

Toxicity, joint toxic effect and resistance monitoring to cypermethrin, alpha-cypermethrin and thiodicarb in pink bollworm *pectinophora gossypiella* (saunders).

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ABSTRACT

Toxicity of two dichlorovinyl pyrethroids, cypermethrin and alpha-cypermethrin, and the oxime-carbamate insecticide, thiodicarb, were determined against the laboratory and field male moth populations of pink bollworm, *Pectinophora gossypiella* (Saunders) and also, the toxicity of their mixtures at the ratio of 1:5 were measured to the field population. Toxicity data from laboratory and field populations, demonstrated that, the LC₅₀'s of cypermethrin were 0.14 and 2.85 ppm, respectively and the corresponding LC₅₀'s of alpha-cypermethrin were 0.125 and 2.96 ppm, while thiodicarb LC₅₀ values were 5.9 and 91.5 ppm. Mixtures of pyrethroid – thiodicarb in mixing ratio of 1:5 were more effective against field population than that of their individuals. The pyrethroid toxicity in the mixture was increased from 5.18 fold for cypermethrin to 5.69 fold for alpha-cypermethrin with co-toxicity factor of 77.7 and 79.6, respectively. On the other hand, pyrethroid – thiodicarb mixtures were highly increased the toxicity of thiodicarb alone. Monitoring resistance data showed high resistance levels in the field population against cypermethrin and alpha-cypermethrin each one alone. Based on comparing with LC₅₀ values of the laboratory population, the resistance ratio values were 20.36 and 23.68 fold, respectively, while mixing with thiodicarb decreased these levels to 3.93 fold for cypermethrin and 4.16 fold for alpha-cypermethrin.

Key words: Resistance, monitoring, mixtures, resistance management, joint toxicity, pink bollworm, pyrethroids, oxime-carbamates.

INTRODUCTION

Since the first described case of resistance (Melander 1914), resistance had increased to 400 species in 1983 (Georghiou and Mellon 1983) and the estimations were made that, by 1993, the number would be dangerously close to 1500, which are major pests of world agriculture (Gazzoni 1998). Pink bollworm, *Pectinophora gossypiella* (Saunders), which is considered as mid- and late-season pest of cotton and one of the most destructive pests of cotton in most of the cotton producing countries in the world (Osman et al., 1991) has developed resistance to most or may be to all insecticides used in conventional control programmes.

The efficacy of an insecticide is due to its binding at target molecules in the insect. However, the insect may modify the molecule, leading to decreased toxicity and increased polarity, which results in better elimination of the insecticide. Generally, resistance results from one of a number of phenomena, including target molecule modification, increased metabolism, and decreased penetration, or from a combination of several of these (Chalvet-Monfray et al., 1998).

Resistance management can help to reduce the harmful effects of pesticides, delay widespread development of insecticide resistance by pink bollworm and reduce the probability of severe yield loss by using more than one insecticide including sequences, mixtures, rotations and mosaics and prolonging the efficacy of environmentally safe pesticides (Tabashnik 1989 and Leonard et al., 1994). So, for resistance management tactics to be effective, resistance must be detected in its early stage (Roush and Miller 1986).

Overall, if two insecticides are jointly applied to an insect, one may interfere with the activation of the other, here antagonism would occur, or may interfere with its detoxication reactions or with the detoxifying enzyme of the other in this case potentiation would occur, or with both, this is the third case in which additive effect would occur, depending on the degree of interference with different reactions (Watson et al., 1986).

In this investigation the search for new insecticides from other groups to act as pyrethroid synergists which might increase activity sufficiently to allow use of lower dose of pyrethroids and thus decrease cost. Also, the possibility of using binary combinations of cypermethrin and alpha-cypermethrin with thiodicarb in resistance management strategy to overcome partially the problem of resistance in pink bollworm was studied.

MATERIALS AND METHODS

I) Attracticide Resistance Monitoring Technique(ARMT):

This technique eliminates insects handling, allow a rapid determination of the adult population response and enable the collection of large field sample without laboratory rearing. It is also repeatable, usable for field and greenhouse populations and provided stable LC_{50} 's with low control mortality (Haynes *et al.*, 1986 and 1987).

To detect toxicity, joint toxic effect and resistance levels of the two tested dichlorovinyl-pyrethroids and the oxime-carbamate insecticide, thiodicarb, The attracticide resistance monitoring technique as described by Miller (1986) and modified by Shekeban (2000) was used.

a) Insect used:

Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders)
(Lepidoptera : Gelechiidae).

1- Field male moth population of PBW was locally used in Kafr El-Dawar cotton fields, El-Behera Governorate, Egypt, late 2003 cotton season using pheromone baited delta traps.

2- Laboratory male moth population was supplied by the Bollworm Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt, where it has been reared for several years under laboratory conditions. The rearing procedure was as reported by Abdel Hafez *et al.*, (1982) .

b) Insecticides used :

They were used in formulated form and supplied by the Ministry of Agriculture

*Cypermethrin : Polytrin 20% E.C. [(R,S)- α - cyano - 3 - phenoxybenzyl - 2,2- dimethyl - (1R,1S) - cis - trans - (2,2 - dichlorovinyl) cyclopropane carboxylate].

*Alpha-Cypermethrin : Fastac 10% E.C.[(1R cis) S and (1S cis) R enantiomerisomer pair of α - cyano - 3 - phenoxybenzyl - 2,2- dimethyl - (2,2 - dichlorovinyl) cyclopropane carboxylate].

*Thiodicarb (oxime – carbamate) : Larvin 80 % WG. [3,7,9,13 - tetramethyl - 5,11 - dioxo - 2,8,14 trithia - 4,7,9,12 - tetra - azapentadeca - 3,12 - diene- 6,10 - dione].

c) Toxicity procedure :

Delta traps were used with a sticky adhesive – coated cards insert containing the insecticide concentration or insecticides mixtures placed in the trap bottom. A rubber septa with 1mg gossyplure (ZZ, ZE -7, 11-hexadecadienyl acetate) acted as the source of pheromone. Toxicity of the tested individuals and resistance ratio were measured.

II) Joint toxic effect procedure:

Combinations of different concentrations from each tested pyrethroid and thiodicarb were prepared at the ratio of 1:5 , this ratio was selected according to Metcalf (1967). The experiments were designed on assumption that thiodicarb play a role as an esterase inhibitor which may result in an increase in insecticidal activity against pink bollworm which was subjected for this study using ARMT. The toxicity of the binary mixtures, its relative toxicity and co-toxicity factor were measured.

III) Statistical analysis :-

a- Regression equation, LC_{50} , LC_{95} and confidence limits were calculated according to Finney (1971) probit analysis computer program.

b- Relative Toxicity (R.T) : These values were measured according to the equation of Metcalf (1967) as follow : $R.T = LC_{50}$ of the lowest toxic insecticide / LC_{50} of the tested insecticide or LC_{50} of insecticide alone / LC_{50} of insecticide in the mixture (fold).

c- Resistance Ratio (RR) was calculated according to the following equation :- $R.R = LC_{50}$ of the field population / LC_{50} of the laboratory one (fold).

d- A procedure for analysis of the joint action of insecticide mixtures was developed by Salem (1970) using co-toxicity factor as a criterion, the latter term was expressed as follows: Co-toxicity factor (CTF) = $[1 - (\text{actual dose of A in mixture} / \text{estimated dose of A single} + \text{actual dose of B in mixture} / \text{estimated dose of B single})] \times 100$. This factor was used to differentiate the results into three categories; A positive factor of 25% or more was considered potentiation, a negative factor of 25% or more means antagonism, and any intermediate values between -25% and +25% indicate only additive effect.

e- Reduction of the resistance ratio (RR Red.) = RR of the field population – RR of the field one after joint action.

RESULTS AND DISCUSSION

1- Toxicity and Resistance Studies Using ARMT:-

Data in Table (1) presented the toxicity of the two tested dichloromovinyl-pyrethroids; cypermethrin and alpha-cypermethrin, and the oxime- carbamate insecticide, thiodicarb, against the laboratory and field populations of pink bollworm (PBW) *Pectinophora gossypiella* (Saunders). These data indicated that, the pyrethroid insecticides were highly toxic compounds with LC₅₀ values of 0.14 and 2.85 ppm for cypermethrin and 0.125 and 2.96 ppm for alpha- cypermethrin, while thiodicarb was less toxic with LC₅₀ values of 5.9 and 91.5 ppm for laboratory and field populations, respectively. These results are in a good agreement with those reported by El-Dahan (1983), Shekeban (1989) and Marei *et al.*, (1991). They showed that, cypermethrin and the other tested pyrethroids exhibited highest toxicity among the other tested insecticides against the cotton leafworm (CLW).

Table (1): Toxicity parameters of cypermethrin, alpha-cypermethrin and thiodicarb against male moths of laboratory and field populations of pink bollworm, *P. gossypiella* and resistance levels using ARMT.

Insecticide	Population	Reg. Equation Y=a + bx	LC ₅₀ (ppm) (95% C L)	LC ₉₅ (ppm) (95% C L)	*RR ₅₀	**RR ₉₅
Cypermethrin	Lab.	Y= 0.88+1.05x	0.14 (0.19-0.11)	5.37 (10.37-3.03)	-	-
	Field	Y= -0.67+1.48x	2.85 (3.96-2.0)	36.87 (99.1-15.4)	20.36	6.87
Alpha-cypermethrin	Lab.	Y= 0.91+1.01x	0.125 (0.17-0.09)	5.33 (10.94-2.95)	-	-
	Field	Y= -0.74+1.57x	2.96 (3.74-2.33)	32.7 (68.0-17.51)	23.68	6.14
Thiodicarb	Lab.	Y=-1.51+1.97x	5.9 (7.40 – 4.60)	40.3 (58.0 – 28.4)	-	-
	Field	Y=-3.22+1.64x	91.5 (118.2-70.8)	917.7 (2155-395)	15.51	22.77

* RR₅₀: Resistance ratio at LC₅₀ level.

** RR₉₅: Resistance ratio at LC₉₅ level.

The previous toxicity results pointed out to high increase in the LC₅₀ values of the tested pyrethroids against PBW male moths field population compared to that of laboratory one, while the levels of LC₉₅ values of tested

pyrethroids indicated moderate increases. These toxicity parameters and the calculated resistance ratio values were also tabulated in Table (1). Relative to LC_{50} values in the laboratory population, the resistance ratio values were 20.36 and 23.68 fold against cypermethrin and alpha-cypermethrin, respectively. While against thiodicarb it was 15.51 fold. Based on LC_{95} values, the resistance levels were much lower than that based on the LC_{50} values especially for the tested pyrethroids. These values were: 6.87 fold for cypermethrin and 6.14 fold for alpha-cypermethrin, while it was higher than that based on the LC_{50} values for thiodicarb (RR= 22.77 fold). The high resistance levels observed from comparing LC_{50} values and the decreased levels of resistance upon LC_{95} values, can be supported by the results of Shekeban *et al.* (2003 a & b) when tested mixtures of pyrethroids +chlorpyrifos and pyrethroids + thiodicarb against the male moths of both laboratory and field populations of pink bollworm using ARMT. Also, El-Bassiony (2001) reported that, comparing LC_{50} values of different pyrethroids in early and late 1996 and 1997 cotton seasons both from pheromone or insecticides treated areas, pink bollworm and spiny bollworm accepted high resistance ratios reached to 119.6 and 144.8 fold, respectively at different intervals. Where the resistance values detected on the basis of LC_{90} at different intervals were decreased, for example, resistance ratio to cyfluthrin was 20 fold at LC_{90} instead of 58.5 fold at LC_{50} . This variation can be attributed to the change in the slope of the regression lines reflecting change in susceptibility.

2- Joint Toxicity Studies Using ARMT:-

Using ARMT at late 2003 cotton season to show the joint toxic effect of the esterase inhibitor, thiodicarb, on the toxicity of the tested pyrethroids was evaluated against male moths of pink bollworm and the results are presented in Table (2). The toxicity of the pyrethroid + thiodicarb mixtures could be arranged as follow: alpha-cypermethrin + thiodicarb followed by cypermethrin + thiodicarb with LC_{50} values of 0.52 and 0.55 ppm, respectively and the corresponding relative toxicity values were 5.69 and 5.18 fold. It is of interest to note that thiodicarb was found to potentiate the two tested pyrethroids and the co-toxicity factor values were 79.6 and 77.7 %, for alpha-cypermethrin and cypermethrin respectively. On the other hand, both of the tested pyrethroids potentiated the toxicity of thiodicarb strongly with RT values of 34.92 and 33.89 fold, respectively.

The role of thiodicarb as potentiator for deltamethrin and fenpropathrin against pink bollworm was supported with the results of El-Dahan (1983) who reported that methomyl, the oxime-carbamate insecticide, showed the

maximum potentiation effect when mixed with all tested pyrethroids against susceptible strain of CLW. Marei *et al* (1991) indicated that propoxur when combined with deltamethrin, tralomthrin, cypermethrin, permethrin, *cis*-permethrin, *trans*-permethrin, flucythrinate or fenvalerate at 1:5 level to the 4th instar larvae of CLW resulted in potentiation. Also, Shekeban (2003 a & b) found that, thiodicarb synergized both deltamethrin and fenpropathrin and chlorpyrifos potentiated all tested pyrethroids against PBW using ARMT. So, these mixtures allowed to use the lower doses of pyrethroids and thus decrease its cost.

Table (2): Joint toxic effect of pyrethroid insecticides with thiodicarb in mixing ratio of 1 : 5 against pink bollworm, *P. gossypiella* using ARMT.

Insecticide alone or in mixture	Reg. Equation $Y = a + bx$	LC ₅₀ (ppm) (95% C L)	LC ₉₅ (ppm) (95% C L)	¹ R.T	² CTF
Cypermethrin	$Y = -0.67 + 1.48x$	2.85 (3.96-2.0)	36.87 (99.1-15.4)	-	-
Cypermethrin + Thiodicarb	$Y = 0.24 + 0.95x$	0.55 (0.76-0.38)	29.4 (73.2-15.4)	5.18	77.7
Alpha-cypermethrin	$Y = -0.74 + 1.57x$	2.96 (3.74-2.33)	32.7 (68.0-17.51)	-	-
Alpha-cypermethrin + Thiodicarb	$Y = 0.26 + 0.94x$	0.52 (0.73-0.36)	29.4 (74.1-15.4)	5.69	79.6
Thiodicarb	$Y = -3.22 + 1.64x$	91.5 (118.2-70.8)	917.7 (2155-395)	-	-
Thiodicarb + Cypermethrin	$Y = -0.41 + 0.95x$	2.7 (3.78-1.9)	144.9 (336.1-72.74)	33.89	77.7
Thiodicarb + Alpha-cypermethrin	$Y = -0.39 + 0.94x$	2.62 (3.67-1.83)	147.25 (315.99-73.35)	34.92	79.6

¹RT= Relative toxicity ²CTF= Co-toxicity factor

Table (3) presented the joint toxicity of each tested pyrethroid with the esterase inhibitor, thiodicarb, at the ratio of 1:5 and showed a remarkable decreasing in the pink bollworm male moths, field population, resistance levels. So, the resistance ratios based on LC₅₀ after joint toxicity were 3.93 fold for cypermethrin with resistance reduction rate of 16.43 fold and 4.16 fold for alpha-cypermethrin with resistance reduction rate of 19.52 fold. The corresponding resistance ratios based on LC₉₅ after joint toxicity were 5.47 and 5.52 fold for cypermethrin and alpha-cypermethrin, with resistance

Table (3): Effect of joint toxicity on insecticide potentials and resistance levels using ARMT against pink bollworm *P. gossypiella* two populations

Insecticide	Population	Reg. Equation $Y=a+bx$	LC ₅₀ (ppm) (95% C L)	LC95 (ppm) (95% C L)	RR ₅₀	*RR Red	RR ₉₅	RR Red
Cypermethrin	Lab.	$Y=0.88+1.05x$	0.14 (0.19-0.11)	5.37 (10.37-3.03)	-	-	-	-
	Field	$Y=-0.67+1.48x$	2.85 (3.96-2.0)	36.87 (99.1-15.4)	20.36	-	6.87	-
	Field after JTE	$Y=0.24+0.95x$	0.55 (0.76-0.38)	29.4 (73.2-15.4)	3.93	16.43	5.47	1.4
Alpha- cypermethrin	Lab.	$Y=0.91+1.01x$	0.125 (0.17-0.09)	5.33 (10.94-2.95)	-	-	-	-
	Field	$Y=-0.74+1.57x$	2.96 (3.74-2.33)	32.7 (68.0-17.51)	23.68	-	6.14	-
	Field after JTE	$Y=0.26+0.94x$	0.52 (0.73-0.36)	29.4 (74.1-15.4)	4.16	19.52	5.52	0.62
Thiodicarb	Lab.	$Y=-1.51+1.97x$	5.9 (7.4-4.6)	40.3 (58.0-28.4)	-	-	-	-
	Field	$Y=-3.22+1.64x$	91.5 (118.-70.8)	917.7 (2155-395.3)	15.51	-	22.8	-
	Field after JTE with cyper.	$Y=-0.41+0.95x$	2.7 (3.78-1.9)	144.9 (336.1-72.74)	0.46	15.05	3.6	19.2
	Field after JTE with Alpha-	$Y=-0.39+0.94x$	2.62 (3.67-1.83)	147.25 (315.99-73.35)	0.44	15.07	3.65	19.15

*RR Red.= resistance ratio reduction rate (fold) = field population RR – observed RR after joint toxic effect.

reduction rates of 1.4 and 0.62 fold, respectively. Mixing thiodicarb with the tested pyrethroids at the ratio of 5:1 increased the power of its toxicity to the degree that the field population become susceptible more than the laboratory reference population. The resistance ratios based on LC₅₀ after joint toxicity were 0.46 fold for thiodicarb + cypermethrin mixture (with resistance reduction rate of 15.05 fold) and 0.44 fold for thiodicarb + alpha-cypermethrin mixture (with resistance reduction rate of 15.07 fold). These results may be supported by Busvine (1971) who reported that, not only the mixing of chemicals offers many possibilities in the search for better and

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more potent uses of toxicants but also it could theoretically prevent the emergence of resistant populations.

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التنبؤ بالسمية والفعل السام المشترك ورصد المقاومة للسيبرمثرين والألفاسيبرمثرين والثيوديكارب في دودة اللوز القرنفلية

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تم تقدير سمية اثنين من المبيدات البيرثرويدية (الداي كلورو فينيل) وهما السيبرمثرين والألفاسيبرمثرين وكذلك المبيد الأوكسيم كاربامتي ، ثيوديكارب ، كلا بمفرده ، ضد عشيرتي فراشات الذكور الحقلية والمعملية لدودة اللوز القرنفلية ، بكتينوفورا جوسيببلا (ساندرز) وكذلك مخاليطهما بنسبة خلط ١:٥ ضد العشيرة الحقلية. ولقد أشارت نتائج دراسة السمية ضد كلا من العشيرتين المعملية والحقلية إلى أن قيم LD_{50} لمبيد السيبرمثرين هي ٠.١٤ و ٢.٨٥ جزء في المليون ، على التوالي بينما كانت قيم LD_{50} المقابلة لمبيد الألفاسيبرمثرين هي ٠.١٢٥ و ٢.٩٦ جزء في المليون في حين اظهر الثيوديكارب (مبيد الأوكسيم كاربامات) قيمة LD_{50} هي ٥.٩ و ٩١.٥ جزء في المليون على التوالي.

لقد كانت مخاليط البيرثرويد - ثيوديكارب عند نسبة خلط ١:٥ ذات فعالية أكبر ضد العشيرة الحقلية عن استخدام كلا منهما بمفرده، فلقد زادت سمية البيرثرويدات في مخاليطها بنسبة ٥.١٨ ضعف للسيبرمثرين و ٥.٦٩ ضعف للألفاسيبرمثرين وذلك عند قيم لمعامل السمية المشتركة (co-toxicity factor) ٧٧.٧ % و ٧٩.٦ % على التوالي. من ناحية أخرى فإن مخاليط البيرثرويد - ثيوديكارب قد أدت إلى زيادة كبيرة في قوة سمية الثيوديكارب .

أشارت نتائج اختبارات التنبؤ بالمقاومة ضد السيبرمثرين و الألفاسيبرمثرين قيما عالية لكلا منهما منفردا فعندما اعتمدت على مقارنة قيم LD_{50} مع العشيرة المعملية كانت قيم المقاومة ٢٠.٣٦ ضعف و ٢٣.٦٨ ضعف على التوالي ، بينما أدى الخلط مع الثيوديكارب الى خفض هذه القيم إلى ٣.٩٣ ضعف للسيبرمثرين و ٤.١٦ ضعف للألفاسيبرمثرين.