MECHANISM OF HOUSE FLY RESISTANCE: I- Toxicity of Cypermethrin, Imidacoloprid and Spinosad to Larvae and Adults of the Laboratory Strain

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ABSTRACT

The house fly, *Musca domestica* L., is a vector for more than 100 human and animal diseases and has the ability to develope resistance to different insecticides. This study evaluated toxicity of Cypermethrin, Imidacloprid and Spinosad, in addition to, their mixtures under laboratory conditions against larvae and adults of *M. domestica* for laboratory strain. The LC_{50}s of the three tested insecticides were 579.30, 415.46 and 16.32ppm, respectively, against larvae 48h post treatment. The toxic effect of these insecticides against *M. domestica* adults was assessed 24h post treatment. The LC_{50}s of Cypermethrin, Imidacloprid and Spinosad were 208.75, 238.3 and 86.05 ppm, respectively, against the laboratory strain. This study also showed efficacy of the binary mixtures of these insecticides, where the mixture of Cypermethrin+Imidacloprid and Cypermethrin+Spinosad showed potentiation at ratios 1:1, 1:2 and 2:1. On other hand, the mixture of Imidaclporid+Spinosad showed an additive effect at all mixing ratios.

INTRODUCTION

The house fly, *Musca domestica* (Linnaeus), is a major domestic, medical and veterinary pest that transmit more than 100 to human and animal diseases, including bacterial infections such as salmonellosis, anthrax ophthalmic, shigellosis, typhoid fever, tuberculosis, cholera and infantile diarrhea; protozoan infections such as amebic dysentery; helminthic infections such as pinworms, hook worms and tapeworms; and both viral and rickettsial infections (Li, *et al*., 2013). Also, house fly plays a role as a vector for *Yersinia pseudotuberculosis,* which results in high avian mortality on poultry farms. So that, the caused infestations reduced feed conversion efficiency, and increased stress levels for young or adult animals, leading up to $200 million in annual production losses (Rinkevich, *et al.,* 2013).

This study discusses the toxicological impact of Cypermethrin, Imidacloprid and Spinosad against the laboratory strain of house fly. In addition to, the efficiency of the binary mixture of these insecticides in controlling house flies.

MATERIALS AND METHODS

Insecticides

Three insecticides in their formulations form Cypermethrin (**Cymbush®** 10% EC**)**, Imidaclopride (**Imidazed®** 20 % SC**)** and Spinosad (**Tracer®** 24% SC**)** were used to calculate their LC_{50} values.

Rearing media.

Larval medium: This medium was prepared freshly according to the method described by Singh and Jerram (1976) with a slight modification. The bran was used instead of the agar. The medium consisted of 40g milk powder, 150g wheat middling (bran), 20g yeast powder, 0.3g methyl β hydroxyl benzoate, and 0.1g streptomycin sulphate. The contents were mixed and wetted with water.

Adult medium: This medium was prepared according to Singh and Jerram, (1976) method with a slight modification. The present medium does not contain egg yolk powder and cholesterol. Two media were used: the first was paper rolls saturated with 2.5% sugar solution; and the second was a solid nutrient mixture consisted of 9g sugar, 9g milk powder and 2g yeast powder.

Rearing cages: Wooden cages with the dimensions of 72cm height, 60cm length, and 54cm width. The front side of each cage has a circular hole which closed with a tube of muslin to provide the adults with the diet. Two sides of the cage were covered with muslin cloth to allow aeration for the cage, and the fourth side was made of glass to permit a follow up of the rearing process.

Rearing of *Musca domestica* L.

A strain of *M. domestica* larvae was collected from the accumulation of garbage places of Fayoum Governorate. This strain was colonized in the previously described cages and provided with the adults' medium. Twenty five grams of larval medium was placed in a small plastic tray as an oviposition site and placed with adults in the rearing cage. The medium containing laid eggs was transferred to an incubator under a constant temperature of 30 ± 2°C. until pupation, the collected pupae were transferred to cages provided with adult medium at room temperature.

The house fly was reared in the laboratory for 30 generations in the insects rearing room at the Plant Protection Department, Faculty of Agriculture, Fayoum University, without any exposure to insecticides.

Treatment of larvae

The method of larvae treatment was described by Siriwattanarungsee, *et al*., (2008). One hundred grams of larvae medium was used for each insecticide concentration and divided into four portions (25g each) in a small plastic tray. Twenty five of the second instar larvae were transferred to the poisonous bait tray and kept in an incubator at a constant temperature of 30 ± 2°C with a relative humidity of 50 - 60%. The mortality percentage calculated after 48h treatment.

Treatment of adults:

The base of the bait consisted of a mixture of black honey and dry yeast powders at the ratio of 1:2, mixed to form a paste. Different concentrations of each tested insecticide were prepared and added to the paste to get poison bait. Each concentration was divided into four replicates and about one gram of the toxic bait was spread over slices of aluminum foil. Twenty five adults were introduced into each plastic jar, then one prepared aluminum foil slice was suspended in the jar opening and fixed by the jar cover. Small holes were made in the cover to allow ventilation. The jars were kept in an incubator at a constant temperature of 28°C±2 and a relative humidity of 50-60%±5 and the mortality rate was recorded at 24h post treatment

Effect of binary mixtures

The calculated values of LC_{25} of Cypermethrin, Imidaclprid and Spinosad were used to make the binary insecticide combination at the ratios of 1:1, 1:2 and 2:1. The prepared mixture was added to the adult's media to get toxic 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 the equation represented by (Mansour, *et al*., 1966 and 2010)**,** as follows,

$$\text{Co-Toxicity factor (C.F)} = \frac{\text{Observed mortality} - \text{Expected mortality}}{\text{Expected mortality}} \times 100$$

A positive value from +20 or more indicates potentiation, a negative factor of -20 or less indicates antagonism, and the intermediate values of > -20 to < +20 indicates an additive effect

Statistical analysis:

The mortality data were corrected by the Abbott formula (Abbott, 1925), and **t**oxicity line was plotted according to Finney analysis **(**Finney, 1971). The software program (Micro Origin) was used for the statistical analysis of the data and plotting the histograms.

RESULTS AND DISCUSSION

1- Toxicity against *M. domestica* larvae.

Spinosad exhibited the most toxic effect on the laboratory strain, while Cypermethrin was the least toxic depending on the value of the LC_{50} and LC_{90} (Table ١ and Fig. 1). The treatments could be arranged in a descending order according to their toxic effect as follows: Spinosad, Imidacloprid and Cypermethrin, with LC_{50}'s of 16.32, 415.46 and 579.30 ppm, respectively. This finding agrees with Kristensen and Jespersen, (2004) and Abo-El-Maged, (2014), who reported that, Spinosad was highly toxic to the larvae of house fly.

Table (1). The LC_{50}, LC_{90} (ppm) and slope values at 48h post treatment of Cypermethrin, Imidacloprid and Spinosad against the laboratory strain of *M. domestica* larvae.

Insecticides	LC_{50}	Confidence Level		LC_{90}	Confidence Level		Slope ±SE*
		Lower	Upper		Lower	Upper	
Cypermethrin	579.30	471.06	697.65	3628.27	2497.67	6573.93	1.61±0.2
Imidacloprid	415.46	351.39	481.09	1748.82	1326.48	2681.51	2.05±0.25
Spinosad	16.32	14.09	18.8	69.09	55.05	93.06	2.05±0.17

*SE = Standard Error

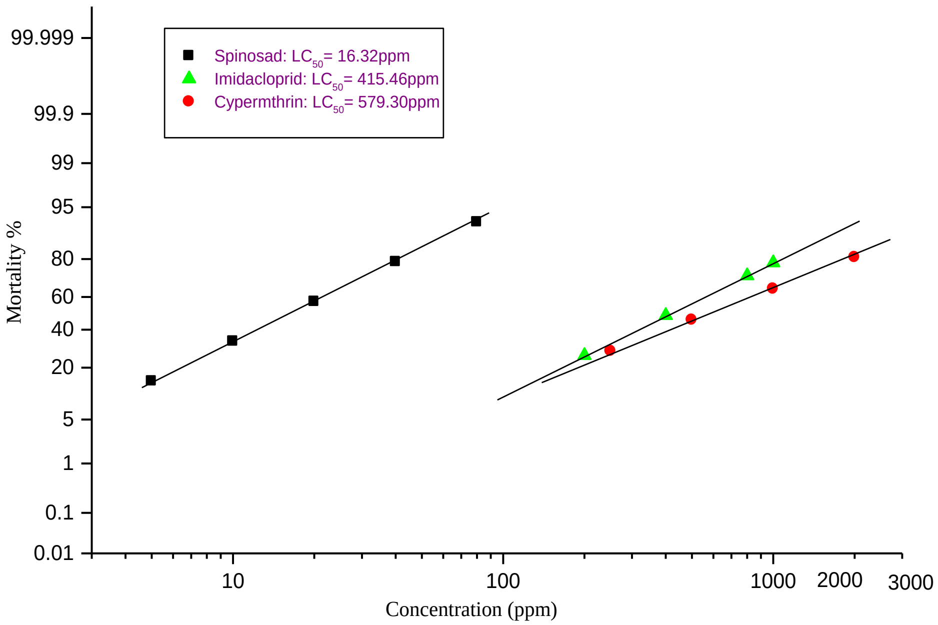

Fig (1) Toxicity Lines of the tested insecticides against the laboratory strain of *M.domestica* larvae

2- Toxicity against *M. domestica* adults.

Table (2) and Fig. (2) show the toxicity of tested insecticides at 24h post treatment against laboratory of *M. domestica* adults. Spinosad was the most toxic insecticide with LC_{50} value was 86.05ppm. The least toxic insecticide to the laboratory strain was Imidacloprid with LC_{50} of 238.3 ppm.

Deacutis, *et al.,*(2006) studied the efficacy of Spinosad assessed by three bioassay methods, topical application, feeding and residual exposure on *M. domestica*, the LD_{50} or LC_{50} was 0.054µg/fly, 2.85µg/g and 0.064µg/cm^2, respectively. In addition, Kaufman, *et al.,* (2006 they showed susceptibility of Imidacloprid to laboratory strain and field population of *M. domestica*, that collected from the United States, their LC_{50} were 30 ppm for each other. Further more, these results agreed with Asid, *et al.,* (2015)**,** who reported that Cypermethrin against laboratory and field strains, gave LD_{50} 0.0223 and 0.0645 ppm, respectively.

Table (2). The LC_{50}, LC_{90} (ppm) and slope values at 24h post treatment of Cypermethrin, Imidacloprid and Spinosad against laboratory strain of *M. domestica* adult.

Insecticides	LC_{50}	Confidence Level		LC_{90}	Confidence Level		Slope ±SE*
		Lower	Upper		Lower	Upper	
Cypermethrin	208.75	176.22	253.46	957.65	668.89	1660.23	1.94 ±0.23
Imidacloprid	238.3	204.93	282.03	809.65	590.78	1373	2.41 ±0.33
Spinosad	86.05	72.39	100.19	302.93	229.1	481.99	2.34 ±0.33

*SE = Standard Error

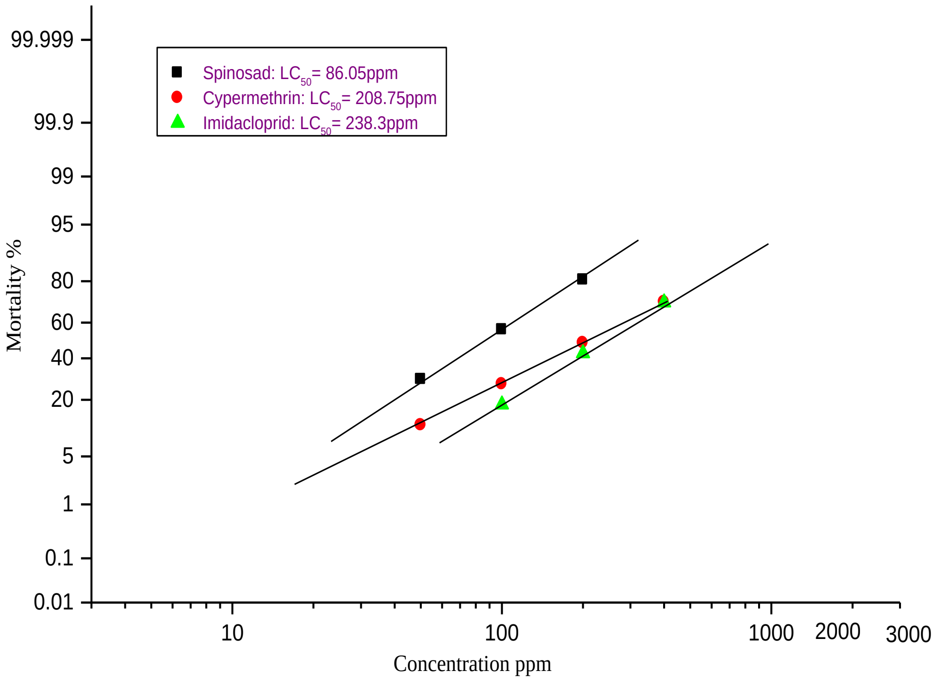

Fig (2) Toxicity Lines of the tested insecticides against the laboratory strain of *M.domestica* adults

Effect of binary mixtures

The interaction effects among the tested insecticides depended on the type of insecticide used, ratios and strains. The LC_{25} of Cypermethrin, Imidacloprid and Spinosad were used to make the binary insecticide combinations, where calculated as 89.5, 125 and 41ppm, respectively against the laboratory strain.

Data in (Table 3) show the effect of the insecticide mixtures against the laboratory strain. The mixture of Cypermethrin+ Imidacloprid showed potentiation at the ratios of 1:1, 1:2 and 2:1 against adults *M. domestica* and it exhibted high potentiation in the laboratory strain at the ratio of 1:1, where the co-toxicity factor was 46. This could be because 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 at the ratio of 1:1, recording 66. In addition, it gave a potentiation in 1:2 and 2:1 ratios, where co-toxicity factors were 24 and 21.3, respectively. This is useful in the control of *M. domestica*. The toxicity of pyrethroids could be enhanced by the addition of new insecticides like Emamectin benzoate, Fipronil and Spinosad. According to this, one toxicant in the mixture interferes with the metabolic detoxification of the other toxicant, Khan, *et al.*, (2013) who demonstrated that the mixture of Cypermethrin+Emamectin gave a synergistic action. These results conversely agree with Abbas, *et al*., (2015) who 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 *S. oryzae.*

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. Also, the mixture containing Fipronil and Acetamiprid had an additive effect on *M. domestica* (Levchenko, *et al*., 2018).

Table (3) Effect of binary mixtures of Cypermethrin, Imidacloprid and Spinosad at the LC_{25} level against *M. domestica.*

Mixing ratio	Co-toxicity factor		
	Cypermethrin+Imidacloprid	Cypermethrin+Spinosad	Imidacloprid+Spinosad
1 : 1	46 P*	66 P	10 Ad**
1 : 2	29 p	24 P	4 Ad
2 : 1	24 P	21.3 p	2.7 Ad

*P. Potentiation effect **Ad. Additive effect

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ميكانيكية مقاومة الذبابة المنزلية: ١- التأثير السام للسيبرمثرين، الايميداكلوبريد والسبينوسات ضد اليرقات والحشرات الكاملة للسلالة المعملية

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الملخص العربى

يعتبر الذبابة المنزلية من الحشرات الناقلة للامراض حيث ينقل اكثر من ١٠٠ مسبب مرضى للانسان والحيوان وتمتلك قدرة عالية على تطور المقاومة للعديد من المبيدات. هذة الدراسة قيمت التأثير السام لمبيدات السيبرمثرين، الايميدكلوبريد والسبينوسات، بالاضافة الى كفاءة الخلط بينهم تحت الظروف المعملية ضد اليرقات والحشرات الكاملة للذبابة المنزلية فى السلالة المعملية. قيمة التاثير النصفى السام للثلاث مبيدات كانت ٥٧٩,٣٠، ٤١٥,٤٦ و١٦,٣٢ جزء فى المليون على التوالى، ضد اليرقات بعد ٤٨ ساعة من المعاملة. بينما ضد الحشرات الكاملة بعد ٢٤ ساعة، كانت قيمة التاثير النصفى السام٢٠٨,٧٥ ٢٣٨,٣ و ٨٦,٠٥ جزء فى المليون على التوالى. ايضا هذة الدراسة اوضحت كفاءة المخاليط المزدوجة لهذه المبيدات، حيث اظهرت مخاليط السيبرمثرين + الايميداكلوبريد والسيبرمثرين + السبينوسات تأثير تقوية على معدلات ١:١، ١:٢ و ٢:١. من الناحية الاخرى، اظهر مخلوط الايميداكلوبريد + السبينوساد تأثير اضافة على كل المعدلات.