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TOXICOLOGICAL AND BIOLOGICAL INDICES OF THE HOUSE FLY, *MUSCA DOMESTICA* AFTER CSI'S TREATMENTS: LUFENURON, FLUFENOXURON AND HEXAFLUMURON

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Abstract

The house fly, *Musca domestica* transmits many diseases to humans, so that controlling it without side effects on human health is extremely important. Therefore, the current study aimed to compare the toxicity of tested chitin synthesis inhibitors (CSIs) lufenuron, flufenoxron, and hexflumuron against 3rd instar larvae of the house fly, and to assess the lethality effects by serial concentrations (1000, 500, 250 & 125ppm) of each tested compound on the developmental parameters and growth indices of the immature stages of *M. domestica*. The least toxicity values of sub-lethal concentrations (LC_{25}, LC_{50} & LC_{75}) displayed for lufenuron (158.23, 332.46 & 698.56, respectively). Also, hexaflumuron scored (64.06ppm at LC_{25}), followed by flufenoxuron (140.95 & 283.62ppm at LC_{50} & LC_{75}, respectively). Flufenoxuron was the most toxic, followed by hexaflumuron and lufenuron. CSIs showed a toxic efficiency on 3rd instar larvae of *M. domestica* by decreasing developmental and growth indices rates at higher concentrations of each one. Flufenoxuron exhibited a marked significant decrease in larval development and growth index rates (15.50 & 2.71% at 1000ppm, respectively), highest water loss (69.79%) compared to hexaflumuron and lufenuron. Flufenoxuron was the most toxic one against pupae as compared to others, it prolonged pupal duration (14.71day) and induced pupal water loss (53.77%), its pupation, developmental and weight rate reduced (17.5 & 6.8%, & 53.77mg respectively). But, growth index did not cause mortality with the highest concentration. Adult emergence displayed non-adult emergence and a high percentage of the malformation rate at 1000ppm when treated with flufenoxuron compared to other compounds.

Keywords *M. domestica*, lufenuron, flufenoxuron, hexaflumuron, larvicidal, pupation, development, growth.

Introduction

The house fly, *M. domestica* (Diptera: Muscidae) is likely the most all-inclusive prevalence and broadly linked with man worldwide (Wiegmann *et al,* 2003; Nazni *et al,* 2005). The houseflies are mechanical vectors of zoonotic bacteria, fungi, viruses, helminthes and protozoa (Macovei *et al,* 2008). They play a role in botulism (El-Bahnasawy *et al,* 2014), nosocomial myiasis (Morsy, 2014) and mechanical transmission of many zoonotic pathogens (Elnakib *et al*, 2018).

Many insecticides were used against *M. domestica*, but with precision to avoid insect resistance (Khan *et al,* 2017). They showed that resistance to most traditional insecticides so, alternatives and environmentally friendly acceptable ones were indicated (Assar *et al,* 2010). There was the bioinsecticides such as insect growth regulators to avoid the hazards of chemical insecticides on man, animals and their environment (Atwa *et al,* 2010). Insect growth-regulators (IGRs) are insect developmental inhibitor prevent regular metamorphosis to adults (Muhammad, 2019). The CSIs are chemical compounds that change the insect's growth with minimal environmental damage. They reduce the insect's ability to produce a new exoskeleton after molting, leaving them without protection and thus reduced survival chances (Yankanchi and Gadache, 2010). The benzoylphenylurea (BPU), Diflubenzuron (Dimilin) a potent compound used against lepidopterous and dipterous larvae (Miyamoto *et al,* 1993). Dimilin and its derivatives were effective without harm to man, animals and beneficial insects (Msangi *et al,* 2011). The mo-

des of action were suggested for BPUs, by blocking the chitin binding into cuticular proteins causing inhibition of cuticle deposition; prevent chitin formation induced by protease inhibition, and activation of phenoloxidases and chitinases (Saenz *et al*, 2006), and connected with chitin catabolism (Khajepour *et al*, 2012).

Lufenuron has incredible effects on the growth and development against various harmful insect species, as the *Drosophila melanogaster* (Bogwitz *et al*, 2005); *M. domestica* (Abo El-Mahasen, 2010; Guneidy *et al*, 2011); *Spodoptera littoralis* (Ivan *et al,* 2011; Essam *et al,* 2014); *Pectinophora gossypiella* (Sabry and Abdou, 2016); *Helicoverpa armigra* (Gogi *et al*, 2006) and *Tribolium castaneum* (Muhammad, 2019). Also, flufenoxuron has been used against many insect species, as *Lobesia botrana* (Bressan *et al*, 2002); *Spodoptera littoralis* larvae (Essam *et al,* 2014) and *Agrotis ipsilon* (Shaurub *et al,* 2018). Hexaflumuron was used to control the subterranean termites (Su and Scheffrahn, 1996); *M. domestica* larvae (Abo-El-Mahasen, 2010); *Ephestia figulilella* (Khajepour *et al*, 2012); *Pectinophora gossypiella* (Kandil *et al,* 2013) and *Helicoverpa armigera* (Mohsen *et al,* 2015).

The present study aimed to evaluate the toxicity of CSIs lufenuron, flufenoxron and hexaflumuron against the 3rd instar larvae of *Musca domestica,* and to assess the lethal effects at different concentrations of on developmental parameters and growth indices of its immature stages.

Materials and Methods

Insect rearing: The house fly, *M. domestica* was obtained from Medical Insect Research Institute, Dokki, Giza. Both sexes were reared in wire cages with wooden frames (30x30x30cm) at 27±2ºC, 60-70% RH, and constant light (Amano, 1985). Adults were fed on 10% sucrose solution in cotton pads. The cotton pads soaked in milk powder dissolved in water put in Petri-dishes for oviposition. Eggs were collected and transferred to larval artificial medium consisted of dry milk powder (30gm), yeast (20gm), wheat bran (300gm) and distilled water (300ml) according to Bell *et al.* (2010). The newly hatched larvae were grown on the same artificial diet in glass jars until pupation. Once pupae appeared, they were transferred by a fine blunt forceps into cages for adult emergence. *M. domestica* were reared for several generations (Elkattan *et al*, 2011).

Chemicals and application: The chemicals were kindly supplied by the Laboratory of Insecticides, Plant Protection Research Institute, Dokki, Giza. They were 1- Lufenuron (Match 10% EC-CAS No. CG A-184699), with formula: N- [2,5-dichlor- o-4-(1,1,2, 3, 3hexa-fluoro-propoxyl) phenyl) amino 2,6 diflubenzamid (CA)], 2- Flufenoxuron (Cascade 10% ECC- AS No.1014-63-69-8), with formula N- [4-2-chloro-4-(trifuloromethyl) phenoxy]-2-fluorophenyl] amino] carbonyl]-2, 6-difluorobenzamide, and 3- Hexaflumuron (Consult 10%, ECCAS No. 86479), with formula: N- (3,5-dichlo-ro-4-(1,1,2,2 tetrafluoroethoxy) phenyl]3-(2,6 difluorobenzoyl) urea.

Each compound was dissolved in distilled water to prepare four concentrations (1000, 500, 250 & 125ppm). Larval artificial diet was mixed with different concentrations of each compound. Forty individuals of newly 3rd instar larvae of *M. domestica* were immediately put into glass jars contained the treated media. Four replicates were used for each concentration, while control larvae fed on an artificial diet mixed with water. Dead larvae & pupae were removed daily until adult emerged. Fresh body weight was daily scored by a digital balance (Gadever). Larval and pupal durations were daily recorded.

Mortalities and lethal effects: Mortality rate was assessed by using the Briggs's formula (Jepson and Thacker, 1990) as follows: (mortalities at end of each stage/total insects no. at beginning of same stage)x100. Mortalities were corrected using Abbott's formula (Abbott, 1925). Sub-lethal concentration values of tested CSIs calculated according to its corrected mortality.

Growth & developmental parameters: Developmental rate was calculated using Richard's equation (1957) (100/mean duration in days). Water loss calculated by the equation: [(initial weight-final weight) /initial weight] x100.

Pupation rate was evaluated by (number of pupae/tested larvae) x100. Adult emergence was counted and estimated by the equation: (Adults no. by Pupae no.) x100. Larval growth index calculated as: (pupation ratio/larval duration). Pupal growth index calculated as: (adult emergence/larval & pupal duration) after (Pretorius, 1976). Survival potential calculated as: (adults normal no./ emerged adults no.) x100. Metamorphosis changed and individual deformations were calculated in %.

Statistical analysis: Data was tabulated and analyzed using (SPSS 19.0) Software. Data entered as (M±SD), differences among CSIs treatments or concentrations were analyzed by one-way ANOVA, followed by Fisher's (LSD) to determine significant differences. A chi-square (x^2) statistic was used to compare the ratios of differences. P-value was considered significant when less than 0.05/ 0.01 (Steel and Torrie, 1984). Sub-lethal concentration values and corresponding regression lines of tested CSIs were evaluated by Probit analysis (Finney, 1978).

Results

The larvae were treated with 1000, 500, 250 & 125ppm with tested CSIs, after 24, 48, 72 & 96hr and then calculated larval, pupal, adult, general and corrected mortalities rate (Tab. 1).

Table 1: Toxicity effects of CSIs compounds after treatment newly moulted 3rd instar larvae of *M. domestica*.

Tested CSIs	Conc. (ppm)	Larval mortal. (%) After:					Pupal (mortal. %)	Adult (mortal. %)	General (mortal. %)	Corrected (mortal. %)
		24 h	48 h	72 h	96 h	Total				
Lufenuron	1000	32.5	30.0	12.5	--	75.00	40.00	--	85.00	82.86
	500	25.0	25.0	--	12.5	62.5	40.00	--	77.50	74.29
	250	--	5.00	20.0	--	25.0	13.33	--	35.00	25.71
	125	--	12.5	--	--	12.5	14.29	10.00	32.50	22.86
Flufenoxron	1000	82.5	--	--	--	82.5	100.0	--	100.0	100.00
	500	50.0	17.5	--	--	67.5	38.46	37.50	87.50	85.71
	250	10.0	22.5	12.5	--	45.0	36.36	14.29	70.00	65.71
	125	--	7.5	25.0	--	32.5	29.63	10.53	57.50	51.43
Hexaflumuron	1000	--	--	27.5	55.0	82.5	42.86	--	90.00	88.57
	500	--	--	15.0	35.0	50.0	40.00	33.33	80.00	77.14
	250	--	7.50	22.5	--	30.0	35.71	27.78	67.50	62.86
	125	2.50	5.00	22.5	--	30.0	10.71	16.00	47.50	40.00
Control	--	2.50	10.0	--	--	12.5	--	--	12.50	0.00

Conc.: Concentration level (ppm), ppm: parts per million, mortal: mortalities%& h: hour

Total larval mortality rate among 3rd instar larvae gave an increase (82.5% at 1000ppm) for flufenoxruon and hexaflumuron, but it decreased (12.5% at 125ppm) for lufenuron. Pupal mortalities rate showed fatality (100% at 1000ppm) for flufenoxruon (10.71% at 125ppm) for hexaflumuron. Adult mortality was high (37.5% at 500ppm) for flufenoxruon and low rate (10% at 125ppm) for lufenuron. General mortality was fatal (100% at 1000ppm) for flufenoxruon, but lowest one was (32.50% at 125 ppm) for lufenuron.

Table 2: Sub-lethal concentration of CSIs compounds after treating newly moulted 3rd instar larvae of *M. domestica*.

Tested CSIs	Lethality values (ppm)			Slope ± SE
	LC_{25} (Lower - Upper) Limit	LC_{50} (Lower - Upper) Limit	LC_{75} (Lower - Upper) Limit	
Lufenuron	158.23 (99.78 – 250.92)	332.46 (209.64 – 527.23)	698.56 (440.50 – 1107.81)	2.210 ± 0.102
Flufenoxron	70.05 (38.34 – 127.99)	140.95 (77.15 – 257.54)	283.62 (155.29 – 518.20)	1.715 ± 0.134
Hexaflumuron	64.06 (34.63 – 118.49)	170.07 (91.94 – 314.60)	451.55 (244.11 – 835.29)	1.591 ± 0.136

Values in brackets =95% confidence limits for $LC_{25, 50 \& 75}$ values, slope values calculated from probity regression lines, SE= standard error.

Sub-lethal toxicity values (LC_{25}, LC_{50} & LC_{75}) for lufenuron were (158.23, 332.46 & 698.56, respectively). Hexaflumuron score was (64.06ppm at LC_{25}), followed by flufenoxuron (140.95 & 283.62ppm at LC_{50} & LC_{75}, respectively). Flufenoxuron was the most toxic, followed by hexaflumuron and then lufenuron. Toxicity effects on 3rd instar larval duration, developmental rate, weight, water loss and growth index of *M. domestica* post-treated larvae with selected concentrations were recorded (Tab. 3).

Table 3: Effects of CSIs compounds on ultimate larvae of *M. domestica* after treating newly moulted 3rd instar larvae.

Treatments	Conc. (ppm)	Duration (M ±SD)	Develop. (%)	Weight (Mean ±SD)	Water loss (%)	Growth index (%)
Lufenuron	1000	$5.25^{a} \pm 0.95$	19.05	$10.16^{e} \pm 0.25$	56.86	4.76
	500	$4.66^{b} \pm 0.83$	21.46	$11.37^{d} \pm 0.30$	48.27	8.05
	250	$3.22^{c} \pm 0.69$	31.06	$14.72^{c} \pm 0.29$	35.30	23.29
	125	$2.87^{cd} \pm 0.39$	34.84	$17.64^{b} \pm 0.50$	21.70	30.49
	Control	$2.53^{d} \pm 0.35$	39.53	$18.27^{a} \pm 0.45$	19.55	34.58
	F/x^2 value	F/43.48	x^2/15.33	F/885.94	x^2/13.45	x^2/16.60
	P-value	0.000**	0.013*	0.000**	0.022*	0.005**
Flufenoxron	1000	$6.45^{a} \pm 0.80$	15.50	$6.28^{d} \pm 0.14$	69.79	2.71
	500	$5.05^{b} \pm 0.55$	19.80	$7.95^{c} \pm 0.26$	65.40	6.44
	250	$4.20^{c} \pm 0.35$	23.81	$9.87^{b} \pm 0.42$	59.11	13.10
	125	$3.15^{d} \pm 0.44$	31.75	$10.05^{b} \pm 0.33$	54.21	21.43
	Control	$2.53^{d} \pm 0.35$	39.53	$18.27^{a} \pm 0.45$	19.55	34.58
	F/x^2 value	F/147.25	x^2/10.79	F/2910.22	x^2/9.31	x^2/12.19
	P-value	0.000**	0.033*	0.000**	0.035*	0.028*
Hexaflumuron	1000	$5.90^{a} \pm 0.65$	16.95	$8.90^{d} \pm 0.21$	59.32	2.97
	500	$4.75^{b} \pm 0.75$	21.05	$10.93^{c} \pm 0.32$	47.93	10.53
	250	$3.90^{c} \pm 0.42$	25.64	$11.22^{c} \pm 0.57$	52.15	17.95
	125	$3.61^{c} \pm 0.40$	27.70	$13.28^{b} \pm 0.62$	39.44	19.39
	Control	$2.53^{d} \pm 0.35$	39.53	$18.27^{a} \pm 0.45$	19.55	34.58
	F/x^2 value	F/93.11	x^2/16.00	F/871.06	x^2/14.85	x^2/17.37
	P-value	0.000**	0.007**	0.000**	0.015*	0.003**

Duration = days± standard deviation. Weight scaled by (mg) and expressed to final weight. Growth index: Pupation rate/larval duration. No significant differences at $P > 0.05$ when repeating same alphabet above means in each category. Significance=*$P < 0.05$, **$P < 0.01$.

The lufenuron prolonged larval duration registered highly significant increase (5.25 days at 1000ppm) than (2.87 days at 125 ppm) compared to control (2.53 days). Developmental and growth index rates significantly decreased (19.05&4.76% at 1000ppm than 34.84&30.49% at 125ppm respectively). Larval weight was highly significant decreased (10.16mg at 1000 ppm than 17.64 mg at 125ppm) compared to control, which retracted to increase larval water loss by increasing concentration levels. Lufenoxuron showed a high significant decrease in larval development and growth index rates (15.50 &2.71% at 1000ppm than 31.75&21.43%, at 125ppm, respectively). The larval durations gave a highly significant prolongation (6.45 days at 1000ppm than 3.15 days at 125ppm). But, larval weights were highly decreased significantly (6.28mg at 1000ppm than 10.05 mg at 125ppm). Water loss significantly depended on concentrations increasing. Hexaflumuron showed that larval duration prolonged consecutively to ascending concentrations. Developmental and growth index rates caused a significant inhibition (16.95% at 1000ppm than 27.70% at 125ppm). Larval weight was highly significant reduced (8.90 mg at 1000ppm than 13.28mg at 125ppm), Water loss drastically increased by concentrations increasing.

Toxicity effects of CSIs on pupation, its duration, developmental rate, weight, percent of water loss and growth index of *M. domestica* after-treating 3rd instar larvae with selected concentrations were given (Tab. 4).

Table 4: Effects of CSIs compounds on pupal stage of *M. domestica* after treating newly moulted 3rd instar larvae.

Treatments	Conc. (ppm)	Pupation (%)	Duration (M ±SD)	Develop. (%)	Weight (M ±SD)	Water loss (%)	Growth index (%)
Lufenuron	1000	25.00	$8.03^{a} \pm 1.07$	12.45	$8.97^{e} \pm 0.07$	24.30	0.84
	500	37.50	$7.50^{a} \pm 0.92$	13.33	$9.64^{d} \pm 0.10$	19.60	1.40
	250	75.00	$6.15^{b} \pm 0.75$	16.26	$10.35^{c} \pm 0.10$	18.57	4.96
	125	87.50	$5.21^{cd} \pm 0.64$	19.19	$11.19^{b} \pm 0.15$	22.18	6.50
	Control	87.50	$4.86^{d} \pm 0.58$	20.58	$12.41^{a} \pm 0.17$	17.98	10.15
	F/x^2 value	$x^2/15.30$	F/36.99	$x^2/29.05$	F/1072.2	$x^2/31.83$	$x^2/37.36$
	P-value	0.027*	0.000**	0.000**	0.000**	0.000**	0.000**
Flufenoxron	1000	17.50	$14.71^{a} \pm 0.83$	6.80	$3.19^{d} \pm 0.01$	53.77	0.00
	500	32.50	$13.42^{b} \pm 0.79$	7.45	$3.32^{d} \pm 0.03$	58.40	1.64
	250	55.00	$12.92^{b} \pm 0.87$	7.74	$5.25^{c} \pm 0.06$	40.74	3.74
	125	67.50	$8.35^{c} \pm 0.69$	11.98	$7.85^{b} \pm 0.09$	21.26	5.88
	Control	87.50	$4.86^{d} \pm 0.58$	20.58	$12.41^{a} \pm 0.17$	17.98	10.15
	F/x^2 value	$x^2/10.80$	F/454.64	$x^2/17.87$	F/1513.7	$x^2/20.00$	$x^2/25.47$
	P-value	0.032*	0.000**	0.008**	0.000**	0.005**	0.000**
Hexaflumuron	1000	17.50	$11.93^{a} \pm 0.88$	8.38	$3.49^{d} \pm 0.03$	58.40	0.47
	500	50.00	$11.35^{a} \pm 0.90$	8.81	$7.74^{c} \pm 0.06$	20.78	1.62
	250	70.00	$9.20^{b} \pm 0.95$	10.87	$9.85^{b} \pm 0.15$	22.38	2.63
	125	70.00	$7.93^{c} \pm 0.80$	12.61	$10.02^{b} \pm 0.12$	16.43	5.43
	Control	87.50	$4.86^{d} \pm 0.58$	20.58	$12.41^{a} \pm 0.17$	17.98	10.15
	F/x^2 value	$x^2/16.00$	F/162.92	$x^2/24.18$	F/4506.3	$x^2/26.12$	$x^2/23.83$
	P-value	0.025*	0.000**	0.001**	0.000**	0.000**	0.003**

Duration = days± standard deviation. Weight scaled by (mg) and expressed to final weight. Growth index: Growth index: Emergence rate / Total developmental period. No significant differences at $P > 0.05$ when repeating same alphabet above means in each category. Significance = *$P < 0.05$, ** $P < 0.01$.

Pupation rate of 3rd instar larvae treated with lufenuron showed significant inhibition (25.00&37.50% at 1000 & 500ppm) compared to control. Pupal duration exhibited a highly significant elevation (8.03 days at 1000ppm than 5.21 days at 125 & 250pm). Developmental and growth index rates decreased with increasing concentrations compared to control. Pupal weights were highly significantly reduced (8.97mg at 1000ppm than 11.19mg at 125ppm). Water loss increased related to ascending concentrations. Pupal stage was disturbed by flufenoxuron, as pupation and developmental rates showed highest decrease (17.50&6.80%, respectively at 1000ppm than 67.50&11.98 %, respectively at 125 ppm) compared to control. Pupal duration showed a highly significant prolongation (14.71days at 1000ppm than 8.35 days at 125ppm). Pupal weight revealed a significant decrease (3.19mg at 1000ppm than 7.85mg at 125ppm). Growth index caused complete inhibition (0% at 1000ppm), & water loss markedly increased 58.40% at 500ppm. Hexaflumuron highly reduced pupation rate (17.50% at 1000ppm than 70.0% at 125ppm) compared to control. Pupal duration was significantly prolonged (11.93 days at 1000 ppm than 9.20 & 7.93 days at 250 & 125ppm, respectively), but developmental rate showed a significant decrease (8.38% at 1000ppm than 12.61% at 125ppm). Growth index caused high inhibition (0.47% at 1000 ppm than 5.43% at 125ppm), and pupal weight significantly decreased (3.49, 7.74 & 9.85mg at 1000, 500 & 250ppm, respectively). Pupal water displayed increase (58.4% at 1000ppm than 20.78, 22.38& 16.43% at 500, 250 & 125ppm, respectively).

High toxicity effects of CSIs on adult emerged (Fig. 1) for hexaflumuron (75 & 65%, respectively) at 125&250ppm. But, flufenoxuron displayed (0%) at 1000 ppm with impaired metamorphosis. High adult survival potential% manifested (Fig. 2) for lufenuron (67.5&66% at 125&250 ppm, respectively). High malformation rate% displayed (Fig. 3) for flufenoxuron (30% at 1000ppm), and it caused no malformation at 125 & 250ppm and hexaflumuron at 125ppm. Morphogenic abnormalities (Fig. 4) showed various shape larval deformation, pupal, and adult emerged from 3rd larval instar post-treatment with the CSIs different concentrations as compared to

control one.

Larval morphogenic abnormalities were irregular and elongated due to lufenuron treatment (Fig. A). Larva was full darkened, body desiccation and curved shape due to flfenouxron (Fig. D). Larva was swelling with patches on the cuticle due to hexaflumuron treatment (Fig. G) compared to control larva (Fig. J). Pupal abnormalities looked dark and compressed pupa with a larval part at its anterior end due to lufenuron treatment (Fig. B). Pupa treated with flfenouxron looked hard, C-shaped, and small sized body (Fig. E). Pupa treated with hexaflumuron was enlarged and with shrinkage body (Fig. H) compared to control one (Fig. K). Emerged adults treated with lufenuron, flfenouxron and hexaflumuron showed many morphogenic malformations, such as adult completely free but possessed crumpled and incomplete wings and legs formations became dwarfism (Fig. C, F & I) compared to control one (Fig. L).

Discussion

IGRs are helpful because they do not remain long in the habitat due to their instant biodegradation and low toxicity. CSIs are ordinarily classified in IGRs, and hence inhibit moulting, or produce a deficient cuticle (Hammock and Quistad, 1981). These substances are efficient oppressors of development for the whole life cycle of insect pests. They also influence the longevity and peritrophic membrane.

Flufenoxuron, hexaflumuron and lufenuron belonged to same group of IGRs and have the same mode of action, although their effects on same species excessively differed. The current study evaluated the tested CSIs impacts on the larvicidal, pupicidal and adulticidal activities. The present results agreed with other studies, which used many different IGRs (Vazirianzadeh *et al*, 2007; Sulaiman *et al*, 2008; Al Ghamdi *et al*, 2014).

The least toxicity values of the sub-lethal concentrations (LC_{25}, LC_{50} & LC_{75}) displayed for lufenuron. Meanwhile, hexaflumuron scored the highest toxicity at LC_{25}, followed by flufenoxuron at LC_{50} and LC_{75}. As a result, flufenoxuron was the most toxic, followed by hexaflumuron and lufenuron. The present study agreed with Kelly *et al*. (1987) but conflicted with Scott *et al*. (2000). Lethal efficacy of the CSI means variations in the levels of potentiation among the test mixtures that may reflect the differences in their mode of action and the tested sub lethal values (Bakr and Tanani, 2018). The current study exhibited that flufenoxuron was highly toxic efficacy at all concentration levels than hexaflumuron and lufenuron inducing larval, pupal and adult mortality, it caused 100% pupal mortality at 1000ppm. This agreed with Ghoneim *et al*. (1992) who found an initial larval mortality in *Muscina stabulans* due to Bay Sir-8514 treatment. The present results agreed with Abo El-Mahasen *et al*. (2010) who found that hexaflumuron and lufenuron compounds caused 100% larval mortalities. Thus, cyromazine has to be applied in the larvicidal program for suppression of the house fly (Vazirianzadeh *et al*, 2007; Donahue *et al*, 2017). But, Ghoneim *et al*. (2004) found that lufenuron led only to pupal & adult mortalities but not the larvae.

The current study showed that flufenoxuron prolonged the larval duration, followed by hexaflumuron and lufenuron, due to the hormone titers disturbance responsible for normal growth and the pupal stage transformation (Sehnal and Bryant, 1993). Moreover, CSIs have inhibited the final step of chitin biosynthesis pathway, and the precursor unconverted into chitin leading to the prolongation of the developmental duration.

The present study indicated that the tested CSIs reduced the larval weight, growth index and developmental rate of the treated larvae. Meanwhile, the rate of water loss elevated as a result of the toxicity efficacy of the tested CSIs on the larval cuticle caused a disturbance in the cuticle evaporation began to increase water loss that caused reduction in larval weight. Flufenoxuron affected on the pupal stage, followed by hexaflumuron then lufenuron as compared to control. Pupal duration was markedly prolonged; mean-

while, the percent of the pupation rate was slightly regressed by increasing concentration levels in all tested compounds. This agreed with Abo El-Mahasen *et al*. (2010) who proved that percent of pupation was highly decreased compared to the control. Also, the current results indicated that pupal body weight, growth index, and developmental rate decreased, while the percent of water loss markedly increased. The present results agreed with Sabry and Abdou (2016) revealed that housefly larval and pupal development was inhibited after-treatment with diflubenzuron, hexaflumuron and teflubenzuron. The current study showed that the reduction in *M. domestica* pupal weight was a result of the decrease in total water content, as well as due to the lack of proper sclerotization of the newly formed puparium, or evaporation of body fluids that led to decrease pupal weight. These results agreed with Abo El-Mahasen *et al*. (2010) who found that hexaflumuron and lufenuron induced a reduction in the house fly pupal weight. Also, in the current study, a high percentage of adult emergences registered for hexaflumuron at the two low concentration levels, and flufenoxuron displayed (0%) non-adult emergence at 1000ppm that revealed the impaired metamorphosis. The high percentage of adult survival potential of *M. domestica* was registered for lufenuron at the low concentration. This agreed with the different effect of the IGRs dimilin TH (diflubenzuron) on house fly (Kocisova *et al*, 2004); triflumuron and cyromazine (Srinivasan and Amalraj, 2003); methoxyfenozide (Assar and Abo-Shaeshae, 2004); CME and IKI (Ghoneim *et al*, 2004); IKI and novaluron (Cetin *et al*, 2006); cyromazine, flufenoxuron, and chlorfluazuron IKI (Vazirianzadeh *et al*, 2007) and cyromazine (Bell *et al*, 2010; Donahue *et al*, 2017).

The present study showed mark high percent of malformation rate of *M. domestica* displayed by flufenoxuron at 1000ppm. Also, lufenuron showed no malformation at 2 low concentrations and hexaflumuron at 125 ppm. These results agreed with Khalil *et al.* (2010) who found that the CSIs caused an inhibition of facilitated diffusion and active transport of nucleosides and amino acids across cell membranes led to insect morphogenesis. The present results showed that the pre-pupa failed to complete metamorphosis program of pauperism, where; CSIs prevented formation of the new cuticle resulting in the production of molting abnormalities. Elkattan (2011) cleared that the malformation in a pre-pupal stage that appeared as larval-pupal intermediate might be deemed to the inability of treated larvae to liberate themselves from their old cuticle. Also, the present study observed melanization patches on the larval-pupal intermediates cuticle treated with flufenoxron and hexaflumuron, due to their good efficiency on the disorganization of light and dark bands of the muscles or the inhibition of melanin synthesis (Gelbič and Němec, 2001). Also, Darvas *et al.* (1998) found that the molting disorders on the thorax of *Aedes aegypti* like dangling larval exuvium, head capsule slippage failure, and more sclerotization were due to the methoxyfenozide effect. Thus, these larval-pupal intermediates failed to molt into pupa that died. Carton *et al.* (1998) found that the treatment of *S. exigua* larvae with methoxyfenozide directed to induce the premature, larval moulting, existence of a paired head capsule and presence of larval-pupal intermediate.

The present study showed that pupal abnormalities as darkening of the pupa and compressed due to treatment with lufenuron, and pupa treated with flfenouxron was dry C-sha ped with small size. Enlarged and shrinkage body pupa treated with hexaflumuron compared to control pupa. The greatest pupae abnormal was its failure to complete the metamorphosis died inside the puparium or changed to incomplete adult.

The current study showed that adult emerged with curled wings, small body size (dwarfism), and malformed legs. Morphological abnormalities resulted due to lysosomal enzyme risky activity (Josephrajkumar *et al,*

1999). Same deformities were reported with *M. domestica* using other CSIs that induced larval-pupal intermediates, pupae and adults, diflubenzuron, and pyriproxfen (Awad and Mulla, 1984) and methoxyfenozide and pyriproxyfen (Assar and Abo-Shaeshae, 2004). Carton *et al*. (1998) found that methoxyfenozide applied to *S. exigua* larvae led to the wings malformation and emerged adult suffered discarding from pupal exuvium. This agreed with Mansour *et al.* (2011) who reported that different morphological deformation as larval development retardation, pupal emergence failure, and incomplete development of adult's wings which died 12 h after emergence.

Conclusion

The CSIs caused several actions on house fly development, metamorphosis, and morphogenesis. Also, they suppressed the insect inhabitance number of this species, directly via their acute toxic effects or indirectly via their associated effects. The CSIs have a marked target efficacy with great potentiality against *M. domestica* especially flufenoxuron which has amplified efficacy against all its developmental stages. These data can be used for integrated pest management programs. Studies of these compounds as man and environmental friend relationship are a must.

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Explanation of figures

Fig. 1: Toxicity effects of tested CSIs on adult emergence.
Fig. 2: Toxicity effects of tested CSIs on survival potential.
Fig. 3: Toxicity effects of tested CSIs on malformation rate.
Fig. 4: Various deformation shapes of *M. domestica* after treatment of 3rd instar larvae by CSIs, (lufenuron, flufenouxron and hexaflumuron). Several aberrations as compared to normal (A, D & G) treated larval-pupal intermediate, (J) normal (control) larva, (B, E & H) treated pupa, normal pupa (K), treated adult (C, F & I) and normal adult (L).