGR on egg- mass and 4th instar larvae of *S. littoralis* field strain

Insecticide	IGR	CF for egg	CF for 4th instar larvae
Chlorpyrifos	+ Chromafenozide (Virto)	-56	+24
	+ Flufenoxuron (Cascade)	+75	0
	+ Hexaflumuron (Consult)	-82	+88
	+ Triflumuron (Alsystin)	+55	+60
	+ Chlorfluazuron (Tobron)	0	+38
	+ Lufenuron (Match)	-9	+14
	+ Tobufenozite (Mimic)	+37	+22
profenofos	+ chromafenozide (Virto)	- 64	-34
	+ Flufenoxuron (Cascade)	+70	-28
	+ Hexaflumuron (Consult)	-77	+58
	+ Triflumuron (Alsystin)	+28	+4
	+ Chlorfluazuron (Tobron)	-50	-16
	+ Lufenuron (Match)	- 43	-52
	+ Tobufenozite (Mimic)	- 48	-25

On the other hand, IGRs exhibited low levels of resistance on the both eggs and larvae, but they were higher toxic action on larvae than that on eggs. Furthermore the mixtures of chorpyrifos with the IGRs tobufenozite or triflumuron were high synergism on the both egg and larval stages. Thus, the efficiency of certain insecticides and insect growth regulators (IGRs) alone or in combinations with chlorpyrifos against *S. littoralis* for integrated pest management are common to control multiple and resistant insect pests. The use of mixtures is potentiating and useful strategy to combat insecticide resistance. The potentiating mixtures are supposed to counteract a mechanism of metabolic detoxification only. Through time mixtures are select other mechanisms of resistance to insecticides. Resistance to mixtures and their components should therefore be monitored regularly. For season-long pest control, mixtures should be rotated with other insecticide classes.

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