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Effect of lambda-cyhalothrin on midgut of larva of *Culex pipiens* (Diptera: Culicidae)

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Abstract
The present study aimed to examine the histopathological effects of synthetic pyrethroid Lambda-cyhalothrin on the midgut of the L4 stages of *Culex pipiens* larvae. Exposure to LC_{50} (0.00001%) concentration resulted in severe midgut damage, including cell lysis, vacuolation, and disruption of epithelial and peritrophic membranes after exposure of insecticide for 24 hours which increased after 48 hours. At 0.000005% similar but less severe alterations were observed. The findings demonstrate that even 0.00001% doses of lambda-cyhalothrin can cause significant midgut damage in *Culex pipiens* larvae.

Keywords: Mosquito, histology, *Culex pipiens* larvae, midgut, lambda-cyhalothrin

Introduction

Culex mosquitoes are one of the most widespread and significant groups within the Culicidae family. They are primarily recognized for their involvement in the transmission of pathogens such as the West Nile virus, St. Louis encephalitis virus, and lymphatic filariasis [1]. The genus *Culex* encompasses over 1,000 species, with *Culex pipiens* and *Culex* quinquefasciatus being among the most studied due to their public health relevance [2]. *Culex* species are found across the globe, with a presence in temperate, tropical, and subtropical regions. Their distribution is influenced by a variety of factors, including climate, availability of breeding sites, and human activities. For instance, *Culex pipiens* is prevalent in urban and suburban areas in temperate regions such as North America, Europe, and northern Asia. *Culex* quinquefasciatus is more common in tropical and subtropical zones, including regions in South America, Africa, South Asia, and Australia [2, 3]. Other notable species, such as *Culex* tarsalis, are found in North America, primarily in the western United States, where they are significant vectors of arboviruses [4]. The biology of *Culex* mosquitoes encompasses their behavior, physiology and ecological interactions. Adult *Culex* mosquitoes are typically active during the evening and night, seeking out hosts for blood meals necessary for egg development. They are known to be opportunistic feeders, often feeding on birds, mammals, and humans. Their feeding preferences can vary; for example, *Culex pipiens* primarily feeds on birds but can also bite humans, especially in urban environment [5]. This opportunistic feeding behavior makes them efficient vectors of zoonotic diseases.

Culex mosquitoes exhibit remarkable adaptability to various environmental conditions. They can breed in a wide range of habitats, from natural bodies of water like ponds and marshes to artificial containers such as discarded tires, gutters, and sewage systems [6]. Their ability to thrive in polluted water sources, in particular, gives them an advantage in urban and peri-urban areas where such breeding sites are common [3].

Lambda-cyhalothrin is employed in mosquito control programs to reduce the spread of diseases such as malaria, dengue fever, and Zika virus. It is used in indoor residual sprays (IRS) and mosquito nets. It is effective against household pests like ants, cockroaches, and termites [7]. Lambda-cyhalothrin is a synthetic pyrethroid insecticide widely used in agriculture and public health for pest control. The chemical formula for lambda-cyhalothrin is C23H19ClF3NO3. Its structure includes a cyclopropanecarboxylate moiety, making it highly effective as an insecticide due to its ability to interfere with the nervous system of insects.

The effects of the pyrethroid insecticide lambda-cyhalothrin and the benzoylphenylurea lufenuron investigated on the total protein, carbohydrate, and lipid levels, as well as on digestive enzymes, transaminases, phosphatases, and chitinase activity in *Culex pipiens* [8]. It revealed that treatment with lambda-cyhalothrin led to a significant reduction ($P < 0.05$) in total protein, whereas lufenuron had no significant impact compared to the control. However, both lambda-cyhalothrin and lufenuron caused a significant decrease ($P < 0.05$) in total carbohydrate levels. Additionally, lufenuron treatment resulted in a significant reduction ($P < 0.05$) in total lipid content in the larvae.

A study examined the effectiveness of encapsulated lambda-cyhalothrin nanoparticles loaded in polyethylene glycol against *Culex pipiens* larvae, comparing their larvicidal activity to the conventional form of lambda-cyhalothrin over 24, 48, and 72 hours [9]. The conventional form exhibited LC50 values of 7 ×10-8, 1.8 ×10-8, and 1 ×10-9 M, respectively, during the exposure periods. In contrast, the nanoparticles at reduced concentrations (4% and 8%) showed higher toxicity, with LC50 values of 4.6 ×10-8, 2.9 ×10-9, and 7.8 ×10-10 M (for 4%) and 1.8 ×10-8, 5.3 ×10-9, and 1.2 ×10-9 M (for 8%) after 24, 48, and 72 hours, respectively. The increased toxicity at lower concentrations may be attributed to the controlled release of cyhalothrin, with diffusion rates of 1.84 ×10-9, 1.16 ×10-10, and 3.12 ×10-11 M (for 4%) and 1.44 ×10-9, 4.24 ×10-10, and 9.6 ×10-11 M (for 8%) after the same exposure periods. These findings suggest that encapsulated lambda-cyhalothrin nanoparticles could be a more effective and safer larvicide against *Culex pipiens* larvae compared to the conventional form.

Materials and methods

Larvae used in this study belong to the species *Culex pipiens*. They were collected from the drain and ponds in and around Aligarh Muslim University and nearby area of Aligarh, Uttar Pradesh. They were bought to the laboratory at Department of Zoology for project work. The larvae were maintained in a glass beaker in BOD at 27±2ºC. The larvae were provided a dog feed in 100 ml of distilled water. 0.1%

stock solution of Lambda Cyhalothrin was prepared in distilled water. Then this concentration was diluted to get desired concentration of 0.00001% and 0.000005%.

4th larval stages were chosen and treated with two different concentration of insecticide Lambda cyhalothrin (0.00001% and 0.000005%). The insecticide was mixed with the feed provided.

Microtomy of whole larva as such was performed to see the effect of the insecticide on the midgut after 24 hrs and 48 hrs for which larvae were fixed immediately in Bouin's solution for 24 hours. After 24 hours, washing was done 2-3 times in tap water to remove excess Bouin's solution and dehydration proceeded in ascending grades of alcohol i.e. 30%, 50%, 70%, 80%, 90% for 5 minutes each while in 96% and 100% for half an hour each followed by mixture off 100% and xylene solution (1:1) for 10 minutes. Incubation was done in 600 C in xylene and paraffin wax (1:1) for 15 minutes and then in pure wax for 2 hours. The whole larvae section was embedded in paraffin wax. 5 micrometer microtome sections were cut from the prepared block. Then the ribbons were placed in glass slide which was lubricated by albumin solution having 5 drops of glycerin. Slides were then stretched on warming table to remove creases. Slides were processed in 2 changes of xylene, then descending grades of alcohol series 100%, 96%, 90%, 80%, 70%, 50%, 30% for 5 minutes each and in distilled water for 5 minutes each. Slides were stained in hematoxylin for 10 seconds, then washed in tap water and counter stained with eosin for 25 minutes followed by upgrade dehydration of alcohol for 5 minutes each and then 2 changes of xylene for 10 minutes. After air drying slides were mounted using D.P.X to observe under compound microscope [10]. Photographs were taken using Nikon eclipse Ci compound microscope using appropriate magnification.

Result

Histology of control samples of *Culex* Larva (Fig.1)

The midgut epithelium of larva of C. pipiens is made-up of columnar cells and apical portion is having striated border. The epithelial cell enclosed within itself, the cytoplasm with vesicles and spherical nucleus in central portion. The epithelial cells have somewhat clear cytoplasm and the whole epithelium showed normal intracellular contact along the whole lateral plasma membrane, normal nuclei, a well developed brush border and a normal adhesive basement membrane. The lumen of the midgut is surrounded by peritrophic membrane line between the epithelial cells and food bolus.

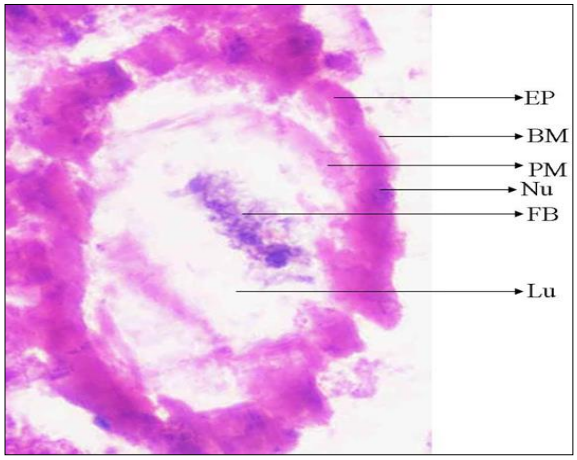

Fig 1: Transverse sections of Midgut of *Culex pipiens*, at 10X (a) Control

Histopathological effect of lambda-cyhalothrin on midgut of larva of *Culex pipiens*.

At 0.000005% concentration of Lambda-cyhalothrin

A. After 24 hours (Fig.2)

The basement membrane show disruption at some points. Intercellular junctions were damaged. The elongated epithelial cells were visible. The midgut had undergone fractional lysis and was separated from basement member. Some damage was also visible in Peritrophic membrane. Most of the cells were having microvilli with a very little degeneration in few cells.

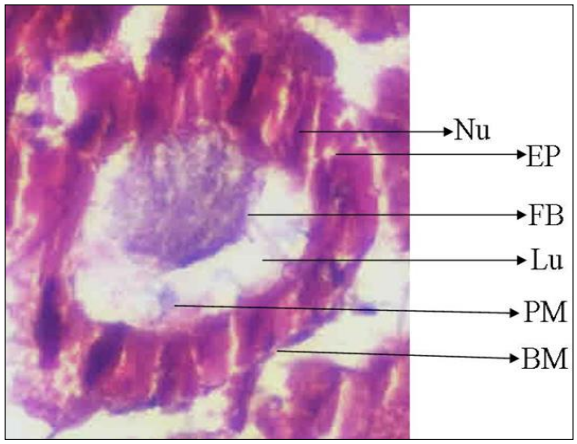

Fig 2: Treated with 0.000005% dose 24 hrs

B. After 48 hours (Fig.3)

A slight hypertrophy in gut cells were observed. Intercellar space in between the epithelial cells was more pronounced. Some cells show deterioration in shape and few of them were having vacuoles. Microvillus appeared more disintegrated. Basement membrane and peritrophic membrane endured more degeneration.

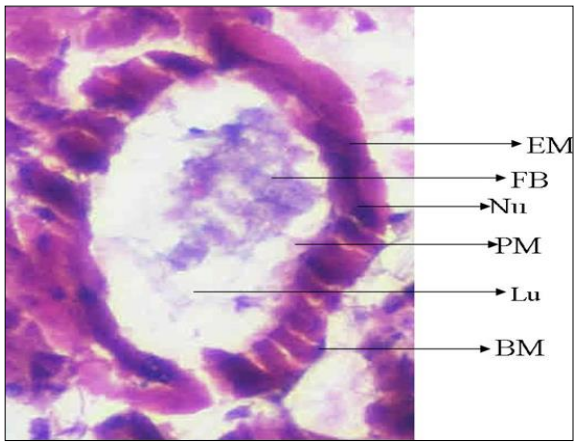

Fig 3: Treated with 0.000005% dose 48 hrs

At 0.00001% concentration of Lambda-cyhalothrin)

A. After 24 hours (Fig.4)

After 24 hours the effects were more severe than the sub lethal concentrations. The midgut show more degree of lysis having lengthened epithelial cells which were partially separated from the underline basement membrane. The basement membrane was wrecked from few areas and the peritrophic membrane was also disrupted. Almost all of the cells show some degeneration in microvilli. A few of the cells had developed vacuoles as well.

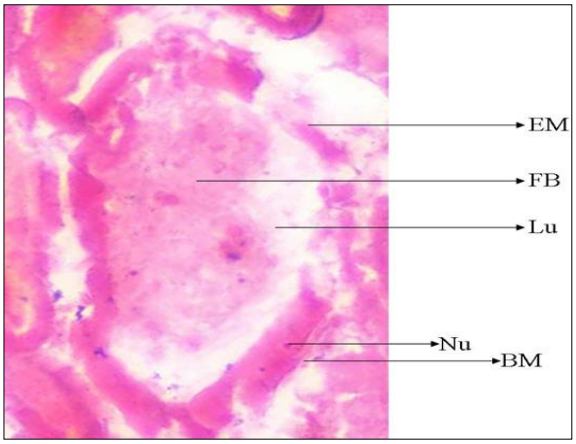

Fig 4: Treated with 0.00001% dose 24 hrs

B. After 48 hours (Fig.5)

The cell lysis was observed with cytoplasmic membrane and debris leaked into the lumen. Major parts of the cells were destroyed. Epithelial cells were disengaged from the basement membrane which itself was showing high degree of disintegration. Peritrophic membrane had also undergone huge disruption. Microvilli also show degeneration. Large size vacuoles were present in most of the epithelial cells.

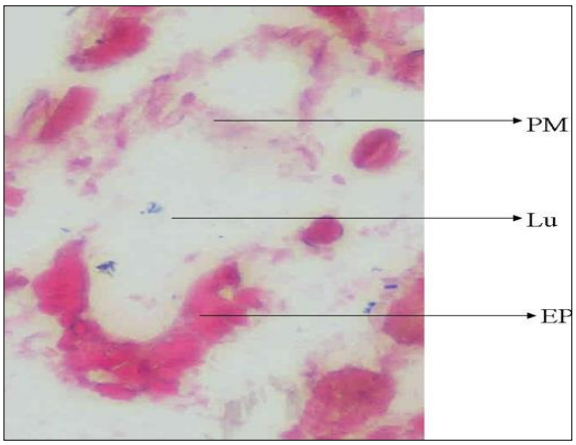

Fig 5: Treated with 0.00001% dose 48 hrs.

[BM = Basement membrane, EC = Epithelial Cells, FB = Food bolus, Lu = Lumen, MT = Malpighian tubule, Nu = Nucleus, PM = Peritrophic Membrane]

Discussion

The present study investigates the histopathological effects of lambda-cyhalothrin on the midgut of *Culex pipiens* larvae at sublethal and LC50 doses. The midgut, a critical organ for nutrient absorption and digestion, reveals significant changes when exposed to this insecticide, providing insights into the potential impacts on larval physiology and survival. At a sublethal dose, lambda-cyhalothrin induces observable but not immediately fatal changes in the midgut of *Culex pipiens* larvae. The basement membrane, which provides structural support and separates the midgut cells from the hemolymph, shows disruption at several points. This disruption can impair the integrity and function of the midgut, potentially affecting the larvae's ability to digest and absorb nutrients effectively.

The damage to intercellular junctions suggests that the communication and barrier functions between epithelial cells are compromised. These junctions are essential for maintaining tissue homeostasis and protecting against pathogens. The visible elongation of epithelial cells might indicate a stress response or an attempt to compensate for the damage by increasing surface area for absorption. Similar observation was reported by Al-Mehmadi andAl-Khalaf [11] in the larvae of *Culex quiquefasciatus* treated with *Melia azedarach* extracts. Toxicity varied according to the concentration and period of exposure.

The fractional lysis and separation of the midgut from the basement membrane further highlight the extent of damage. The peritrophic membrane, which protects the midgut lining from mechanical damage and pathogens, also shows signs of damage. However, most cells still retain their microvilli, essential for nutrient absorption, although slight degeneration is noted in some cells. This suggests that while there is damage, the larvae might still manage some level of nutrient absorption.

The effects of the sublethal dose become more pronounced after 48 hours. Hypertrophy of gut cells indicates an increase in cell size, possibly as a compensatory mechanism or a result of cellular stress. The more pronounced intercellular space suggests further disruption of cell junctions and tissue integrity. The presence of vacuoles in some cells indicates cellular distress or degeneration, possibly due to the accumulation of cellular debris or toxic compounds. The more disintegrated appearance of microvilli implies a progressive loss of absorptive surface area, which can severely impact nutrient uptake. Both the basement and peritrophic membranes show increased degeneration, compromising the midgut's structural and protective functions. This progressive damage underscores the cumulative effect of lambda-cyhalothrin over time, even at sublethal concentration.

The large vacuoles present in most epithelial cells suggest advanced stages of cellular damage, possibly due to autophagic or necrotic processes. This extensive damage to the midgut structure and function highlights the lethal impact of lambda-cyhalothrin at the LC50 dose. De Melo *et al.* [12] found increase in number of vesicles in the midgut cells of *Culex quiquefasciatus* larvae treated with fatty acids and methyl esters.

These findings underscore the potent impact of lambda-cyhalothrin on non-target organisms and highlight the need for careful consideration of its ecological implications. The disruption of midgut structure and function can have cascading effects on larval development, survival, and population dynamics, which are crucial for understanding the broader environmental impact of this insecticide.

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