

SYNERGISED CYPERMETHRIN AND α -CYPERMETHRIN AS RESISTANCE MANAGEMENT STRATEGY IN PINK BOLLWORM *Pectinophora gossypiella* (SAUNDERS).

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ABSTRACT

Two dichlorovinyl pyrethroids and one n-methyl carbamate insecticide, were determined against both laboratory and field male moth strains of pink bollworm *Pectinophora gossypiella* (Saunders). Toxicity data demonstrated that, LC₅₀ values were 0.14 and 2.85 ppm for cypermethrin, 0.125 and 2.96 ppm for alpha-cypermethrin while they were 4.45 and 39.46 ppm for carbaryl against laboratory and field strains respectively.

Pyrethroid-carbaryl combinations were more effective against the field population than that of each individual. Pyrethroid toxicity in the mixture was increased from 13.57 and 5.58 fold with co-toxicity factor of 90 and 75.4 for cypermethrin and alpha-cypermethrin, respectively. On the other hand, carbaryl toxicity was increased in the mixture to 37.94 and 14.95 fold for carbaryl/cypermethrin and carbaryl/ alpha-cypermethrin, respectively.

Determining resistance data based on comparing LC₅₀ values of the laboratory strain showed high resistance levels in the field population. The resistance ratio values were 20.36 and 23.68 fold for cypermethrin and alpha-cypermethrin, respectively; while mixing with carbaryl decreased these levels to 1.5 fold for cypermethrin and 4.24 fold for alpha-cypermethrin. In case of carbaryl, resistance ratio values decreased from 8.87 fold to 0.23 and 0.59 fold in mixture with cypermethrin and alpha-cypermethrin, respectively.

Keywords: Resistance, monitoring, mixtures, resistance management, joint toxicity, pink bollworm, pyrethroid, n-methyl-carbamate.

INTRODUCTION

Melander (1914) described the first case of resistance, while Georgiou and Mellon (1983) declared that, resistance was increased to 400 species in 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 (Shekeban.2002 a,b and Shekeban *et al* 2003 a,b).

Insecticide efficacy is referred to its binding at target molecules in the insect. On this criterion insect try to resist this binding leading to decreased toxicity. 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 factors (Chalvet-Monfray *et al* 1998).

Managing resistance clearly help in decreasing the harmful effects of pesticides, delay widespread development of insecticide resistance by pink

bollworm and reducing the probability of severe yield loss, this can take place by using more than one insecticide in several tactics including sequences, mixtures, rotations and mosaics and prolonging the efficacy of environmentally safe pesticides (Tabashnik 1989, Leonard *et al* 1994).

Carbaryl is used in this study to act as pyrethroid synergist which may increase activity sufficiently to allow use lower dose of pyrethroids and thus decrease cost. Also, the possibility of using binary combinations of cypermethrin and alpha-cypermethrin with carbaryl in resistance management strategy to overcome partially the problem of resistance in pink bollworm is studied.

MATERIAL AND METHODS

1) Attracticide Resistance Monitoring Technique (ARMT):

To detect toxicity, joint toxic effect and resistance levels of the two tested dichlorovinyl-pyrethroids and the n-methyl carbamate insecticide, carbaryl, the attracticide resistance monitoring technique as described by Miller (1986) and modified by Shekeban (2000) was used. This technique eliminates insects handling, allow a rapid determination of the adult population response and it's also repeatable, usable for field and greenhouse populations and provided stable LC₅₀'s with low control mortality (Haynes *et al* 1986 and 1987).

a) Insect used:

Pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera : Gelechiidae) was involved in this study as follow:

1- Field male moth strain 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 strain 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 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 arboxylate].

*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].

*Carbaryl (n-methyl – carbamate) : Sevin 85 % WP. [1-naphthyl N-methyl carbamate].

c) Toxicity procedure :-

Delta traps were used with a sticky adhesive – coated cards insert containing the insecticide or insecticide mixture concentrations 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 insecticides and resistance ratio were measured.

II) Joint toxic effect procedure:

Combinations of different concentrations of each tested pyrethroid and carbaryl were prepared at the ratio of 1:5, this ratio was selected according to Metcalf (1967). The experiments were designed on assumption that carbaryl 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 expressed in 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 expressed in 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, which was expressed as follow: Co-toxicity factor (CTF) = $[1 - (\text{actual dose of A in mixture} / \text{estimated dose of A single}) + (\text{actual dose of B in mixture} / \text{estimated dose of B single})]$ x 100. This factor was used to differentiate the results into three categories; 1- A positive factor of 25% or more was considered potentiation. 2- A negative factor of 25% or more means antagonism. 3- Any intermediate values between -25% and +25% indicate only additive effect.

e- Reduction of the resistance ratio (RR Red.) was determined according to Shekeban 2003a ; $RR \text{ Red.} = \text{Field population RR} - \text{Field population RR after joint action}$.

RESULTS AND DISCUSSION

1- Toxicity and Resistance Studies:-

Dichloromovinyl - pyrethroids; cypermethrin and α -cypermethrin, and the n-methyl carbamate insecticide, carbaryl, were tested against both laboratory and field male moth populations of pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) and the toxicity data are presented in table (1). Data declared that, the pyrethroid insecticides were highly toxic compounds with LC_{50} values of 0.14 and 2.85 ppm for cypermethrin and 0.125 and 2.96 ppm for alpha- cypermethrin, while carbaryl was less toxic with LC_{50} values of 4.45 and 39.46 ppm for laboratory and field populations, respectively. These results are in good agreement with those reported by El-Dahan (1983), Shekeban (1989) and Marei *et al* (1991). They reported that,

cypermethrin and the other tested pyrethroids exhibited the highest toxicity among the other tested insecticides against the cotton leafworm (CLW). On this criterion, both cypermethrin and α -cypermethrin were highly toxic against both field and laboratory male moth populations of PBW than chlorpyrifos or thiodicarb (Shekeban *et al* 2003b and 2004). 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 the tested pyrethroids indicated moderate increases. These toxicity parameters and the calculated resistance ratio values are also tabulated in table (1). Relative to LC₅₀ values of laboratory strain, the resistance ratio values were 20.36 and 23.68 fold for cypermethrin and α -cypermethrin, respectively. While it was 8.87 fold for carbaryl. Based on LC₉₅ values, the resistance levels were much lower than that based on the LC₅₀ values specially for the tested pyrethroids, which were: 6.87 fold for cypermethrin and 6.14 fold for α -cypermethrin, while it was 7.62 fold for carbaryl. These results are in a harmony with the results obtained with Shekeban *et al* (2003a,b and 2004) and El-Bassiony (2001).

Table 1: Toxicity parameters of cypermethrin, alpha-cypermethrin and carbaryl against male moths of laboratory and field strains of pink bollworm *P. gossypiella* and their resistance levels using ARMT¹.

Insecticides	Strain	Reg. Equation Y=a + bx	LC ₅₀ (ppm) (95% C L)	LC ₉₅ (ppm) (95% C L)	RR ₅₀ ²	RR ₉₅ ³
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
α -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
Carbaryl	Lab.	Y=-1.93+2.98x	4.45 (4.96-3.99)	15.86 (19.98-12.70)	-	-
	Field	Y=-5.4+3.39x	39.46 (45.5-34.22)	120.81 (148.3-98.57)	8.87	7.62

1- ARMT: Attracticide Resistance Monitoring Technique.

2- RR₅₀: Resistance ratio at LC₅₀ level.

3-RR₉₅: Resistance ratio at LC₉₅ level.

2- Joint Toxicity Studies:-

Data in table (2) presented the joint toxic effect of the esterase inhibitor, carbaryl, on the toxicity of the tested pyrethroids against the field male moth population of pink bollworm using ARMT at late 2003 cotton season. The toxicity of pyrethroid and carbaryl combinations could be arranged as follow : cypermethrin + carbaryl followed by α -cypermethrin + carbaryl with LC₅₀ values of 0.21 and 0.53 ppm and relative toxicity values of 13.57 and 5.58 fold, respectively. It is of interest to note that carbaryl was found to potentiate the two tested pyrethroids and the co-toxicity factor were 90 and 75.4 %, for cypermethrin and α -cypermethrin respectively. On the

other hand, both of the tested pyrethroids potentiated strongly the toxicity of carbaryl with relative toxicity values of 37.94 and 14.95 fold, respectively.

The role of carbaryl as a potentiator for cypermethrin and α - cypermethrin 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 the n-methyl carbamate when combined with the dichlorovinyl pyrethroids, cypermethrin, *cis*-cypermethrin, *trans*-cypermethrin, permethrin, *cis*-permethrin and *trans*-permethrin, at 1:5 level to the 4th instar larvae of CLW resulted in potentiation. Also, Shekeban (2003a,2004) found that, thiodicarb synergized deltamethrin, fenpropathrin, cypermethrin and α - cypermethrin against PBW using ARMT. So, these mixtures allowed to use lower doses of pyrethroids and thus decrease cost.

Table 2: Joint toxic effect of two pyrethroid mixtures with carbaryl at a ratio of 1:5 and two mixtures of carbaryl with cypermethrin and alpha-cypermethrin at a ratio of 5:1 against pink bollworm *P. gossypiella* using ARMT.

Insecticides	Reg. Equation Y= a + bx	LC ₅₀ (ppm) (95%CL)	LC ₉₅ (ppm) (95%CL)	RT ¹ Fold	CTF ²
Cypermethrin + Carbaryl	Y=0.60+0.88x	0.21 (0.30 -0.13)	15.65 (38.29-8.13)	13.57	90
Cypermethrin alone	Y= -0.67+1.48x	2.85 (3.96-2.0)	36.87 (99.1-15.4)	-	-
α -cypermethrin + Carbaryl	Y=0.29+1.06x	0.53 (0.72-0.37)	19.02 (40.97-10.87)	5.58	75.4
α -cypermethrin alone	Y= -0.74+1.57x	2.96 (3.74-2.33)	32.7 (68.0-17.51)	-	-
Carbaryl+ Cypermethrin	Y=-.016+0.88x	1.04 (1.52-0.67)	78.23 (187.5-40.1)	37.94	90
Carbaryl+ α -cypermethrin	Y=-0.45+1.06x	2.64 (3.62-1.89)	95.1 (194.9-52.5)	14.95	75.4
Carbaryl alone	Y=-5.4+3.39x	39.46 (45.48-34.22)	120.81 (148.28-98.57)	-	-

1- RT= relative toxicity 2- CTF= co-toxicity factor

Table (3) showed the joint toxic effect of the tested combinations of pyrethroid insecticides with carbaryl on resistance levels. The resistance ratios based on LC₅₀ with joint action effect were 1.5 fold for cypermethrin with resistance reduction rate of 18.86 fold and 4.24 fold for α -cypermethrin with resistance reduction rate of 19.44 fold. The corresponding resistance ratios based on LC₉₅ after joint toxicity were 2.91 and 3.57 fold for cypermethrin and α -cypermethrin, respectively; with resistance reduction rates of 3.96 and 2.57 fold, respectively. Mixing carbaryl with the tested pyrethroids at the ratio of 5:1 increased the power of its toxicity to the degree that the field population become more susceptible than the laboratory reference population. The resistance ratios based on LC₅₀ with joint action effect were 0.23 fold for carbaryl + cypermethrin combination with resistance reduction rate of 8.64 fold and 0.59 fold for carbaryl + α -cypermethrin mixture with resistance reduction rate of 8.28 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 more potent uses of toxicants but also it could theoretically prevent the emergence of resistant populations.

Table 3 : Effect of joint toxicity of insecticide mixtures on resistance levels against laboratory and field strains of pink bollworm *P. Gossypiella*, using ARMT.

Insecticide	Strain	Reg. Equation Y=a + bx	LC ₅₀ (ppm) (95% C L)	LC ₉₅ (ppm) (95% C L)	LC ₅₀ RR	RR* Red	LC ₉₅ RR	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 with JTE**	Y=0.60+0.88x	0.21 (0.30-0.13)	15.65 (38.29-8.13)	1.5	18.86	2.91	3.96
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 with JTE	Y=0.29+1.06x	0.53 (0.72-0.37)	19.02 (40.97-10.87)	4.24	19.44	3.57	2.57
Carbaryl	Lab.	Y=-1.93+2.98x	4.45 (4.96-3.99)	15.86 (19.98-12.70)	-	-	-	-
	Field	Y=-5.4+3.39x	39.46 (45.5-34.22)	120.81 (148.3-98.57)	8.87	-	7.62	-
	Field with cyper JTE	Y=-.016+0.88x	1.04 (1.52-0.67)	78.23 (187.5-40.1)	0.23	8.64	4.93	2.69
	Field with α-cyper JTE	Y=-0.45+1.06x	2.64 (3.62-1.89)	95.1 (194.9-52.5)	0.59	8.28	6.0	1.62

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

**JTE = Joint Toxic Effect

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تنشيط السيبرمثرين و الألفاسيبرمثرين كاستراتيجية لإدارة المقاومة في دودة اللوز القرنفلية.

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معهد بحوث وقاية النبات , مركز البحوث الزراعية , الدقي , الجيزة**

تم تقدير سمية اثنين من المبيدات البيروثرويدية ثنائية الكلورو فينيل هما السيبرمثرين و الألفاسيبرمثرين وكذلك احد المبيدات ن-ميثيل كاربامات ،كارباريل، كلا بمفرده ، ضد عشيرتي فراشات الذكور الحقلية والمعملية لدودة اللوز القرنفلية ، بكتينوفورا جوسيبيللا (ساندز) . لقد أشارت نتائج دراسة السمية إلى أن قيم ت ق . لمبيد السيبرمثرين هي ٠,١٤ و ٢,٨٥ جزء في المليون ، على التوالي بينما كانت قيم ت ق . للمبيد الألفاسيبرمثرين هي ٠,١٢٥ و ٢,٩٦ جزء في المليون في حين كانت قيم ت ق . لمبيد الكارباريل هي ٤,٤٥ و ٣٩,٤٦ جزء في المليون على التوالي.

لقد تم خلط البيروثرويدات مع الكارباريل بنسبة خلط ١ : ٥ فكانت المخاليط ذات فعالية أكبر ضد العشيرة الحقلية عن استخدام المبيدات كلا بمفرده. فلقد زادت سمية البيروثرويدات في مخاليطها بقيم ١٣,٥٧ و ٥,٥٨ ضعف مع قيم لمعامل السمية المشتركة (co-toxicity factor) ٩٠ % و ٧٥,٤ % لكلا من السيبرمثرين و الألفاسيبرمثرين على التوالي و من ناحية أخرى فان مخاليط البيروثرويدات مع الكارباريل قد أدت إلى زيادة سمية الكارباريل بدرجة كبيرة جدا .

أشارت نتائج اختبارات تقدير المقاومة اعتمادا على المقارنة مع قيم ت ق . للعشيرة المعملية الى زيادة مستويات المقاومة في العشيرة الحقلية لذكور دودة اللوز القرنفلية وكانت هذه القيم ٢٠,٣٦ و ٢٣,٦٨ ضعف للسيبرمثرين و الألفاسيبرمثرين على التوالي , بينما أدى الخلط مع الكارباريل الى خفض هذه القيم إلى ١,٥ ضعف للسيبرمثرين و ٤,٢٤ ضعف للألفاسيبرمثرين كما انخفضت قيم المقاومة للكارباريل إلى ٠,٢٣ و ٠,٥٩ ضعف في مخلوطي السيبرمثرين و الألفاسيبرمثرين على التوالي بحيث أصبحت العشيرة الحقلية أكثر حساسية من العشيرة المعملية.