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Behaviour and Gill Histological Changes Induced By Cypermethrin (25% EC) On Black Molly, *Poecilia Latipinna*

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Abstract: Nowadays, Aquatic pollution has become a global problem. Fishes are sensitive to a wide variety of pesticide chemicals. The effect of Cypermethrin (25% EC) pesticide on behaviour and histopathology of black molly, *P. latipinna* is studied. The 96hr LC50 concentration is found as 0.035ppm. $1/10^{th}$ of the concentration 0.0035ppm is used for this study. Decreased swimming activity, excessive secretions of mucous around opercular region are observed in Cypermethrin exposed fishes. The gill histopathological changes observed in at 1^{st}, 4^{th}, 8^{th}, 12^{th} and 16^{th} day of 0.0035ppm exposure exhibited gill lamellae includes less affected and less damages in the gill structures, curling and fusion and shortening of gill lamellae, severe necrosis in primary and secondary gill lamellae, club shaped secondary gill lamellae. Cypermethrin (25% EC) pesticide becomes toxic to the aquatic organisms even in low concentration.

Keywords: Cypermethrin, black molly, behaviour, gill.

INTRODUCTION

Pesticides are one of the major classes of toxic substances used in India for management of pest in agricultural lands and control of insect vectors of human disease. For crop preservation from pest and to boost up crop production, in order to meet the ever-increasing food demand of the rising human population usages of pesticides are quite increased [1-3]. The runoff from treated areas enters the river and aquaculture ponds are likely to be contaminated by pesticides [4].

Pesticide affects the whole ecosystem, particularly the aquatic ones get enriched by their aquatic food chain through bioaccumulation, bioconcentration and biomagnifications process [5] leading to unwarranted mortality of aquatic biota especially fish [3]. Nowadays, a synthetic pyrethroid, Cypermethrin insecticides are extensively used due to their least toxic to mammals [6].

The synthetic pyrethroids are structurally similar compounds rendered photostable by additions of various substituent groups such as chlorine, bromine or cyanide on the basic structure. Because of their low mammalian toxicity, high insecticidal potency and lack of persistence in the environment, they have achieved widespread usage in agriculture as a household insectide and in wood preservation [7].

The acute toxicity (LC50) bioassay helps to assess the toxicity of the substances within short intervals and helps to define the safe levels threshold concentration by means of an application factor. Histopathological studies are very sensitive and crucial parameter, which reflects the effect of toxicants on organ [8]. The present study is framed to identify the acute toxicity (LC50) of Cypermethrin (25% EC) on aquarium fish black molly *Poecilia latipinna* and also to study their behavioural changes and gill pathological changes due to Cypermethrin pesticide exposure.

MATERIALS AND METHODS

Fish collection and maintenance

Aquarium fish black molly *Poecilia latipinna* (Figure 1), are procured (n=120; length=5 to 7cm) from Kolathur, Chennai and transported to the laboratory for acclimatization (15days). The fishes are fed with formulated fish feed and boiled egg and the tank water is exchanged every alternative days.

Fig-1: Experimental fish *Poecilia latipinna*

Solution preparation

Cypermerthin (25% EC) pesticide purchased from Sicorin 25 Scientific fertilizer Co. Pvt. Ltd, Pudukkottai. Stock solution is prepared by diluting 1ml of pesticide in 100ml of distilled water. From the stock, different concentrations of working solutions as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.08ppm are prepared by diluting the respective milliliter of the stock solution in 1L of distilled water. Feeding is stopped one day prior to the experiment and also during the experiment.

Bioassay test

Healthy fishes with average weight of 2gm and length of 5cm are selected for each group (n=10). The test solutions are added to the respective plastic troughs (10L). Fishes are observed for the next 4 days. The LC50 values are found by arithmetic graph method [9].

Behavioral and Histological techniques

From the LC50 values of 96hrs exposure, $1/10^{th}$ of the concentration was taken for the experiment. The duration of the experiment is 16days. Behaviour of the fishes is observed throughout the experiment. Tissues are taken for histological studies at the interval of 1^{st}, 4^{th}, 8^{th} and 16^{th} day. Dissected Gill from control and experimental groups are fixed in Bouin's fluid. Fixed tissues processed as per the standard histological procedure [10]. The tissues are examined under light microscope.

RESULTS

3.1. Lethal concentration (LC50 96hrs)

The LC50 value for 96 hours exposure of Cypermerthrin to *P. latipinna* fish is found to be 0.035ppm. The arthimetic graph is plotted and delinated in Figure 2. For the experiment, the $1/10^{th}$ of the concentration, i.e. 0.0035ppm is used.

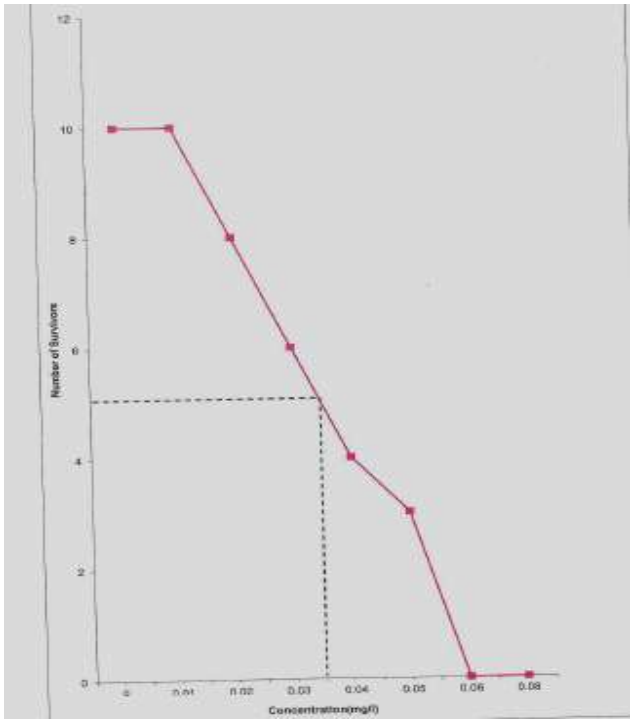

Fig-2: LC50 (96hrs) for Cypermethrin in *P. latipinna* by arithmetic graph method

Behavioural responses

The effect of Cypermethrin pesiticide on the fish *P. latipinna* behaviour is varied to various concentration of the pesticide. The control fish showed normal swimming activity, normal opercular movements and rare surfacing. No behavioural response is observed in 0.01ppm and 0.02ppm groups. A significant change in swimming behaviour and opercular beat are noticed in 0.03, 0.04, 0.05 and 0.06ppm groups. To avoid the toxicant, fishes showed irregular swimming, jerky movement, fast opercular movements, frequent surfacing, gulping of air and erratic movement but later on the fishes try to adjust with their medium with normal activity. Fishes exposed to higher concentration are discharge high mucous at the gill surface.

Gill Histology

Control fish gill

Teleost fish *P. latipinna* have pair of gill arches, slender gill filaments joined at the base by gill spectrum. Primary gill lamellae (PGL) consist of centrally placed rod like supporting axis where the secondary gill lamellae (SGL) are highly vascularized. Semicircular secondary gill lamella present on both sides of gill filament where covered by simple squamous epithelial cells (EC) and many capillaries separated by pillar cells. Cells contains single nucleus, which are flattened in appearance. Inter lamellar region the central axis (CA) is present between two adjacent gill lamellae (Figure 3).

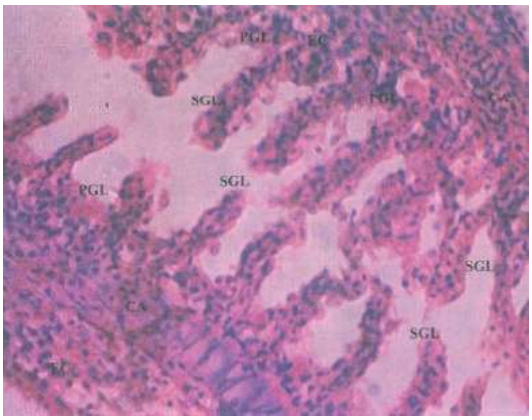

Fig-3: L.S. of *P. latipinna* gill tissues showing normal structure

Cypermethrin exposed gill

Cypermethrin (0.0035ppm) exposed gill tissues are studied on various days. Gills showed less affected gill structure in 1st day, with disturbed primary gill lamellae (PGL) and secondary gill lamellae (SGL) with central axis (CA) (Figure 4). Fused secondary gill lamellae (FSGL) and curled secondary gill lamellae (CSGL) are observed in 4th day gill tissues (Figure 5). Destruction of secondary gill lamellae (DSGL) observed in 6th day gill tissues (Figure 6). The gill of the *P. latipinna* exposed to 0.0035ppm Cypermethrin for 12 days showed necrosis (NEC) in primary and secondary gill lamellae (Figure 7). Severe necrosis in the secondary gill lamellae and club shaped secondary gill lamellae are observed in 16 days 0.0035ppm Cypermethrin exposed gill tissues (Figure 8).

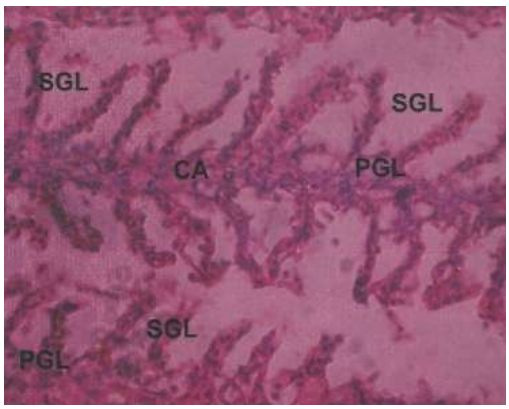

Fig-4: L.S. of *P. latipinna* gill tissues exposed to 1 day sublethal exposure of 0.0035ppm Cypermethrin

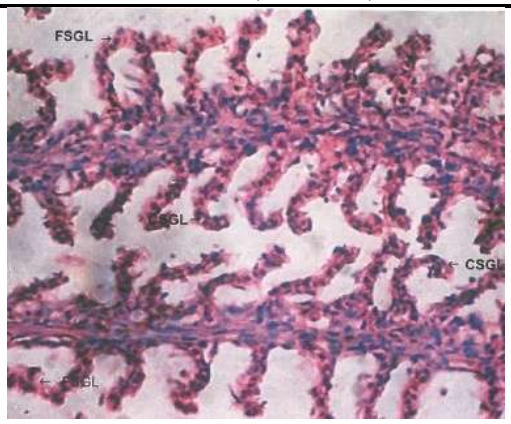

Fig-5: L.S. of *P. latipinna* gill tissues exposed to 4 days sublethal exposure of 0.0035ppm Cypermethrin

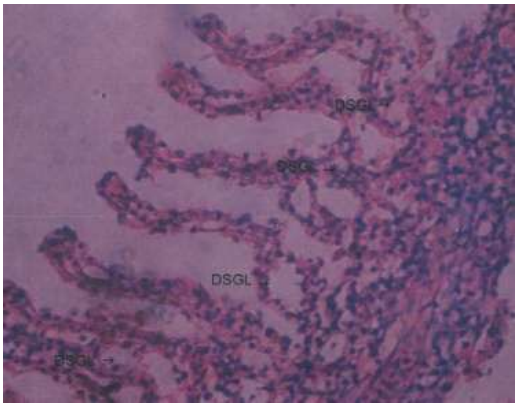

Fig-6: L.S. of *P. latipinna* gill tissues exposed to 6 days sublethal exposure of 0.0035ppm Cypermethrin

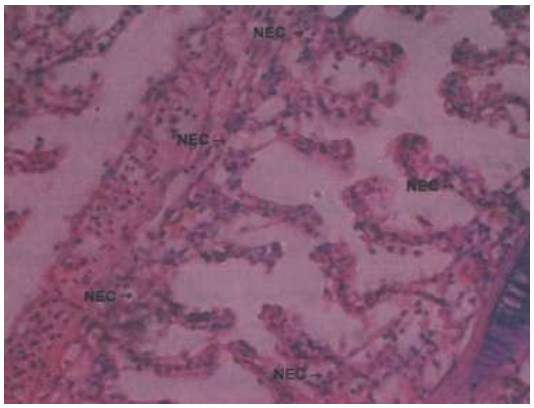

Fig-7: L.S. of *P. latipinna* gill tissues exposed to 12 days sublethal exposure of 0.0035ppm Cypermethrin

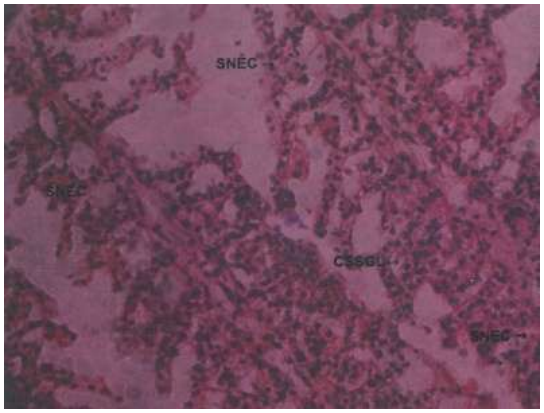

Fig-8: L.S. of P. latipinna gill tissues exposed to 16 days sublethal exposure of 0.0035ppm Cypermethrin

DISCUSSION

Rapid gill movement, erratic swimming, swimming at the water surface and gulping of air for prolonged time and motionless activities are observed in Deltamethrin exposed guppy fish, *Poecilia reticulate* [11], common carp, *Cyprinus carpio*, silver catfish [12]. El-Sayed and Saad [13] described the abnormal behavioural response in Deltamethrin exposed Nile tilapia, *Oreochromis niloticus*. Reduced swimming ability in Deltamethrin exposed rainbow trout, *Oncorhynchus mykiss* [14].

Gills are the most important vital organ for respiration, become the first target organ to come in contact with external toxicant. Pesticide exposure alters the normal histology of fish gill [15]. A synthetic pyrethroids Fenvalerate exposure to *Cirrhinus mirgala* exhibits separation of epithelial layer from the central sinus of filaments and the degree of dilation in primary gill lamellae is more when compared to primary gill lamellae [16]. Progressive degeneration, bulged tips of primary gill lamellae, club shaped secondary gill lamellae are observed in Fenvalerate exposed *Ctenopharyngodon idellus* [17].

Similar to our results, Rainbow trout administered with Cypermethrin both directly through water and indirectly through food evidenced distortion of secondary gill lamellae, cells separation, and gill necrosis, mucous cell hyperplasia are due to the partial metabolized toxicant in gills reached through circulatory pathways resulted inflicted lamellar aneursysm with separated gill filaments [18] whereas the increased duration of pesticide exposure increases the severity in the fish organ [19].

CONCLUSION

The effect of Cypermethrin (25% EC) pesticide on behaviour and histopathology of black molly, *P. latipinna* is studied. The 96hr LC50 concentration is found as 0.035ppm. Decreased swimming activity and excessive secretion of mucous around opercular region in pesticide exposed fishes. The histopathological changes observed in *P. latipinna* exposed to 0.0035ppm at 1^{st}, 4^{th}, 8^{th}, 12^{th} and 16^{th} day in gill lamellae includes less affected and less damages in the gill structures, curling and fusion and shortening of gill lamellae, severe necrosis in primary and secondary gill lamellae, club shaped secondary gill lamellae. Cypermethrin (25% EC) pesticide becomes toxic to the aquatic organisms even in low concentration.

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