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Lethal toxicity of deltamethrin and histological changes in the vital organs of fingerlings of *Labeo rohita*

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Abstract
The investigation was carried out to find out the lethal concentration (LC_{50}) and safe concentration for deltamethrin on fingerlings of *Labeo rohita* by acute toxicity test along with the observations on associated histological alterations in the vital organs upon sub lethal exposure. The static bioassay methodology was followed to find out the 24h, 48h, 72h and 96 h LC_{50} values for deltamethrin. 3 replications were taken for each treatment. The safe application factors for deltamethrin were 1.00µg/l and presumable harmless concentration were 0.117 µg/l. Sub lethal studies were conducted by exposing the fishes to the $1/10^{th}$ of the LC_{50} values for deltamethrin separately for a period of 30 days with provision of food, aeration and water replenishment. The histopathological alterations observed includes degenerated hepoto-pancreatic tissue, appearance of blood streak among hepatocytes, formation of blood cells among hepatocytes, swelling of the hepatocytes, diffused necrosis in liver. Histopathological changes observed in gill includes swollen branchial arch with accumulation of infiltrating cells, etc.

Keywords: Toxicity, lethal concentration, sub-lethal concentration, deltamethrin, *Labeo rohita*, Histological

1. Introduction
With the increase in population there is tremendous pressure on the land in order to feed the population. Pesticides are frequently used in agriculture for the eradication of pests and weeds and to increase production. They are also used to prevent disease-spreading insects like mosquitoes, flies, and termites. Due to injudicious and indiscriminate use of these pesticides, water bodies like ponds, lakes, river and low lying water areas are continuously getting polluted through surface run off, sediment transportation from treated soil and direct application as spray for controlling pests which lead some serious problems to the non-target organisms such as fishes, mammals and birds [13]. Widespread use of various pesticides and their impact on environment are now a worldwide phenomenon [29]. It has been estimated that only about 1 % of applied pesticides land on the target organisms and the rest contaminate the environment [15]. Synthetic pyrethroids, modified derivatives of pyrethrins, natural substances obtained from flowers of pyrethrum species [14] have emerged as an alternative for long term ecological problems associated with the use of organochlorine, organophosphate and carbamate pesticides. Pyrethroids have a high rate of gill absorption due to their lipophilic nature, which would be a contributing factor for fish sensitivity to aqueous pyrethroid. Among pyrethroids, deltamethrin are used widely in agriculture and pisciculture purposes. Deltamethrin are manufactured analogues of naturally occurring pyrethrins found in the flowers of *Chrysanthemum cinerariaefolium* [18]. Deltamethrin is popular not only because of its effectiveness, but also for characteristics that allow the insecticide to work efficiently at low doses. Deltamethrin is a type-II pyrethroid compound that is highly toxic to fish, which are the most abundant aquatic organisms, while it is less toxic to birds and mammals. Fishes, the most diverse group of vertebrate fauna are important component of the food chain and any effect of toxicant may have adverse influence on the nutritional value of fish and on human being through their consumption [12]. They are excellent experimental models for toxicological investigations [30] and are often used as sentinel organisms to assess the biological impacts of contaminants and environmental quality because of their responses to low concentrations of toxic substances [1]. Histopathological alterations have been widely used as biomonitoring tools or biomarkers of health status of fish exposed to chemical compounds both in laboratory experiments [2] and field studies [21].

Histopathological changes in fish organs have been increasingly studied as biomarkers for assessing aquatic contamination in environmental monitoring studies [10]. Histopathology may therefore prove to be a cost effective tool to determine the health status of fish populations, hence reflecting the health of the entire aquatic ecosystem in the biomonitoring process [17]. Owing to the fact that, *L. rohita* inhabiting the freshwater sources, is widely cultured in ponds and lakes of this region. It is highly edible, has great economic importance and one of prime cultured Indian major carp. This freshwater teleost species is highly amenable to laboratory conditions besides its wide availability (freshwater tanks, ponds, rivers and reservoirs) in India. It is representative of the ecologically widespread cyprinidae. In fact fish are generally regarded as sentinels of bio indicators for aquatic pollution and indispensable experimental models in ecotoxicological studies. Hence *Labeo rohita* (Hamilton) was considered and selected for the present investigation. Therefore the main objectives in the present investigation are as follows

1. To standardize the lethal concentration (LC_{50}) for, deltamethrin on fingerlings of *Labeo rohita*.
2. To study the histological alterations made to the vital organs like kidney, liver and gill of the test animals under sub lethal concentration of deltamethrin.
3. To find out the safe concentrations for deltamethrin depending upon the LC_{50} value.

2. Materials and method

Healthy and active *Labeo rohita* fingerlings weighing 7.80±0.40 g, measuring 6.5 ± 0.25 cm were procured from the Instructional farm of College of Fisheries, Rangailunda in april. 2015 and acclimatized in FRP tank under laboratory condition after providing a dip treatment in 0.1% potassium permanganate solution to prevent infection. The acclimatization process continued for 20 days under laboratory condition prior to initiation of the actual experiment. They were fed commercial pelleted feed twice daily during acclimatization period with exchange of rearing media in every 24 hours. The test medium for the above study against fingerlings of *Labeo rohita* at 24, 48, 72 and 96 h under acute toxicity and chronic exposures up to 30 days containing synthetic pyrethroids i.e. deltamethrin. To commence with assays of deltamethrin, common stock solution was prepared by dissolving in one litre of distilled water. For the preparation of common stock solution following formula was used

N1V1=N2V2

Where N1=concentration of availability percentage.

V1= volume of available pesticide.

N2= Required concentration of pesticide to be prepared.

V2 = Volume of solution required

Series of different concentration of deltamethrin was prepared as microgram per litre which were prepared by adding the common stock solution into the measured distilled water with the help of pipette.

The bioassay studies was conducted at College of Fisheries; Rangailunda for which the fresh water was collected from the fish pond located in the instructional farm. The collected pond water was filtered and stored in 500l FRP tanks for 24 h with aeration. The acclimatisation was done in FRP tank of 200L capacity and Glass aquariums of 25 L capacity were used as test container. After cleaning with appropriate detergents, rinsed with acetone and properly washed with tap water prior to initiation of the acute toxicity test. After each test the containers were cleaned properly. Each experimental container was covered with nylon screen to prevent fishes from escaping.

Range finding static bioassay for the fingerlings of *Labeo rohita* was conducted as per APHA, 1985 with test organisms exposed to different range of concentrations. Before initiation of the range finding tests, the animals are starved for 24 hours. No feed and aeration were given during the experiments. The percentage of mortality was recorded at an interval of 24, 48, 72 and 96 h. It is worth to mention here the concentration between 0.36 µg/l and 0.40 µg/l were selected for the present static bioassay study of deltamethrin. For determination of LC 50 deltamethrin, a series of five test concentrations 0.36, 0.37, 0.38, 0.39 and 0.40 µg/l a. Each concentration was run in triplicate with a control. The percentage of mortality at the end of every hour was recorded and then pulled to 24, 48, 72 and 96 hours. Test medium was renewed for every 24 h with their respective test solution and dead fishes were removed immediately after the experiment period. Lethal concentrations were determined by adopting short term static bio-assay technique recommended [32, 33, 34]. The data gathered during the present investigation were analysed by probit regression analysis [9] for determination of LC_{50} values for deltamethrin. The percentage mortality against log concentration was plotted in probability paper to get LC_{50} values graphically

The Safe application factor was calculated using the formula

$C = 48\ h\ LC_{50} \times 0.3 / S^2$

Where, C = presumable harmless concentration

$S = 24\ h\ LC\ 50 / 48\ h\ LC_{50}$

2.1 Chronic exposure test

Sub-lethal concentration ($1/10^{th}$ of 96 h LC_{50}) values for the selected pyrethroids were selected separately for chronic exposure study for a period of 30 days with replacement of test medium at an interval of two days. During the period of sub-lethal test, the experimental animals were fed with artificial diet once a day. Continuous aeration of the experimental units was carried out throughout the experimentation period. At the end of 30 days, the fishes were sampled out from the treatment as well as from control for histological studies.

2.2 Histopathology

Fingerling samples from the control group as well as from the sub-lethal test units were collected and samples like gill, liver and kidney were collected for histological studies. The vital organs were collected carefully from the sampled fish and preserved in 10% neutral buffered formalin for 48 hours before its further processing. The preserved samples were washed in running tap water overnight then dehydrated with ascending grades of alcohol starting from 50% to absolute alcohol. Paraffin blocks were prepared in water bath using paraffin wax (BDH) 58 to 60^0C melting points. Sections were cut out at 3-5 µ thickness using a rotary microtome and were stained by Haemotoxylene Eosine for further study. The stained sections were examined under a research binocular compound microscope to note the changes in the cell configuration of the vital organs exposed to sub-lethal

concentrations of deltamethrin separately.

2.3 Statistical analysis - In lethal toxicity studies, death is taken as the main criteria for assessing the lethal effects. Lethal concentrations were arrived at by adopting short term static bio-assay technique recommended [31, 33, 34]. Cumulative mortality at every 12 h intervals was recorded. The mortality percentage of fingerlings observed at every hour computed to 24, 48, 72 and 96 h under different concentrations and analysed by probit regression analysis [9] for obtaining LC 50 values at 24, 48, 72 and 96 h. The percentage mortality against log concentration was plotted in probability paper to get LC_{50} values too. These values were further tested at 95% confidence limit level to ascertain their statistical significance.

3 Results

3.1 Lethal toxicity

The result pertaining to the lethal toxicity of deltramethrin exposed to different concentrations from0.36 µg/l to0.40 µg/l indicated that death of the fingerlings was not observed for first twelve hours in any of the concentration. and no mortality was observed till the termination of the experiment in control. 50% mortality was observed at a deltramethrin concentration of 0.38µg/l at the end of 72 h whereas, mortality of 50% test animals were observed at a deltamethrin concentration of 0.39 µg/l and 0.40 µg/l at the end of 60 h and 36 h respectively.

Table 1: Test for lethal toxicity of *Labeo rohita* fingerlings exposed to deltamethrin

Conc. (µg/l)	Conc. × 100	Log Conc.	Total No. of fish	Percentage mortality				Response			
				24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0.36	36.0	1.5563	10	0	10	20	30	0	0.1	0.2	0.3
0.37	37.0	1.5682	10	10	20	30	40	0.1	0.2	0.3	0.4
0.38	38.0	1.5797	10	20	30	40	60	0.2	0.3	0.4	0.6
0.39	39.0	1.5910	10	30	40	50	70	0.3	0.4	0.5	0.7
0.40	40.0	1.6020	10	40	50	80	100	0.4	0.5	0.8	1.0

The mortality of fish increased with the increase in the concentration of the toxicant, depicting a direct correlation between the mortality and the concentration. The Lc 50 values for 24 h, 48 h, 72 h and 96 h along with its 95% lower and upper confidence limits (ML and MU) for synthetic pyrethroid deltamethrin is presented in Table 2.

Table 2: Lethal toxicity (LC_{50}) values for *Labeo rohita* fingerlings exposed to different concentrations of deltamethrin

Duration	24 h		48 h		72 h		96 h	
	LC_{50} (µg/l)	Slope 'b'	LC_{50} (µg/l)	Slope 'b'	LC_{50} (µg/l)	Slope 'b'	LC_{50} (µg/l)	Slope 'b'
Fingerlings	0.39 (0.38 – 0.40)	1.93	0.39 (0.36 – 0.42)	3.21	0.38 (0.35 – 0.40)	3.95	0.37 (0.36 – 0.38)	3.98

Values in parenthesis represent 95% confidence limit

The safe application factor of the treated synthetic pyrethroids to fingerlings of rohu is presented in the Table-3. The safe application factors for deltamethrin were in the order of 0.117 µg/l.

Table 3: Safe application factor of the treated synthetic pyrethroids to fingerlings of *Labeo rohita*.

Name of the Pyrethroids	S value = 24 h LC_{50} / 48 h LC_{50} (µg/l)	C = 48 h LC_{50} × 0.3 (µg/l) / S^2
Deltamethrin	1.00	0.117

The probit regression analysis indicates the 24 h, 48 h, 72 h and 96 h LC_{50} value for *L. rohita* to be 0.39 (0.38 to 0.40) µg/l; 0.39 (0.36 to 0.42) µg/l; 0.38 (0.35 to 0.40) µg/l and 0.37 (0.36 to 0.38) µg/l respectively.

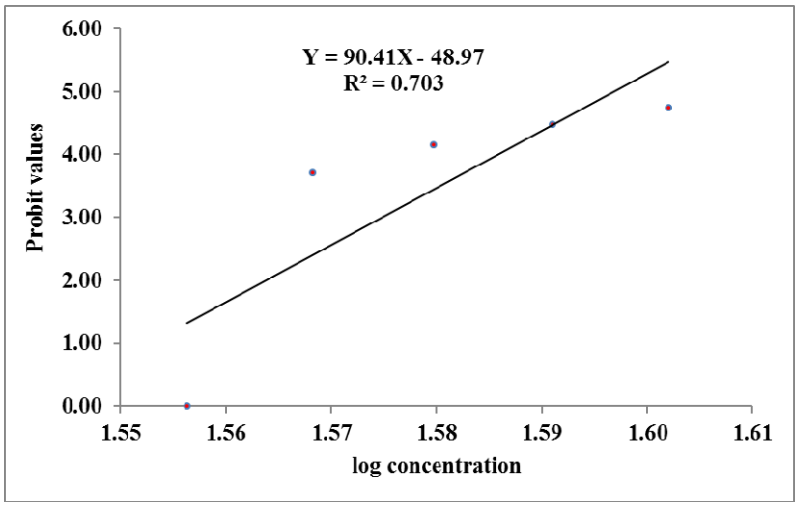

Fig 1: Linear curve between probit mortality and log concentration on 24h exposure to deltamethrin in fingerlings of *Labeo rohita*

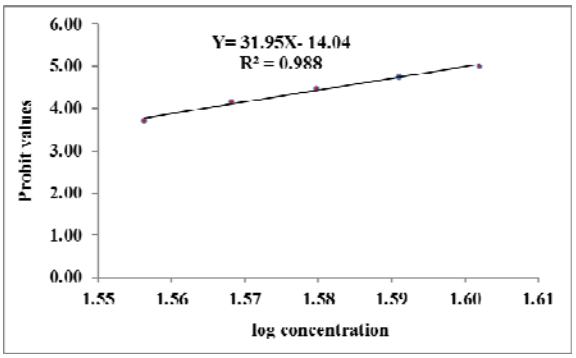

Fig 2: Linear curve between probit mortality against log concentration on 48h exposure to deltamethrin in fingerlings of *Labeo rohita*.

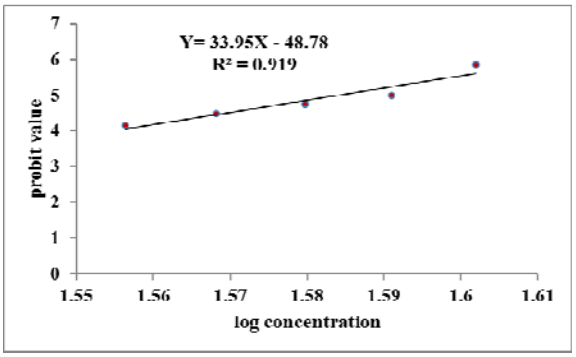

Fig 3: Linear curve between probit mortality against log concentration on 72h exposure to deltamethrin in fingerlings of *Labeo rohita*.

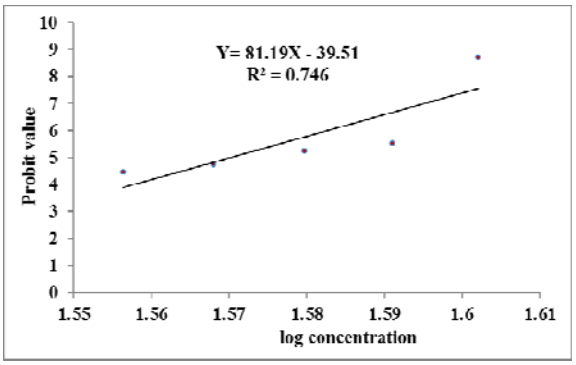

Fig 4: Linear curve between probit mortality against log concentration on 96h exposure to deltamethrin in fingerlings of *Labeo rohita*.

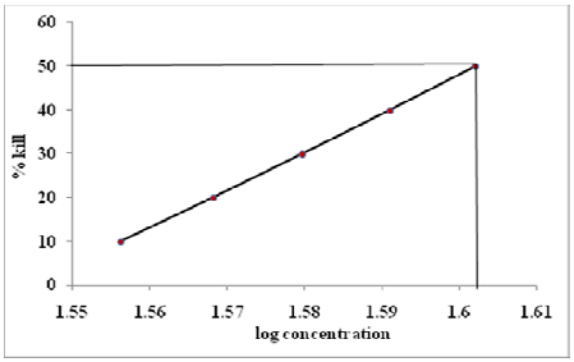

Fig 5 Linear curve between probit mortality against log concentration on 48h exposure to deltamethrin in fingerlings of *Labeo rohita*.

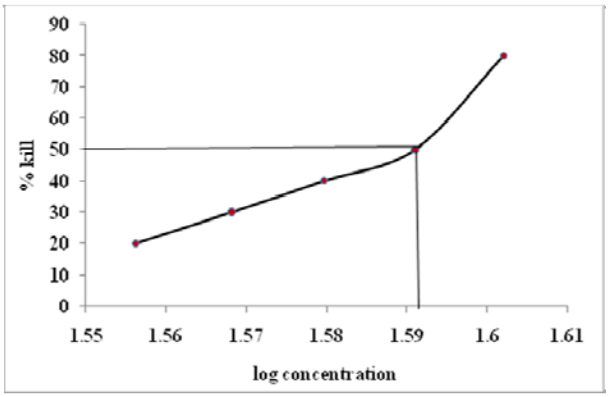

Fig 6: Linear curve between probit mortality against log concentration on 72h exposure to deltamethrin in fingerlings of *Labeo rohita*.

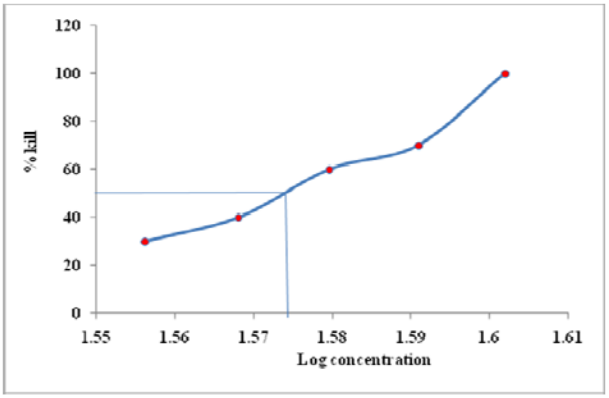

Fig 7: The percentage mortality against log concentration on 96h exposure to deltamethrin in fingerlings of *Labeo rohita*

3.2 Histopathology

Sub-lethal concentration ($1/10^{th}$ of 96 h LC_{50}) values for the selected pyrethroids deltramethrin were selected for chronic exposure study for a period of 30 days with replacement of test medium at an interval of two days. During the period of sub-lethal test, the experimental animals were fed with artificial diet once a day. Continuous aeration of the experimental units was carried out throughout the experimentation period. At the end of 30 days, the fishes were sampled out from the treatment as well as from control for histological studies. The histological alterations observe under the influence of sub-lethal concentrations of chemicals are mentioned below.

3.2.1 Liver under control group

Hepatocytes retains the polygonal structures, distinct vesicular nuclei located centrally, cytoplasm with vacuolation, hepatopancreatic cells were seen surrounding the hepatic portal vein as darkly stained large cells. The typical radiating type of hepatic cells arrangement surrounding the central vein were not seen in the liver tissue of fingerlings of rohu.

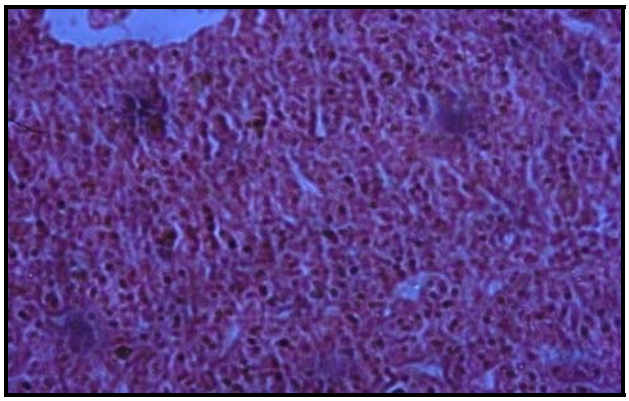

Fig 8: Untreated liver of *L. rohita* fingerlings (Control) (H&E, 400X)

3.2.2 Liver under sub-lethal concentration of deltamethrin

Changes in the liver were characterized by degenerated hepatopancreatic tissue, appearance of blood streak among hepatocytes, Formation of Blood cells among hepatocytes. There was swelling of the hepatocytes at places and diffused necrosis.

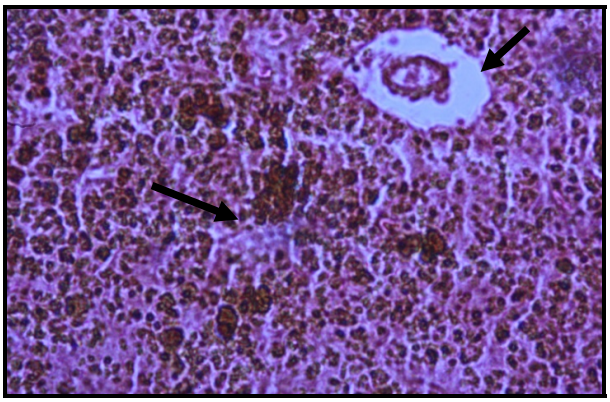

Fig 9: Liver of *L. rohita* fingerlings treated with sub-lethal concentration of deltamethrin showing swelling of the hepatocytes at places and diffused necrosis (H&E, 400X).

3.2.3 Kidney under control group

Kidney showed renal corpuscles and renal tubules, there was distinct differentiation of the head and trunk kidney with relation to the cellular structure, components and their arrangements. Glomeruli were more abundant in trunk kidney.

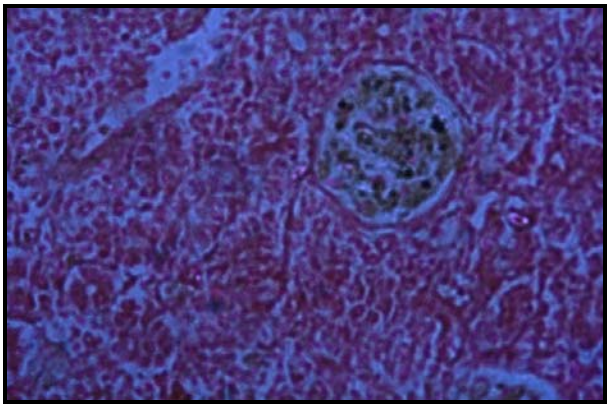

Fig 10: Untreated Kidney of *L rohita* fingerlings (Control) (H&E, 400X)

3.2.4 Kidney under sub-lethal concentration of deltamethrin

Highly degenerative changes were observed in haemtopoetic tissue, severe necrosis, cloudy swelling in renal tubules and granular cytoplasm.

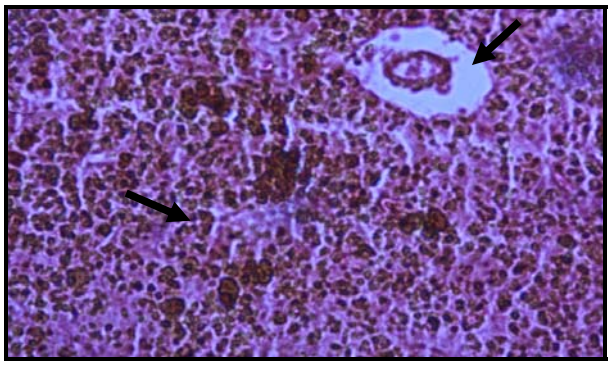

Fig 11: Kidney of *L. rohita* fingerlings treated with sub-lethal concentration of deltamethrin showing severe necrosis and swelling of renal tubules. (H&E, 400X).

3.2.5 Gill under control group

Gill arch appears normal, primary and secondary lamellae showed disrupted uniformly arranged cells, cartilage support to gill filament, organized way of arrangement of stratified squamous epithelium and mucoid cells.

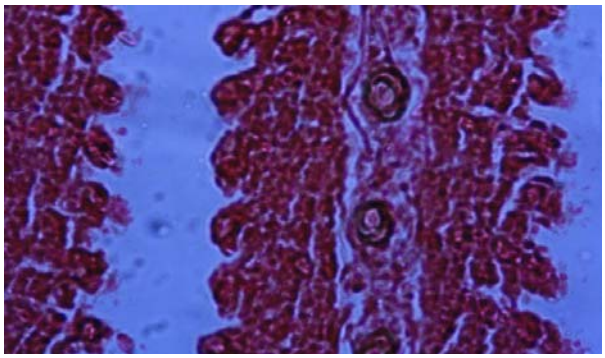

Fig 12 Untreated gill of *L. rohita* fingerlings (Control) (H&E, 400X**)**

3.2.6 Gill under sub-lethal concentration of deltramethrin

Branchial arch swollen with accumulation of infiltrating cells, necrosis in the primary lamella, Fusion of adjacent secondary gill lamella. Vacuolization and degeneration of epithelial cells and pillar cells.

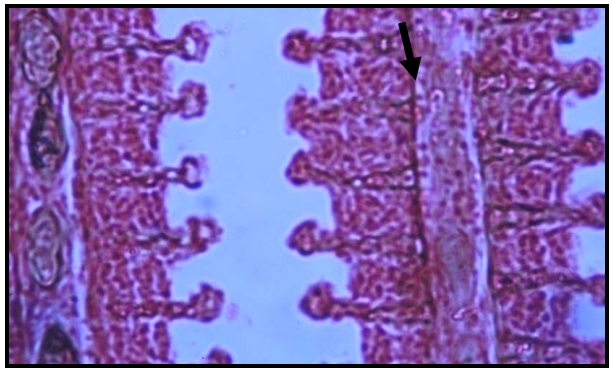

Fig 13: Gill of *L. rohita* fingerlings treated with sub-lethal concentration of deltamethrin showing swollen branchial arch with infiltrating cells and necrosis in the primary lamella. (H&E, 400X).

4. Discussion

4.1 Lethal toxicity

According to WHO [26], the 96 h LC_{50} of deltamethrin to fish is ranging between 0.4 and 2.0 g/l. [23] who determined 96 h LC_{50} value for *Cyprinus carpio* as 0.058 µg/ l. On the other hand, 24 h LC_{50} value of deltamethrin was 0.015 µg/ l in *Clarias gariepinus* [7] and 0.016 ppm in *Poecilia reticulate* [16]. In general, the LC_{50} value varies with respect to species and size of fish. Studies that indicate low level of deltamethrin (0.005 µg/L) in the aquatic environment may have a significant effect on carp populations [22].

In the present investigation the LC_{50} of fingerlings of rohu exposed to different concentrations of deltamethrin after the 24 h, 48 h, 72 h and 96 h LC_{50} value for *L. rohita* to be 0.39 (0.38 to 0.40) µg/l; 0.39 (0.36 to 0.42) µg/l; 0.38 (0.35 to 0.40) µg/l and 0.37 (0.36 to 0.38) µg/l respectively. Which is agreement with the result of Yonar and Sakin [28] who reported the LC_{50} values of deltamethrin for 24 and 96 h to be 0.438 and 0.38 mg/l respectively in *Labeo rohita.*

In previous studies, the 96 h LC_{50} values of deltamethrin to *Sarotherodon mossambica*, *Gambussia affinis*, and *Oncorhynchus mykiss* were in the range of 0.50 and 1.97 µg/ l [3]. Svobodova *et al.,* [23] determined 96 h LC_{50} value for *Cyprinus carpio* as 0.058 µg/ l. On the other hand, 24 h LC_{50} value of deltamethrin was 0.015 µg/ l in *Clarias gariepinus* [7] and 0.016 ppm in *Poecilia reticulate* [16]. In the present investigation the safe concentration and presumable harmless concentration of *L. rohita* fingerlings against deltamethrin were 1.00 µg/l and 0.117 µg/l respectively, which confirm the result of Gautam and Gupta (2008) who recorded range of safe dischargeable and presumable safe concentrations of cypermethrin as 1.04 to 1.09 µg/l and 45.18 to 75.25 µg/l respectively for the juveniles of *Poecilia* at selected levels of temperature, hardness, pH and salinity Studies that indicate low level of deltamethrin (0.005 µg/l) in the aquatic environment may have a significant effect on carp populations [22]. The LC_{50} values obtained in the present investigation for deltamethrin on *Labeo rohita* fingerlings is at par with the earlier findings with slight variations which is mainly attributed due to the differences in test conditions, species specificity of test chemicals, external factors influencing pesticide toxicity like dissolved oxygen, ambient temperature of water, pH, hardness of water etc. However, the results obtained for LC_{50} values and safe application levels of Deltamethrin in the present investigation are in agreement with the earlier findings; [22, 23, 28, 26, 7, 3].

4.2 Histopathology

Liver is an established organ and plays a fundamental role in the uptake, biotransformation and detoxification of foreign compounds [11] in the body and is thus a target organ of xenobiotics. Swelling of hepatocytes and diffused necrosis observed in the present study are in agreement with Deka and mohanta [8] in malathion treated *Heteropneustes fossils.*

In the present investigation the sub lethal effect of deltramethrin on liver of rohu fingerlings showed that changes in the liver were characterized by degenerated hepatopancreatic tissue, appearance of blood streak among hepatocytes, Formation of Blood cells among hepatocytes, swelling of the hepatocytes at places and diffused necrosis, which confirm the result of Singh [20] who studied the effect of dimethoate (30% EC) on liver of common carp, *Cyprinus carpio* reveals the degenerated hepoto pancreatic tissue, appearance of blood streak among hepatocytes and swelling of the hepatocytes at places and diffused necrosis. Sarkar *et al*. [19] reported significant changes such as hyperplasia, degeneration of hepatic mass, focal coagulative necrosis in *Labeo rohita* exposed to synthetic pyrethroids. Hepatic lesions in the liver tissues of fish *Gambusia affinis* exposed to deltamethrin were reported such as hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis, narrowing of sinusoids [5]. Although liver is considered as the primary site of detoxification, it is observed from the present study that, at lower concentrations of the toxicant stress the degenerations to the liver tissue caused is of lower magnitude whereas at the degree of tissue damage at higher concentration is more. Similar types of observations were also reported by the earlier authors.

The extent of histopathological damages caused to kidney in the present study under sub-lethal concentration appeared to be more pronounced than liver and includes degenerated haemtopoetic tissue with erythrocyte, shrinkage of glomuruli and lumen of tubules diminished, necrosis, cloudy swelling in renal tubules and granular cytoplasm. Which is similar with the result of Cengiz [5] who reported that the histopathological effect of deltamethrin on the kidney (degeneration in epithelial cells of renal tubule, dilation of glomerular capillaries, degeneration of glomeruli, intracytoplasmic vacuoles, narrowing of the tubular lumen) of common carp exposed to a concentration of 0.029 and 0.041 mg/l. Tilak *et al* [25] observed severe necrosis, cloudy swelling, cellular hypertrophy and granular cytoplasm were reported in *Channa punctatus* exposed to sub lethal concentration of butachlor and machete, which are agreed with the present study.

The histopathological alterations of gill exposed to deltamethrin under sub lethal concentration in the present study on gill indicates swollen branchial arch with accumulation of infiltrating cells, degenerated secondary gill lamellae, hypertrophy and hyperplasia of nuclei, degeneration of epithelial cells which creates acute respiratory distress in the test animal. This also agreed with the finding of Das and Mukherjee [6] and Tilak *et al* [24] who reported that histopathological alterations like hydropsy, vascular degeneration, bulging and necrosis in the gill tissues of *Labeo rohita* have been reported when exposed to sub lethal concentrations of malathion and chloropyrifos, Cengiz [5] observed necrosis of gill, aneurysm in secondary gill lamellae, lifting of the lamellar epithelium, epithelial hyperplasia were observed in common carp when exposed to sub lethal concentrations of deltamethrin. These findings are similar with the present observation.

The observations in the present study indicates that, exposure to sub lethal concentrations of deltamethrin cause a serious alterations in the tissue level organisations in the vital organs like liver, kidney and gills of *Labeo rohita* affecting the physiological process of the test animals leading to death which coincides with the findings of the earlier authors establishing a correlation between the pesticide exposure and histopathological alterations.

5. Conclusion

The present investigation was carried out in the College of Fisheries, Rangailunda in order to find out the acute toxicity effect of deltamethrin on the fingerlings of *Labeo rohita* along with observations on histopathological alteration caused at the tissue level in the vital organs like liver, kidney and gill. The probit regression analysis for 24 h, 48 h, 72 h and 96 h LC_{50}

value for fingerlings of *L. rohita* was calculated to be 0.39 (0.38 to 0.40) µg/l; 0.39 (0.36 to 0.42) µg/l; 0.38 (0.35 to 0.40) µg/l and 0.37 (0.36 to 0.38) µg/l respectively for deltamethrin. The safe application factor for delatamethrin was calculated to be 0.117µg/l. Swelling of the hepatocytes, diffused necrosis in liver, showing sever necrosis and swelling renal tubules in kidney, swollen branchial arch with accumulation of infiltrating cells, degenerated secondary gill lamellae in the gill were common histological alterations when exposed to deltamethrin. A series of pesticide or chemical products are now used in the aquaculturel practices instead of the biological or plant derivatives to get desired result by incurring low expenditures. Hence, the farmer needs to be provided with the abreast knowledge on the adverse impact of these products prior to their use

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