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Biochemical effects of lambda-cyhalothrin and lufenuron on *Culex pipiens* L. (Diptera: Culicidae)

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Abstract
The present study aimed to compare between the action of the pyrethroid insecticide lambda-cyhalothrin and the benzoylphenylurea lufenuron on total protein, carbohydrate and lipid content, and on digestive enzymes, transaminases, phosphatases and chitinase activity of *Culex pipiens* L. late third-instar larvae compared to the control. Both lambda-cyhalothrin and lufenuron significantly reduced total carbohydrate content, and carbohydrases, glutamic oxaloacetic acid transaminase and phosphatases activity. Lambda-cyhalothrin significantly decreased total protein content, and protease and glutamic pyruvic acid transaminase activity, whereas it significantly increased chitinase activity. Lufenuron significantly decreased total lipid content, and lipase activity. It can be concluded that lambda-cyhalothrin and lufenuron induced significant metabolic alterations in *Cx. pipiens* late third-instar larvae by acting on different secondary targets.

Keywords: Mosquitoes, pyrethroids, benzoylphenylureas, main metabolites, enzymes activity

1. Introduction
In Egypt, *Culex pipiens* L. is the primary vector for bancroftian filariasis [1] and Rift Valley fever [2]. Conventional insecticides such as organophosphates and pyrethroids have been the most favorite mosquito control method throughout the world [3]. However, their indiscriminate usage has led to the development of insecticide resistance in several mosquito species [4], in addition to negative impacts on environment, human health and non-target organisms. These problems forced to search for safe and selective compounds as an alternative control measure; among these measures is the class benzoylphenylureas (BPUs); a class of insect growth regulators [5].

The classical mode of action of the pyrethroid insecticide lambda-cyhalothrin and the BPU lufenuron is well known. Lambda-cyhalothrin is a member of the type II pyrethroids that cause slow depolarization of nerve membrane, which reduces the amplitude of the action potential leading to a loss of electrical excitability [6]. While, lufenuron, as other BPUs, acting as a chitin synthesis inhibitor; leading to abortive larval molt [6]. The present study aimed at elucidating the non-conventional mode of action of lambda-cyhalothrin and lufenuron against *Cx. pipiens* late third-instar larvae. This may be achieved by studying their effects on total protein, carbohydrate and lipid content, and on digestive enzymes, transaminases, phosphatases and chitinase activity compared to the control.

2. Materials and Methods
2.1 Insect rearing
The stock culture of *Cx. pipiens* was started from larvae obtained from a colony maintained in Entomology Department, Faculty of Science, Cairo University, Egypt, for several generations without exposure to insecticides. The culture was maintained in the laboratory at 25–27 °C and 14 L: 10 D photoperiod according to Djeghader *et al.* [7]. Larvae were reared in dechlorinated water and fed on a mixture of biscuit and dry yeast (3: 1) (w/w). Adults were offered 10% sucrose solution for a period of 3–4 days after emergence. The females were then allowed to take a blood meal from a pigeon for 2–3 h for egg production.

2.2 Lambda-cyhalothrin and lufenuron
Technical grade lambda-cyhalothrin (2.5% EC): (*RS*)-α-Cyano-3-phenoxybenzyl 3-(2-chloro-3, 3, 3-trifluropropenyl)-2, 2-dimethylcyclopropanecarboxylate, and lufenuron (5% EC): (*RS*)-1-

[2, 5-dichloro-4-(1, 1, 2, 3, 3, 3-hexafluropropoxy) Phenyl]-3-(2, 6-diflurobenzoyl] Urea, used in this study was obtained from Sumitomo Chemical Co.

2.3 Bioassay

Larvicidal bioassay was performed essentially following the standard World Health Organization protocol [8]. One ml of each desired concentration of lambda-cyhalothrin in acetone solution (0.005, 0.01, 0.02, 0.04, 0.08 and 0.16 ppm) was infiltrated to 249 ml dechlorinated water in 300 ml plastic cup, while lufenuron was diluted directly in the rearing water up to 250 ml (v/v) to give the final desired concentrations (0.005, 0. 01, 0.02, 0.04 and 0.08 ppm). Twenty five *Cx. pipiens* late third-instar larvae were released into each concentration and were fed as described above. Two control experiments were run concurrently. The first control experiment included 1 ml acetone in 249 ml dechlorinated water for the bioassay of lambda-cyhalothrin, while the second control experiment contained 250 ml dechlorinated water for the bioassay of lufenuron. Each concentration was repeated four times. All the experiments were incubated at 25 °C. The number of dead and alive larvae was recorded 24 h post-treatment.

2.4 Biochemical analyses

After 24 h of treatment *Cx. pipiens* late third-instar larvae with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm), as determined in this study, surviving larvae were homogenized separately in distilled water (50 mg/ml) using a chilled glass–Teflon tissue homogenizer. Each homogenate was centrifuged at 8000 rpm for 15 min at 4 °C. A parallel control was also run. The supernatant was stored at -20 °C for further biochemical analyses. Total protein, carbohydrate and lipid were determined according to Singh and Sinha [9], using Folin–phenol, anthrone and phosphovanillin reagents, respectively. Also, the supernatant was analyzed for the estimation of carbohydrases (trehalase, invertase and amylase) activity according to Ishaaya and Swirisk [10], using 3, 5-dinitrosalicylic acid reagent. Protease activity was determined based on the methods of Tatchell *et al.* [11], and the increase in free amino acids split was measured following the procedures of Lee and Takabashi [12], using ninhydrin reagent. Lipase activity was determined according to Tahoun and Abdel-Ghaffar [13], with slight modifications, using triolein substrate. Glutamic oxaloacetic acid transaminase (GOT) and glutamic pyruvic acid transaminase (GPT) activity was quantified according to Reitman and Frankel [14], using α-ketoglutarate-*dl*-aspartate and α-ketoglutarate-*dl*-alanine substrates, respectively. Alkaline and acid phosphatases activity was estimated according to Laufer and Schin [15], using *p*-nitrophenol phosphate substrate, with alkaline buffer (pH 10.5) and acid buffer (pH 4.8), respectively. Colloidal chitin was prepared according to Bade and Stinson [16], and the reaction mixture was prepared according to Ishaaya and Casida [17]. Each assay was replicated five times.

2.5 Statistical analyses

The percentage mortality was corrected using Abbott's formula [18]. The LC_{50} was determined with POLO-PC software [19], based on the Probit analysis [20]. Data obtained were analyzed using one-way analysis of variance (ANOVA). When ANOVA statistics were significant ($P < 0.05$), the means were compared by Duncan's multiple range test [21].

3. Results

3.1 Total protein, carbohydrate and lipid

The results obtained indicated that total protein was significantly decreased ($P < 0.05$) due to treatment with lambda-cyhalothrin, whereas it was insignificantly affected post-treatment with lufenuron compared to the control. On the other hand, both lambda-cyhalothrin and lufenuron significantly decreased ($P < 0.05$) total carbohydrate. Total lipid was significantly decreased ($P < 0.05$) in lufenuron-treated larvae, whereas it was insignificantly changed following treatment with lambda-cyhalothrin (Fig. 1).

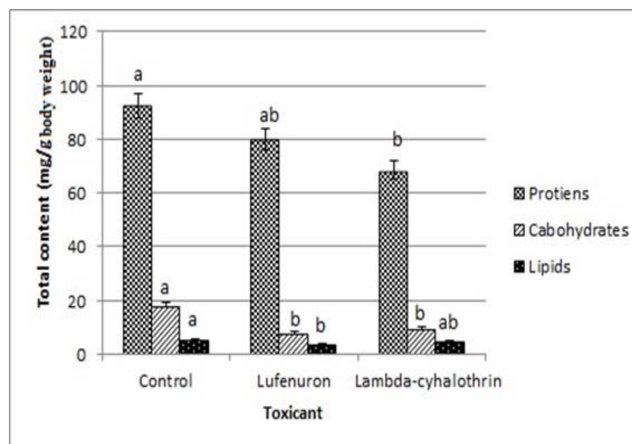

Fig 1: Total protein, carbohydrate and lipid content of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test.

3.2 Digestive enzymes activity

Both lambda-cyhalothrin and lufenuron significantly decreased ($P < 0.05$) carbohydrases (trehalase, invertase and amylase) activity compared to the control (Fig. 2). Protease activity was significantly reduced ($P < 0.05$) in larvae treated with lambda-cyhalothrin (Fig. 3).

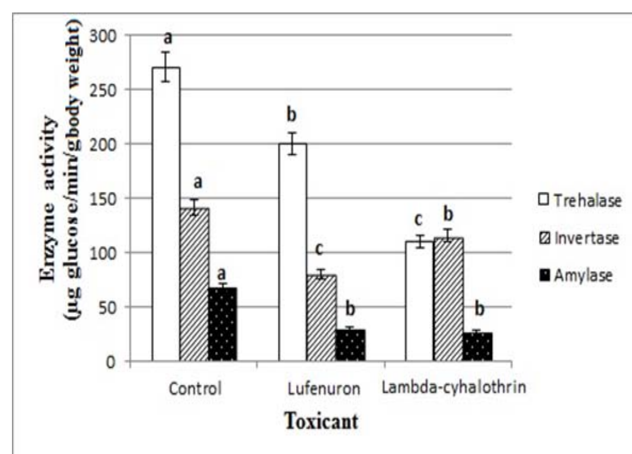

Fig 2: Trehalase, invertase and amylase activity of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test.

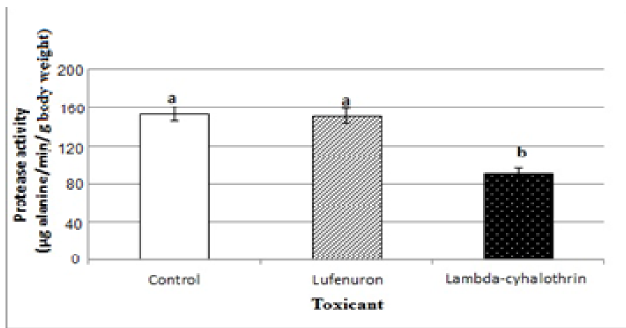

Fig 3: Protease activity of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test.

On the contrary, protease activity was insignificantly changed after larval exposure to lufenuron compared to the control (Fig. 3). Unlike to the pattern of protease activity, treatment with lambda-cyhalothrin insignificantly changed lipase activity, whereas lufenuron significantly declined ($P < 0.05$) lipase activity (Fig. 4).

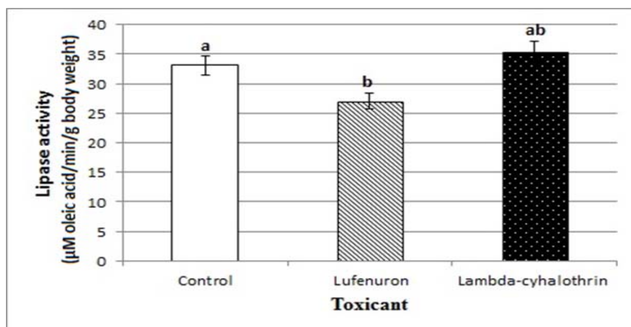

Fig 4: Lipase activity of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test.

3.3 Transaminases activity

Lambda-cyhalothrin significantly depleted ($P < 0.05$) the activity of both GOT and GPT compared to the control. On the other hand, lufenuron significantly reduced ($P < 0.05$) GOT activity, while it was insignificantly affected GPT activity (Fig. 5).

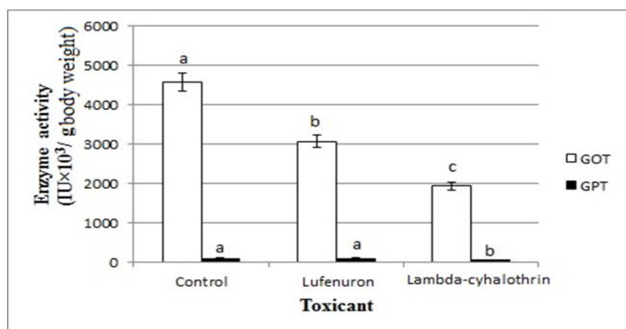

Fig 5: Glutamic oxaloacetic acid transaminase (GOT) and glutamic pyruvic acid transaminase (GPT) activity of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test; IU: international unit.

3.4 Phosphatases activity

The activity of both alkaline and acid phosphatases was significantly suppressed ($P < 0.05$) after larval treatment with either lambda-cyhalothrin or lufenuron compared to the control (Fig. 6).

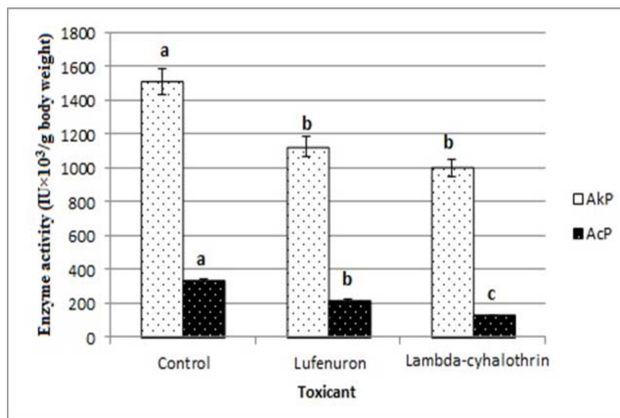

Fig 6: Alkaline phosphatase (AkP) and acid phosphatase (AcP) activity of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test; IU: international unit.

3.5 Chitinase activity

Lambda–cyhalothrin significantly enhanced chitinase activity compared to the control. In contrast, this activity was insignificantly increased after treatment with lufenuron (Fig 7).

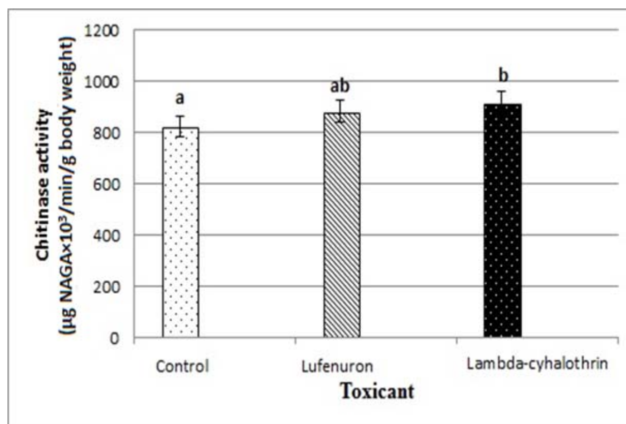

Fig 7: Chitinase activity of *Cx. pipiens* late third-instar larvae 24 h post-treatment with the LC_{50} of lambda-cyhalothrin (0.045 ppm) and lufenuron (0.025 ppm). Different letters above the bars (SE) indicate significant differences ($P < 0.05$); using Duncan's multiple range test; NAGA: *N*-acetyl glucosamine.

4. Discussion

The exposure of an organism to a xenobiotic can modify the synthesis of certain metabolites and disturb the functionality of the organism [22]. In this context, total main metabolites (protein, carbohydrate and lipid) in the whole body of *Cx. pipiens* late third-instar larvae in the present study decreased under the stress of lambda-cyhalothrin and lufenuron. In agreement with our results, the BPU novaluron significantly decreased total protein content of *Culiseta longiareolata* (Macquart) larvae [23], whereas it significantly increased total

carbohydrate and lipid content of the same mosquito species [23] and of *Cx. pipiens* larvae [7]. Ali *et al*. [24, 25] demonstrated that breakdown of protein into free amino acids as well as the decrease in RNA, due to the insecticidal stress, could result in the decrease in total protein. Moreover, insecticidal stress reduces the hemolymph volume; thereby total protein content decreases [26]. Protein depletion may constitute a physiological mechanism and might play a role in the compensatory mechanisms under the insecticidal stress [27]. There is an adverse correlation between the amount of protein and uric acid in hemolymph, i.e., larvae with lowered protein would have higher uric acid where the use of protein for the recovery of the damaged tissues would result in the production of uric acid as a side product of protein catabolism [28].

Carbohydrates as energy elements play a crucial role in the physiology of the insects; the rate of glycogen in tissues are closely related to several physiological events such as the reproduction, the molt and the flight [29]. Accordingly, under stress conditions, more sugars might be metabolized to meet the energy expenses; and this may account for the decrease in total carbohydrate of *Cx. pipiens* larvae treated with both lambda-cyhalothrin and lufenuron. Shakoori *et al*. [30] suggested that disturbance in carbohydrate metabolism may be the result of a long chain effect originating primarily from the inhibition of chitin synthesis. Canavoso *et al*. [31] documented that quantity of lipids available for the energy reserve seems to be the result of a balance between the catch of food and the request for reserve by processes such as reproduction, maintenance and growth, and this balance is disturbed by any toxicant. Therefore, reduction of total lipid in the present study may be due to the effect of lambda-cyhalothrin and lufenuron on lipid metabolism, in addition to the utilization of lipid reserve for energy generation.

Suzuki *et al*. [32] reported that carbohydrases activity was under the control of ecdysone. This finding may explain the depletion of carbohydrases activity in the present study post-treatment with lufenuron, where BPUs might affect the insect hormonal site; thereby resulting in alteration in carbohydrases activity [33, 34]. Acid phosphatase is a lysosomal enzyme [35]; thus the ingestion of any toxic xenobiotic which can affect the lysosomes might affect its activity as evident in this study. According to Shakoori *et al*. [36], the reduction in alkaline phosphatase could be attributed to the reduced enzyme synthesis and/or binding of insecticide at the active site of enzyme. Ali *et al*. [24, 25] showed that decline in GOT and GPT activity was concomitant with decreased protein biosynthesis. This finding extends to the results obtained in this study, where GOT and GPT activity, as well as total protein content decreased after exposure of *Cx. pipiens* late third-instar larvae to lambda-cyhalothrin and lufenuron. Reduction in GOT and GPT activity can be related to reduction in transamination, which in turn can be related to increased soluble protein and free amino acids [24].

Similar to our results, Osman [37] reported that lufenuron significantly increased chitinase activity of *Spodoptera littoralis* (Boisduval) larvae. Abdel-Aziz *et al*. [38] attributed the increase in chitinase activity post-treatment with BPUs to a secondary effect for the reduced activity of β-ecdysone metabolizing enzymes, followed by β-ecdysone accumulation which results in hyperchitinase activity [39]. Chitin synthetase is not the primary biochemical site for the reduced level of chitin since BPUs do not inhibit their activity in cell-free systems [40, 41].

5. Conclusion

It can be concluded that both lambda-cyhalothrin and lufenuron can affect *Cx. pipiens* late third-instar larvae by targeting different biochemical and regulatory molecules in different metabolic pathways.

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