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Investigation of the Lethal Impact of Some Novel Pesticides on House Fly, *Musca domestica* L.

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ABSTRACT

The house fly, *M. domestica* L., is a nuisance insect pest that transmits many pathogens. In this study, four pesticides belong to four different groups “Neonicotinoid (Imidacloprid /Kita Pride® 35% SC), Pyrethroid (Beta-cyfluthrin /Blendo spicial® 10% EW), Organophosphate (Primiphos-methyl /Primi one® 50% EC) and Phenylpyrazole (Fipronil /EG-Fipro® 5% SC)” were evaluated for controlling the house fly. Toxicologically, the result showed that the LC50 of fipronil is 0.0064 mg/kg, followed by Imidacloprid 0.0033 mg/kg, then Beta cyfluthrin and primiphos-methyl 0.0002 and 0.00017mg/kg, respectively. Bio-chemically, the insecticides used affected the total protein content and the highest effect was for Beta-cyfluthrin (54.53±1.34, 41.33±1.26mg/g.b.wt) in adult and larvae, respectively. It also affected the activity of some enzymes (Acetylcholinesterase, Glutathione S-transferase and Non-specific esterase) compared to the control. Therefore, Primiphos-methyl was the most toxic of the other insecticide formulations in the case of adult insects, while fipronil was the most toxic in the case of larvae. And the highest effects on enzyme activity were for beta-cyfluthrin and fipronil, both in larvae and adults.

INTRODUCTION

The order Diptera presents an array of insects that more than any other group pose the greatest challenge to human and veterinary health as vectors of diseases, one such insect, which shares a close ecological niche with man is the house fly *M. domestica* L. (Mansour *et al.*, 2012), is a common insect widely distributed at waste sites such as livestock farms and garbage dumps (Li *et al.*, 2018).

In addition to being a nuisance pest, it is a carrier of over 100 pathogenic organisms including organisms for diseases, such as typhoid, cholera, bacillary dysentery, tuberculosis, anthrax, ophthalmia neonatorum and infantile diarrhea as well as parasitic worms (Fotedar *et al.*, 1992; Sasaki *et al.*, 2000) from garbage, sewage and other sources of filth and transferred to human food either mechanically from contaminated external body parts or after consumption by houseflies through vomiting and defecation while feeding on food (Tilak *et al.*, 2010). To overcome this problem, chemical control has been resorted to with several insecticides such as, Neonicotinoid “imidacloprid” (White *et al.*, 2007; Memmi, 2010; Murillo *et al.*, 2015), Pyrethroid “beta-cyfluthrin” (Burgess *et al.*,

2020), Organophosphorus “pirimiphos-methyl” (Xu *et al.*, 2021; Alzabib *et al.*, 2023) and Phenyl-pyrazole “fipronil” (Levchenko and Silivanova 2019; Levchenko *et al.*, 2019; Levchenko *et al.*, 2020; Khan, 2020; Shumilova *et al.*, 2021; Kinareikina and Silivanova, 2023).

Acetylcholinesterase (AChE) terminates nerve impulses at cholinergic synapses by degrading the neurotransmitter acetylcholine (Trevor *et al.*, 1978). Quantitative and qualitative changes in AChE are usually associated with insecticide resistance (Fournier *et al.*, 1992). On the other hand, the hydrolysis of insecticides by esterase is considered a biochemical mechanism for developing insecticide resistance (Li *et al.*, 2007; Zhang *et al.*, 2020; Gong *et al.*, 2022). In addition, glutathione-S-transferase contributes to protecting insects against insecticides (Li *et al.*, 2007), and glutathione-S-transferase activities may not differ in insecticide-resistant insects (Low *et al.*, 2013; Amelia-Yap *et al.*, 2019), or increase compared to insecticide-sensitive insects (Low *et al.*, 2013; Aponte *et al.*, 2019). On the other hand, the impact of pesticides on the total protein content in house flies (*M. domestica*) is significant, with variations observed across different generations (Abdel-Haleem *et al.*, 2018).

The current study investigated the lethal impact of four insecticide formulations on the laboratory strain of house fly adults and larvae, and also its effect on some biochemical aspects (Acetylcholinesterase, Glutathione S-transferase and Non-specific esterase).

MATERIALS AND METHODS

1-Insects Rearing:

A laboratory strain of the house fly *M. domestica* was obtained from the Research Institute of Medical Entomology Cairo, Egypt (30°02'51.3"N; 31°12'44.4"E), using the entomological cage (50×50×50 cm.) to the rearing of house fly (Ahmed *et al.*, 2004), it was covered with mesh screen with cloth sleeve opening at the front, and provided with saturated cotton by powdered milk dissolved in water, at same Institute at 28-30±1°C, and 55-60% relative humidity. After laid the egg by the female house fly was transfer the cotton on artificial diet (made up 200g wheat bran and 150ml distilled water) was adopted to provide the adults of the house fly to use for running bioassay tests.

2-Pesticides:

Four insecticide formulations were used to study their lethal impact on adult house flies.

Table 1: List of insecticide formulations used in the experiment

Trade name	Common name	Formulation	Group
Kita Pride®	Imidacloprid	SC 35%	Neonicotinoid
Blendo spicial®	Beta-cyfluthrin	EW 10%	Pyrethroid
Primi one®	Primiphos-methyl	EC 50%	Organophosphate
EG-Fipro®	Fipronil	SC 5%	Phenylpyrazole

Those insecticide formulations were obtained from the Research Institute of Medical Entomology Cairo, Egypt.

3-Larvae Bioassay (residual film technique):

Larval A larval bioassay was performed according to (Chere *et al.*, 2018) to determine the impact of different doses of pesticides on 3rd instar larvae of the house fly, Petri dishes (Fig. 1.b.) were prepared by washing and sterilizing them in a sterilization oven for 30 minutes at 80° C. The dishes were numbered before use with an adhesive tape containing the name of the insecticide, its concentration, and the repeat number. The filter

paper was saturated with 1 ml of ethanol or water was to the control dishes, and 1 ml of the pesticide formulation after dilution, either with alcohol with Primiphos-methyl and Beta-cyfluthrin or distilled water with Imidacloprid and Fipronil, then it was left to dry.

The filter paper was placed in Petri dishes and then larvae were added (n=10), after that the dishes were left at room temperature for 24 hours and the number of dead or live larvae was to calculate the mortality percentage by the following:

$$\%mortality = \frac{\text{number of treated flies that died}}{\text{total number of control flies}} \times 100$$

The observed mortality in insecticide-exposed flies must be corrected using Abbott's formula:

$$\text{Corrected treatment mortality (\%)} = \frac{(\% \text{ treatment mortality} - \% \text{ control mortality})}{100 - \% \text{ control mortality}} \times 100$$

4-Adult Bioassay (CDC bioassay technique):

Recording to WHO and CDC protocol (CDC, 25 March 2022), the CDC bottles (Fig. 1.a.) were washed in warm soapy water and rinsed, then placed in an oven (50°C) for 15-20 minutes to dry completely and using adhesive tape the bottles are numbered before use, it has the name of the insecticide and its concentration. 1 ml of acetone or ethanol or distilled water was added to the control bottles, and 1 ml of the pesticide formulation after dilution, either with alcohol with Primiphos-methyl and Beta-cyfluthrin or distilled water with Imidacloprid and Fipronil. The bottles were covered with their lids, then they were rotated on the sides to cover all the inner sides with the pesticide, and then they were tilted toward the lid to cover the inner part of the lid with the pesticide, after that, the lid is opened, and the bottles are left to dry. Using an aspirator, 15-30 insects obtained from diet A were introduced into the control and test bottles (exact number of insects does not matter). The bottles were examined at time 0, and the number of dead flies was counted. If dead flies were found at time 0, they were excluded. After that, record how many insects are dead or alive every 15 min. until all are dead or up to 2hours, to calculate the mortality percentage as previously described.

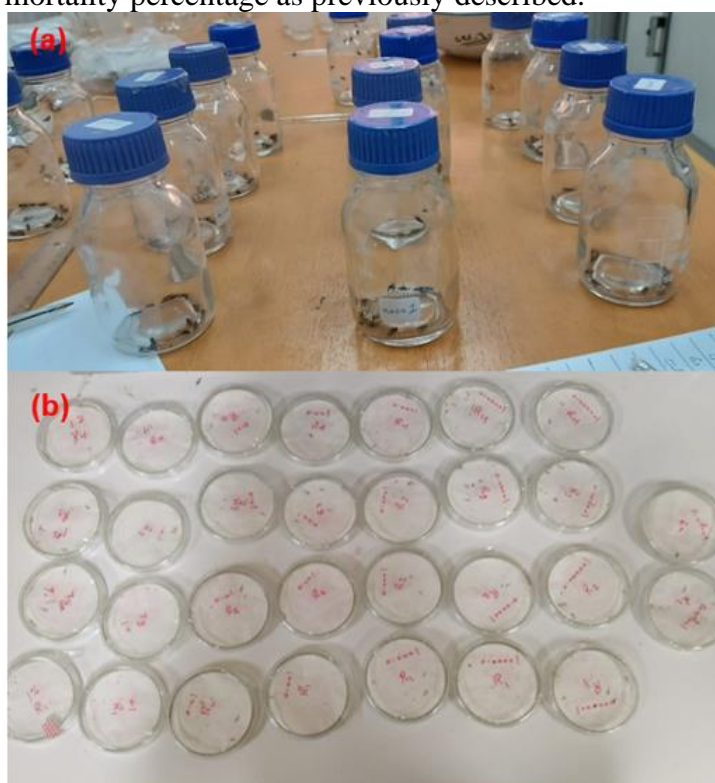


Fig. 1. CDC pottles which are used for adult's bioassay technique (a) and Petri dishes which are used for the larvae bioassay technique (b).

5-Biochemicals Analysis:

5.1-Enzymes Activity:

- **AchE:** AchE (acetylcholinesterase) activity was measured according to the method described by (Simpson *et al.*, 1964), using acetylcholine bromide (AchBr) as substrate.
- **GST:** Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione could be detected as described by the method of (Habig *et al.*, 1974).
- **Non-specific esterases:** α -esterases and β -esterases were determined according to Van Asperen (1962) using α -naphthyl acetate or β -naphthyl acetate as substrates, respectively.

5.2.Total Protein:

Total proteins were determined by the method of Bradford (1976).

6-Statistical Analysis:

The toxicity or efficacy of the insecticide formulations used was determined by calculating the LC₅₀, 90 and 95 (the concentration required for the death of 50, 90 and 95% of insects) using a computerized probability analysis, SAS SOFTWARE (Rodriguez, 2011). Biochemical determinations were pooled from triplicate determinations. The results were analyzed by one-way analysis of variance (ANOVA) using costate statistical software (cohort software, Berkeley). When the ANOVA statistics were significant ($P > 0.05$), means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

1-Lethal Impact of Insecticide Formulation on Larvae and Adults' Stages:

The LC value is widely used to assess toxicity. The result in Table (2), showed that the LC₅₀ and LC₉₀ of fipronil are (0.0061 and 0.1043mg/kg), followed by Imidacloprid (0.0028 and 0.1724mg/kg), then Beta cyfluthrin (0.0002 and 0.0023mg/kg) and pirimiphos-methyl (0.0002 and 0.0112mg/kg), respectively. Regarding larvae, the LC₅₀ and 90 were (0.001 and 0.9084mg/kg) for Beta-cyfluthrin, Imidacloprid (0.0001 and 0.5181mg/kg), Primiphos-methyl (0 and 2.128mg/kg) and Fipronil (0.0001 and 0.3546mg/kg), respectively.

Those results indicated that the pirimiphos-methyl was more effective than the other insecticide formulations on the larvae and adults of house flies. In the same trend, results of this study are consistent with (Asid *et al.*, 2017 & Subekti *et al.*, 2024) they found that the Pirimiphos methyl insecticide was very toxic to flies and very effective in controlling larvae and adults of house flies. In some other studies, it have shown high mortality rates in numerous insect species (Stevens, 1992 & Agossa *et al.*, 2014). It has been reported to induce over 80% mortality in treated populations (Agossa *et al.*, 2014 & Tchicaya *et al.*, 2014). While environmentally, pirimiphos-methyl exhibited a strong residual effect, maintaining its insecticidal properties over extended periods, which is crucial for controlling house flies' populations, particularly in environments where resistance to other insecticides is prevalent (Tchicaya *et al.*, 2014).

Table 2: Toxicity data for the tested insecticides against laboratory strain of the larvae and adults' stage of *Musca domestica*, as projected after the duration of 2 hours exposure times by using CDC bioassay technique for adults, and 24 hours exposure times by using residual film technique for larvae:

Insecticide	LC ₅₀ (Low-Up.)	LC ₉₀ (Low-Up.)	LC ₉₅ (Low-Up.)	Slope ±SE	Chi-square	P-value	r (0.8780)	d.f.
<i>Larval stage</i>								
Beta-cyfluthrin 10%	0.001 (0.0003-0.0033)	0.9084 (0.2718-8.8168)	6.203 (1.6142-93.5875)	0.4354 ±0.0301	11.5168	0.042	0.9654	7
Imidaclopride 35%	0.0001 (0-0.0001)	0.5181 (0.1512-2.7057)	6.9686 (1.4645-59.0034)	0.3219 ±0.0280	3.0241	0.0696 3	0.9915	7
Primiphos-methyl 50 %	0 (0-0.0001)	2.128 (0.3282-36.0566)	52.6867 (4.7509-2145.0326)	0.2607 ±0.0298	4.0752	0.3959	0.9764	6
Fipronil 5%	0.0001 (0-0.0004)	0.3546 (0.1026-5.7676)	3.7661 (0.8539-106.6465)	0.3666 ±0.0287	11.5664	0.0412	0.9615	7
<i>Adult stage</i>								
Beta-cyfluthrin 10%	0.0002 (0.0001-0.0002)	0.0023 (0.0014-0.004)	0.0048 (0.0028-0.0096)	1.1078 ±0.0876	3.7850	0.2856	0.9687	5
Imidaclopride 35%	0.0028 (0.0019-0.0044)	0.1724 (0.0843-0.4368)	0.5522 (0.2366-1.6861)	0.7187 ±0.0557	3.5352	0.3162	0.9891	5
Primiphos-methyl 50 %	0.0002	0.0112	0.0366	0.7075 ±0.0677	33.3446	0.0000	0.9080	4
Fipronil 5%	0.0061	0.1043	0.2333	1.0393 ±0.0763	27.0396	0.0000	0.8864	5

2-Biochemical Analysis:

2.1-Total Protein:

Regarding adults' data presented in Table (3), showed the highest content of protein was in insects not treated with pesticides (control group) of $1018.00 \pm 17.09 \text{ mg/g}$, while the protein content decreased in the groups treated with the tested insecticide formulations of 535.00 ± 11.36 , 211.00 ± 4.58 , 81.70 ± 3.75 and $54.53 \pm 1.34 \text{ mg/g}$ in Primiphos-methyl, Fipronil, Imidaclopride and Beta-cyfluthrin, respectively. While in the larvae the protein contents were $195.33 \pm 4.51 \text{ mg/g}$ in the control group, $239.67 \pm 8.33 \text{ mg/g}$ in Primiphos-methyl, $166.00 \pm 4.58 \text{ mg/g}$ Imidaclopride, $41.33 \pm 1.26 \text{ mg/g}$ in Beta-cyfluthrin and $33.77 \pm 2.80 \text{ mg/g}$ in Fipronil.

The impact of pesticides on the total protein content in house flies (*M. domestica*) is significant. Exposure to insecticides like imidacloprid reduces total protein levels in adult house flies (Abdel-Haleem *et al.*, 2018). Also, found resulting for treated with imidacloprid to lower protein content (Abdel-Haleem *et al.*, 2018). Additionally, sublethal concentrations of fipronil also affected enzyme activities, suggesting biochemical changes that could correlate with protein content alterations (Kinareikina & Silivanova, 2023).

2.2-Acetylcholinesterase (AChE):

AChE is crucial for terminating nerve impulses by hydrolyzing the neurotransmitter acetylcholine at cholinergic synapses. In adult insects and larvae **table (3)**, the highest activity of AChE was in insects not treated with insecticides, while the activity decreased in groups treated with the tested insecticide preparations. In the larval stage, AChE least activity was in the larvae treated with fipronil ($34.70 \pm 1.91 \mu\text{g AchBr}/\text{min/g.b.wt}$), while in the adult insects, its last activity was in the larvae treated with Beta-cyfluthrin ($57.20 \pm 0.82 \mu\text{g AchBr}/\text{min/g.b.wt}$).

Research indicates that these insecticides inhibit Ca-ATPase activity in susceptible strains, suggesting a direct effect on the nervous system, while resistant strains show lower sensitivity to this inhibition (Yun-zhuan *et al.*, 2001). Furthermore, the interaction of pyrethroids with nicotinic acetylcholine receptors suggests that their action may extend beyond AChE inhibition, impacting synaptic transmission and overall motor

function in these insects (Abbassy *et al.*, 1983 & Bloomquist and Miller, 1985). On the other hand, Exposure to fipronil at sublethal concentrations led to a notable decrease in AChE activity in female house flies. This suggests that even sublethal doses can impact enzymatic functions and potentially contribute to resistance development over time (Kinareikina and Silivanova, 2023).

2.3. Glutathione S-transferase:

Glutathione S-transferases (GSTs) play a crucial role in detoxifying harmful compounds. The GSTs activity was lower in insects treated with insecticides formulation compared to untreated insects (control group) Table (3). The activity of GSTs was lower in adults treated with Imidaclopride (58.10 ± 2.01 mmol/ min/g.b.wt), while the activity was lower in larvae treated with fipronil (25.50 ± 3.04 mmol/ min/g.b.wt).

Exposure to imidacloprid leads to increased GST activity in house flies, with studies indicating a 15- to 237-fold increase in gene expression of detoxification enzymes in resistant strains (Farooq *et al.*, 2018). In another study, imidacloprid treatment resulted in a significant up-regulation of GST mRNA levels, suggesting enhanced detoxification capacity in response to the insecticide (Sillapawattana & Schäffer, 2017). On the other hand, exposure to insecticides like fipronil leads to altered GST activity, particularly in larvae (Kinareikina and Silivanova, 2023). In other study, Exposure to fipronil has been shown to induce GST activity in house flies (Miao and Minsheng, 2011 & Monteiro *et al.*, 2019).

2.4. Non-Specific Esterase (α and β esterases):

The activity of α - and β -esterases in house flies (*M. domestica*) is significantly influenced by exposure to various insecticides. These enzymes play a crucial role in the detoxification process, helping insects metabolize and resist the effects of harmful chemicals.

In Table (3), the most effective insecticide formulation on α -esterases and β -esterases enzymes activity in both larvae and adult insects was fipronil, compared to the control, which had high Non-specific esterase enzymes activity.

Fipronil is noted to be highly toxic to house flies. Also, Studies have demonstrated that exposure to fipronil can lead to alterations in both α - and β -esterase activities (Levchenko *et al.*, 2018). Also, one study reported a decrease in CarE activity by approximately 33.20% in female house flies after exposure to fipronil at a concentration of 0.001% (Kinareikina and Silivanova, 2023). This suggests that fipronil may impair the detoxification capabilities of house flies by inhibiting key enzymes involved in metabolizing xenobiotics.

Table 3: The effect of tested insecticides on some biochemical parameters (Acetylcholinesterase, Glutathione S-transferase and Non-specific esterase) for treated house flies' larvae and adults:

Parameters	Total protein (mg/g.b.wt)	AchE (ug AchBr/ min/g.b.wt)	GST (mmol sub. conjugated/ min/g.b.wt)	Non-specific esterases	
				α -esterases (ug α -naphthol/ min/g.b.wt)	β -esterases (ug β -naphthol/ min/g.b.wt)
<i>Larval stage</i>					
B-cyfluthrin	41.33±1.26 ^d	36.87±2.01 ^d	29.37±1.42 ^d	40.07±0.51 ^d	46.07±2.06 ^d
Fipronil	33.77±2.80 ^d	34.70±1.91 ^d	25.50±3.04 ^d	16.60±1.31 ^e	40.23±3.16 ^d
Imidaclopride	166.00±4.58 ^c	139.67±1.53 ^c	151.33±3.21 ^c	133.33±4.04 ^c	143.00±8.89 ^c
Primiphos	239.67±8.33 ^a	240.67±9.02 ^a	203.00±8.54 ^a	161.33±3.21 ^b	408.33±23.63 ^a
Control	195.33±4.51 ^b	179.67±7.23 ^b	188.33±7.64 ^b	169.00±5.57 ^a	206.00±12.49 ^b
LSD at 0.05	8.92	9.75	10.06	6.28	23.12
<i>Adult stage</i>					
B-cyfluthrin	54.53±1.34 ^e	57.20±0.82 ^e	66.33±0.76 ^d	61.27±0.87 ^c	61.37±3.27 ^d
Fipronil	211.00±4.58 ^c	206.33±4.16 ^c	179.00±7.81 ^c	49.67±5.69 ^c	203.33±4.16 ^c
Imidaclopride	81.70±3.75 ^d	90.17±2.36 ^d	58.10±2.01 ^d	52.83±1.76 ^c	54.50±1.80 ^d
Primiphos	535.00±11.36 ^b	455.67±18.18 ^b	548.00±15.72 ^b	196.33±7.23 ^b	401.00±13.53 ^b
Control	1018.00±17.09 ^a	847.00±13.89 ^a	956.33±34.93 ^a	480.33±13.05 ^a	704.67±18.45 ^a
LSD at 0.05	17.41	19.03	31.85	13.09	19.16

a, b & c: There is no significant difference ($P>0.05$) between any two means for each stage, within the same column have the same superscript letter.

Conclusion:

This study highlighted the differences between the LC values of the four insecticide treatments. It showed that primiphos-methyl was more effective on adults and fipronil on larvae than the other insecticide formulations. However, fipronil had the highest effect on enzyme activity, whether on larvae or adults. Therefore, we recommend using one of the three insecticides (primiphos-methyl, Beta-cyfluthrin and fipronil) due to their effectiveness in controlling house fly larvae and adults.

Declarations

Ethical Approval: Not applicable

Competing Interests: The authors declare no conflicts of interest.

Authors Contributions: All authors contributed to methodology, validation, investigation, resources and data curation, manuscript writing, review and editing.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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