

## Biochemical effects of a newly synthesized cyclohexyloxy compound JHA on the lipids of fourth-instar larvae of *Culex pipiens quinquefasciatus* Say

Synthetic juvenile hormone analogues (JHAs) behave like endogenous juvenile hormone (JH) of insects and thus are insect-growth regulators. The newly synthesized compound 1-(3'-methyl-6'-isopropyl-cyclohexyl-oxy-3,7-dimethyl-2 (E)),6-octadiene has been found to be a JHA<sup>1</sup>. Shortly before ecdysis, wax (consisting of long chain of hydrocarbons and the esters of fatty acids and alcohols) is secreted on the surface of the new cuticle and the layer adjacent to the cuticulin – the oriented monolayer<sup>2</sup>. The importance of lipids in insects has been reviewed<sup>3</sup>. Obvious success of insects on the planet has been their ability to utilize lipids efficiently as substrate for reproduction, embryogenesis, metamorphosis and flight<sup>4</sup>. This study was undertaken to know the effect of synthetic test JHA on the lipids of treated fourth-instar larvae

of *Culex* along with control. Thin layer chromatography (TLC) and biochemical studies on lipids revealed that the JHA causes decrease in different lipids. These changes seem to be responsible for formation of intermoult and mortality of developing stages.

Fourth-instar larvae were selected because this is the transition stage between larvae and pupae of mosquitoes. It has a critical period when the ecdysiotropin secreted by neurosecretory cells is very low and this period is the most suitable one for synthetic JHA treatment for insect control<sup>5</sup>. Lipids are important constituents of the fat body cells in insects<sup>2,3</sup>. These cells contribute lipids to the new cuticle during metamorphosis through oenocytes<sup>2</sup>. Keeping in view the importance of lipids in metamorphosis in mosquitoes, the present study was undertaken to know

the mode of action of test compound in metamorphosis.

Fourth-instar larvae of pure colony of *Culex pipiens quinquefasciatus* were reared in 1 ppm, 2 ppm and 3 ppm aquatic solutions of JHA having molecular formula C<sub>20</sub>H<sub>36</sub>O along with controls. Total lipids, phospholipids and cholesterol were estimated following the method of Folch *et al.*<sup>6</sup>, and lipid fractions were separated using the TLC technique. Developed spots were estimated following the methods of Mangold<sup>7</sup>, Zlatkis *et al.*<sup>8</sup>, Lowry and Tinsley<sup>9</sup>, Sidney and Bernard<sup>10</sup>.

Biochemically estimated values of lipids (total lipids, phospholipids and cholesterol) are given in Table 1. A percentage decrease in total lipids, phospholipids and cholesterol was observed after 1 ppm, 2 ppm, 3 ppm doses compared to their con-

Table 1. Quantitative changes in lipids in the fourth-instar larvae of *Culex* treated with JHA

S. no.	Dose	Total lipids	Phospholipids	Cholesterol
1.	N (0 ppm)	51.68 ± 0.280	13.698 ± 1.166	3.231 ± 0.727
2.	C1 (1 ppm)	51.12 ± 0.740	12.016 ± 0.5999	2.764 ± 1.871
3.	T1 (1 ppm)	50.88 ± 3.458***	10.376 ± 1.028	2.528 ± 1.650
% Change		- 0.47%	- 13.65%	- 8.54%
4.	C2 (2 ppm)	50.09 ± 1.089	11.529 ± 0.734	2.530 ± 0.148
5.	T2 (2 ppm)	49.44 ± 3.315	08.710 ± 1.205***	2.255 ± 0.148*
% Change		- 12.98%	- 24.45%	- 10.87%
6.	C3 (3 ppm)	49.95 ± 2.645	11.346 ± 0.754	2.203 ± 1.102
7.	T3 (3 ppm)	38.96 ± 0.841***	04.501 ± 0.491***	1.194 ± 1.136**
% Change		- 22%	- 60.33%	- 45.0%*

\**P* < 0.05. All values (mg/g) are expressed as mean ± SD of six replicates.

\*\**P* < 0.01

\*\*\**P* < 0.001.

N = normal water, C = Acetone in water, T = JHA in acetonetic water.

Table 2. Quantitative changes in TLC fractions of phospholipids in fourth-instar larvae with JHA

	Phospholipids							Cholesterol ester	
	Tri-glycerides	Phosphatidyl serine Phosphatidyl inositol	Lyso-phosphatidyl choline	Sphingo-myelin	Phosphatidyl choline	Phosphatidyl ethanolamine	Free fatty acids		
C	2.235	3.3	1.9	2.123	2.3	3.3	3.5	3.23	1.117
1 ppm	1.647	1.9	1.4	1.169	1.82	3.0	3.26	2.456	1.03
2 ppm	1.294	1.5	1.1	1.63	0.981	2.2	2.82	2.212	0.867
3 ppm	0.941	1.1	0.983	1.199	0.732	2.0	2.72	1.166	0.829

All values are in mg/g.

trols. Increased dose caused increased decrease in lipids. Quantitative estimation of TLC spots of individual lipid classes is given in Table 2. These values showed a decrease in phospholipids (phosphatidyl serine, phosphatidyl inositol, lysophosphatidyl choline, sphingomyelin, phosphatidyl, ethanolamine), cholesterol and its ester, triglycerides and free fatty acids after 1 ppm, 2 ppm and 3 ppm compared to their control.

The fat body is the important site for the storage and biosynthesis of lipids<sup>2,3</sup> and contributes lipids to the different layers of new cuticle of insects during metamorphosis through oenocytes<sup>2</sup>. It seems that JHA interferes in biosynthesis of lipids by the fat body and thus the formation of new cuticle is impaired as reported by Mittal *et al.*<sup>1</sup>. Decrease of cholesterol ester and cholesterol seems to be responsible for decreased ecdysone biosynthesis which causes incomplete moulting as observed after treatment with

test compound<sup>2</sup>. As the phosphatides are present in different tissues in insects, free fatty acids are required for the growth of larvae, and triglycerides are major reserve food lipids in developing stages of insects<sup>2,3</sup>; their decrease seems to be responsible for mortality of developing stages, incomplete metamorphosis and formation of intermoult observed after treatment<sup>1</sup>.

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