MECHANISM OF HOUSE FLY RESISTANCE: II- BINARY MIXTURES OF CYPERMETHRIN, IMIDACLOPRID AND SPINOSAD AND THEIR IMPACT TO BREAK DOWN THE LABORATORY DEVELOPED RESISTANCE.

Doaa El-Sherif[1], Makram Sayed[1], Zaki El-Fiky[2] and Ahmed Etman[1]

1. Plant Prtectin Dept, Faculty of Agric. Fayoum Univ.
2. Genetic Dept. Faculty of Agric., Faayoum Univ.

ABSTRACT

The present study indicated that the repeated exposure of the laboratory strain of the house fly, *Musca domestica* (Linnaeus) to Cypermethrin, Imidacloprid and Spinosad lead to developing resistance against these insecticides. The resistance level of the selected strains reached after 30th generation to 24.8, 15.53 and 9.56 folds for Cypermethrin, Imidacloprid and Spinosad compared with the laboratory strain.

This study also showed the breakdown of resistance using insecticide binary mixtures, where the mixture of Cypermethrin+ Imidacloprid exhibited potentiation at the ratio of 1:1 and 1:2 against all the resistant strains. Also, the combination of Cypermethrin with Spinosad gave potentiation at the ratios of 1:1, 1:2 and 2:1 against Imidacloprid and Spinosad resistant strains.

Key words: *Resistance, Joint action, cypermethrin, imidacloprid, spinosad*

INTRODUCTION

Management of the house fly requires multiple applications of different insecticides. Unfortunately, the house fly has a well-documented history of developing resistance to many insecticides, including pyrethroids, neonicotinoids, organophosphates (OPs), carbamates, organochlorines and the triazine cyromazine. The house fly has been found to be resistant to 62 unique insecticide active ingredients, with 337 documented cases worldwide, and is listed as the world's No. 1 resistant urban insect pest (Zhu, *et al.*, 2016 and Li, *et al.*, 2013). Increased metabolic detoxification and decreased target site sensitivity of the insect nervous system are two of the major mechanisms involved in the development of insecticide resistance in house flies (Li, *et al*., 2011).

The current investigation is curried out using the insecticides formulation **Cymbush®** 10% EC (Cypermthrin), **Imidazed®** 20 % SC**)** (Imidacloprid) and **Tracer®** 24% SC (Spinosad) insecticides on *M. domestica*. The adult house fly resistance to Cypermethrin, Imidacloprid and Spinosad was developed in the laboratory through 30 generation. The aim of this study is to using the binary mixtures of the tested insecticides to breakdown the laboratory developed resistance.

MATERIAL AND METHODS

Development of resistant strains

The laboratory strain of *M. domestica* was reared in the insects rearing room, Plant Protection Department, Faculty of Agriculture, Fayoum University using different media according to (Singh and Jerram, 1976 and El-Sherif, *et al*., in press). The laboratory strain was used to build up the resistance against the insecticides, Cypermethrin, Imidacloprid and Spinosad. Adults were treated with the calculated LC_{50} values of Cypermthrin, Imidacloprid and Spinosad (El-Sherif, *et al*., in press). Survivors after 24h treatment were used to establish a new generation for each tested insecticide. Each generation was exposed to the same LC_{50} until mortality reached a rate less than 35%, at this point a LC_{50} was estimated from a new dose-response curve. This procedure was followed for 30 generation, and the resistance ratio (Fold) was calculated using the following formula (Georghiou 1962) until the thirty generation.

$$\text{Fold} = \frac{\text{LC50 of resistant strain}}{\text{LC50 of laboratory strain}}$$

Effect of binary mixtures of different insecticides

The adult's medium (black honey and dry yeast powders at the ratio of 1:2) were applied to evaluate the efficiency of the binary mixtures of different insecticides to investigate their role in breakdown of the house fly resistance against insecticides.

The calculated values of LC_{25} of the Cypermethrin, Imidaclprid and Spinosad were used to make the binary mixtures at the rate of 1:1, 1:2 and 2:1. The prepared mixture were added to the adult's media to get poison bait, which introduced to the house fly adults. Percent mortality was recorded at 24h post treatment and the co-toxicity factor was estimated according to Mansour, *et al*., (1966 and 2010) equation.

The co-toxicity factor with a positive value from 20 or more indicates potentiation, a negative factor of -20 indicates antagonism, and the intermediate values of > -20 to < 20 indicates an additive effect.

RESULTS AND DISCUSSION

Resistance to Cypermethrin

Data in Table (1) and Fig. (1) show the development of resistance to Cypermethrin expressed as LC_{50} values and resistance ratio (fold). The selection process by Cypermerthin in the laboratory was carried out until the thirtieth generation. The house fly resistance increased against Cypermethrin and recorded as 24.8 fold in the generation 30 compared with the laboratory strain.

Development of Cypermethrin resistance in the current study was slowly compared with the other studies. High levels of resistance to the pyrethriod detected by Abbas and Shad, (2015), where they reported that,

house fly developed 445 fold resistances against Lambda-Cyhalothrin after 26 generations. Also, Deltamethrin selected strain for six generations built a resistance ratio (RR) of 176.34-fold, when compared with the lab-susceptible strain, (Khan, *et al.,* 2015).

Table (1). The values of LC_{25}, LC_{50} (ppm), slope and fold values of resistance in laboratory and the three resistant strains at 30th generation

Insecticides	Strains	LC_{25}	LC_{50}	Slope	RR* (Fold)
Cypermethrin	Laboratory	89.5	208.75	1.94	-
	Resistant	2200	5177.09	2.33	24.8
Imidacloprid	Laboratory	125	238.3	2.41	-
	Resistant	1740	3700.59	1.95	15.53
Spinosad	Laboratory	41	86.05	2.34	-
	Resistant	410	822.78	2.13	9.56

***Resistance ratio (RR)** = LC_{50} of resistant strain / LC_{50} of laboratory strain

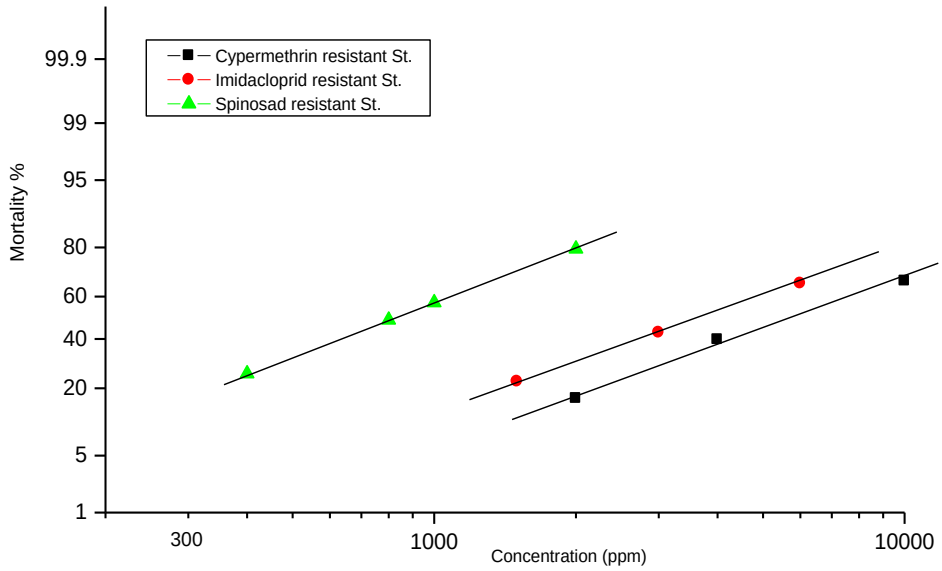

Fig. (1) Toxicity Lines of resistant strains to the tested insecticides at the 30th generation against the adult of *M. domestica*

Resistance Imidacloprid in *M. domestica*

Data in Table (1) and Fig. (1) show that the Imidacloprid selected population developed 15.53 fold resistance in the thirtieth generation compared with the laboratory population. This means that *M. domestica* showed moderate resistance to Imidacloprid. In contrast, Khan, *et al.,* (2014)

found that after 13 generations of laboratory selection with Imidacloprid, the resistance ratio increased to 106 fold compared with the susceptible strain and Kaufman, *et al.,* (2010) found that high level of resistance after the fifth generation selection, was 331-fold at the LC_{70} were recorded.

Ma, *et al*., (2017) reported that, Imidacloprid-resistant strain was established from the field strain by their selections with Imidacloprid for 21 generations in laboratory, showing 80.15-fold compared with the susceptible strain.

Resistance Spinosad in *M. domestica*

Low level resistance was observed for Spinosad in *M. domestica,* where the resistance ratio did not reach 10 fold after thirtieth generation under Spinosad selection (Table 1 and Fig. 1). This finding could be due to the absence and or inactive metabolic and genetic factors, which develop the Spinosad resistance. Also, this fact could promote the using of Spinosad in the control process against the house fly. Building up of resistant to Spinosad increased slowly, where the resistance ratio recorded 9.56 fold at thirtieth generation. Shi, *et al.,* (2011) reported that the house fly exhibited 279 fold after 27 generation compared with the susceptible strain. In addition, Shono and Scott, (2003), found that the selection of the houseflies produced 150 fold against Spinosad after 10 generations of selection.

Effect of binary mixtures of the tested insecticides against resistant strains after 24h of exposure.

The interaction effects among the tested insecticides depended on the type of insecticides used, ratios and strains. The values LC_{25} of the Cypermethrin, Imidacloprid and Spinosad were used to make the binary insecticide combinations, where calculated in the resistant strains after the 30^{th} generation, 2200, 1740 and 410 ppm, respectively.

Data in (Table 2 and Fig. 2) show the mixture insecticides in different strains; the mixture of Cypermethrin+Imidacloprid showed potentiation when used at the ratio of 1:1 and 1:2 in all the strains and it gave high potentiation. While at a ratio of 2:1 it showed an additive effect in the Cypermethrin and Imidaclporid resistant strains and potentiation in Spinosad resistant strain. This could be because the result of different modes of action they poses, or because the binding of monooxygenase enzymes with Imidacloprid insecticide would prevent or delay the degradation, and enhance the toxicity of Cypermethrin by competitive substrate inhibition mechanism. As is the case in some organophosphate insecticides which bind to the active site associated with esterase enzymes responsible for detoxification of pyrethroid-based insecticides (Cloyd, 2011 and Ahmad, 2009).

The mixture of Cypermethrin with Spinosad gave the highest co-toxicity factors Imidacloprid and Spinosad resistant strains at the ratio of 1:1, recording 54 and 36, respectively. In addition to, it gave potentiation in the other ratios in Imidacloprid and Spinosad resistant strains, and also, showed

potentiation at a ratio of 1:2 against the Cypermethrin resistant strain, but it showed an additive effect when used at the ratio of 1:1 and 2:1 in the same strain. This is useful in preventing the development of resistance in *M. domestica* to the tested insecticides. The toxicity of pyrethroids could be enhanced by the addition of new insecticides like Emamectin benzoate and Fipronil and Spinosad. According to this, one toxicant in the mixture interferes with the metabolic detoxification of the other toxicant, Khan, *et al.,* (2013) they demonstrated that the mixture of Cypermethrin+Emamectin gave a synergistic action. These results conversely agree with Abbas, *et al*., (2015) whom revealed that combination indices for Lambda-Cyhalothrin+Emamectin benzoate and Lambda-Cyhalothrin+ Spinosad mixtures were significantly less than 1, demonstrating an antagonistic effect. Thus, Vayias, *et al*., (2010), revealed that the combination of Spinosad with Deltamethrin did not appear to be compatible with the rice weevil, *Sitophilus oryzae* (Linnaeus).

The potentiation effect was also demonstrated in the case of the combination between pyrethroids and organophosphates by (Zahidul and Khalequzzaman, 2002 and Asid, *et al*., 2017).

On the other hand, the mixture of Imidaclporid+Spinosad showed an additive effect at all mixing ratios and also all the tested strains expect in the Imidacloprid resistant strain at the ratio of 1:1 which gave a potentiation effect. Also, the mixture containing Fipronil and Acetamiprid have an additive effect on *M. domestica* (Levchenko, *et al.*, 2018).

Table (2): Efficacy of binary mixtures against Cypermethrin, Imidacloprid and Spinosad resistant strains of *M. domestica.*

Combinations	Mixing ratio	Co-toxicity factor		
		Cypermethrin	Imidacloprid.	Spinosad
Cypermethrin+Imidacloprid	1 : 1	36 P*	38 P	32 P
	1 : 2	29.3 P	29 p	22.7 p
	2 : 1	6.7 Ad**	16 Ad	21.3 P
Cypermethrin+Spinosad	1 : 1	8 Ad	54 P	36 P
	1 : 2	22.7 P	29.3 P	25.3 P
	2 : 1	4 Ad	22.7 P	25.3 P
Imidacloprid+ Spinosad	1 : 1	20 Ad	22 P	8 Ad
	1 : 2	5.3 Ad	18.7 Ad	20 Ad
	2 : 1	4 Ad	-6.7 Ad	1.3 Ad

***P. Potentiation effect** ****Ad. Additive effect**

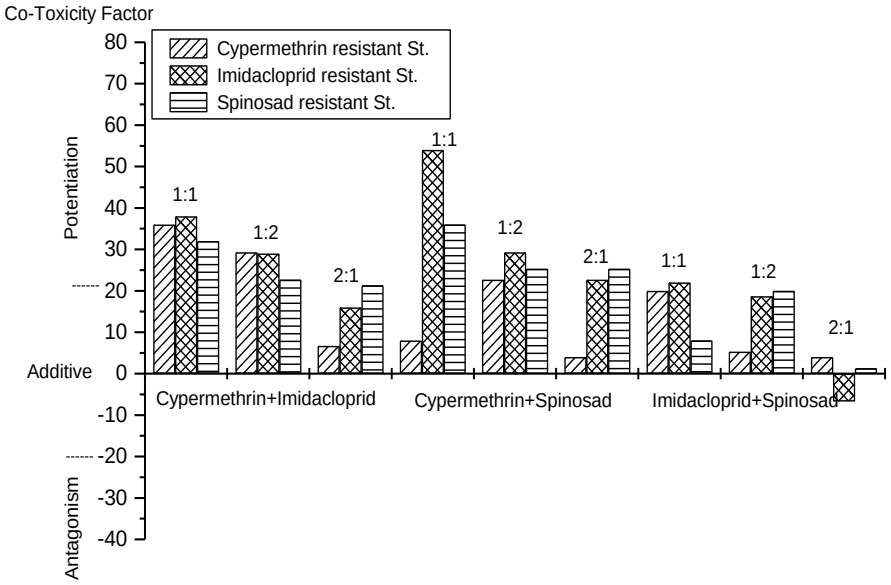

Fig. (2) The joint action of the tested insecticides against the Cypermethrin, Imidacloprid and Spinosad resistant strains of house fly.

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ميكانيكية مقاومة الذبابة المنزلية:٢- تأثير المخاليط المزدوجة للسيبرمثرين، الايميداكلوبريد والسبينوسات ودورهم فى كسر المقاومة المعملية

دعاء الشريف، ا.د/ مكرم سيد، أ.د/ زكى الفقى و ا.د/ احمد عتمان

الملخص العربى

الدراسة الحالية اوضحت ان بتكرار المعاملة بالمبيدات فى السلالة المعملية للذبابة المنزلية بمبيدات السيبرمثرين، والايميداكلوبريد والسبينوساد ادى الى تطور المقاومة ضد هذه المبيدات. مستوى المقاومة للسلالت المنتخبة وصل عند الجيل الثلاثين الى ٢٤,٨، ١٥,٥٣ و ٩,٥٦ ضعف للسيبرمثرين، الايميدكلوبريد و السبينوساد على التوالى مقارنة بالسلالة المعملية. هذه الدراسة ايضا اوضحت امكانية كسر المقاومة ياستخدام المخاليط المزدوجة للمبيدات، حيث اظهر مخلوط السيبرمثرين + الايميداكلوبريد تأثير تقوية على معدلات ١:١ و١:٢ ضد كل السلالات المقاومة. ايضا اعطى مخلوط السيبرمثرين مع السبينوساد تأثير تقوية على معدلات ١:١، ١:٢ و ٢:١ ضد السلالة المقاومة الايميدكلوبريد والسلالة المقاومة للسبينوساد.