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Evaluation of Deltamethrin Induced Stress on Teleost Model, Common Molly *Poecilia sphenops* (Valenciennes, 1846)

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Abstract

Deltamethrin, a synthetic pyrethroid insecticide contaminating aquatic ecosystem as a potential toxic pollutant is under investigation in the present study of chronic toxicity. Bony fishes were selected for the bioassay experiment. The 96hr LC_{50} value, behavioural assessment post treatment and evaluation of stress parameters by studying the histopathology of the fish tissues were the primary components of the study. For the experimental setup, a commonly available teleost fish, Common Molly *Poecilia sphenops* (Valenciennes, 1846) was obtained and kept in well aerated aquarium tanks for a period of 7 days to acclimatize. The water parameters like pH, temperature and dissolved oxygen were regularly checked and observed every day. Fishes were divided in groups (low concentration, medium concentration, high concentration and control group). We recorded a continuous observation of changes in fish behaviour after giving different dose of deltamethrin. After exposure of the different concentrations of deltamethrin, random samples were collected from the each of the group and were sacrificed for their regular histopathological studies for gills and muscles.
Keywords: Insecticides, fish, LC_{50}, behaviour, histology.

1 Introduction

Insecticides are the second-largest group of pesticides used worldwide. The synthetic organic insecticides category has traditionally been classified into one of four main chemical groups: organochlorines (e.g. DDT and chlordane), organophosphates (e.g. parathion and diazinon), carbamates (e.g. carbaryl), and pyrethroids (e.g. natural and synthetic) (Johnson et al., 2010). Insecticides are considered as the most powerful and financial mechanism for controlling the risks caused by undesirable

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vermin in agriculture. Over utilization of such insecticides causes impact on non-target creatures and results in physiological and biochemical changes that would also affect the tertiary consumers, the human beings as well. In this manner, insecticides have picked up an overall acclaim and also concern as well. Additionally, the insecticides affecting the non target life form can result into numerous erratic poisonous impacts like nerve tissue damage and numerous respiratory and hematological ailments (WHO, 1990). Insecticides exposed by agricultural run-off pollute aquatic bodies and also remain persistently in water for a considerable period which adversely affects fishes and other aquatic animals. Being present, in such situations the diversity of organisms are harmed. The research of diverse fauna especially on the fishes will play a role for determining the effect of chemical substances of pollutant, which is also diverse on aquatic ecosystems (Kayhan et al., 2013). The ecological hazards associated with the use of organochlorine, organophosphate, and carbamate pesticides pushed the presentation of another generation of pesticides with a lower level of persistence. So the synthetic pyrethroids have risen as viable substitutes. The synthetic pyrethroids are lesser important and less poisonous to mammals and birds (Smith and Stratton, 1986). One of the pyrethroids that has discovered wide scalesuitability is deltamethrin (S)a-cyano-3-phenoxybenzyl-(1R)- cis- 3- (2.2-dibromovinyl)- 2,2-dimethylcyclopropane carboxylate). It has been broadly used in agriculture and forestry because of its high activity against a large spectrum of insect pests (Villarini et al., 1998). Deltamethrin is a pyrethroid insecticide, synthesized in 1974 and was marketed in 1977. Pyrethroids pose a serious probable hazard to fish and other aquatic organisms because of the use in many aquatic larvicidial programs (Smith and Stratton, 1986). Pyrethroid insecticides are very much used compounds because of their (1) discerning toxicity, (2) towering potency, (3) ability to lessen disease transmission, (4) relative permanence in the environment and ease of degradation in vertebrates, and (5) small cost. Emulsifiable pyrethroid concentrate formulations are usually two to nine times more toxic than the technical grade, most likely because of synergistic interactions (Sanchez-Fortun and Barahona, 2005). Deltamethrin products are among the most popular and widely used insecticides in the world. Deltamethrin have been used worldwide to control a variety of insect pest in different environmental conditions. In last two decades, the use of deltamethrin is increasing in farming practices. Deltamethrin is also widely used as an insecticide in major agricultural crops like cotton, groundnut, rice etc. It is used as one of the components of insecticides in managing malarial vectors, particularly Anopheles gambiae, and while being the majority employed pyrethroid insecticide, can be used in combination with, or as a substitute to permethrin, cypermethrin and other organophosphate-based insecticides, such as malathion and fenthion. This insecticide is extremely toxic to aquatic life, particularly fish, and therefore must be used with intenseconcern having water bodies around.There are many uses for deltamethrin, from agricultural usage to home pest control. Deltamethrin has been involved in preventing the increase of diseases carried by tick-infested prairie dogs, rodents and other burrowing animals (Wishart, 2018). It is useful in eliminating and preventing a wide variety of household pests,likespiders, fleas, ticks, carpenter ants, carpenter bees, cockroaches and bedbugs.

Deltamethrin is considered as one of the most important toxins among the insecticides which target the non-target organisms in ponds, lakes, and streams bounded by agricultural area. Lakes, ponds, rivers, seas have become the illegal end point of discharge of the agrochemicals and also waste products. The agrochemical may exert lethal effect to the environment and the physiological and biochemical process in the fish by getting accumulated in their body. The fish exhibits numerous symptoms of stress when treated with deltamethrin (Datta and Kaviraj, 2003). The tissue damages realized by such pollutants can be easily observed because the fish gills come into instantaneous contact. Deltamethrin inhibits the gills and heart Na+K+-ATPase activity of A. multispinnis (Assis et al., 2009). Cipermethrin, also a pyrethroid, caused ATPase reticence in brain, liver and kidney of carps exposed during 45 days of initial contact period (Das and Mukherjee, 2003).

The high rate of absorption of deltamethrin through gills also makes fish a susceptible target of its toxic conditions (Srivastav et al., 1997). In fish, the internal condition is alienated from the external condition by just a couple of microns of delicate gill epithelium and thus the branchial capacity is very responsive to any environmental changes orpollution. Henceforth, fish servesas an amazing bioassay animal for studying the toxicological impact studies and have been broadly used for this purpose for many compounds. The present work can be summarized upon the finding out the analyzing the effects of Deltamethrin on the gills and muscles of a fish by studying the behavioral changes and histo-pathological evaluation.

2 Materials and Methods

Study model

The common Molly, *Poecilia sphenops*, belonging to family Poeciliidae (Order: Cyprinidontiformes) a common freshwater aquarium fish is found in streams, pond, lake and brackish water habitats was chosen as an animal model for the experiment. They are omnivorous in nature and feeds on various aquatic invertebrates, such as insects, worms, plant and other organic debris in natural condition as well as in aquarium condition, in the deficiency of natural foods, nutritional artificiallyproduced feeds that contain all essential nutrients must be fed to fish. Moreover, mollies are very easily available with required sizes at the local aquarium market.

Test Chemical

A widely used formulated product, Deltamethrin (DECIS 2.8 W/W) manufactured by Bayer Crop Science Ltd. It was selected as the test chemical for the current study and was procured from the local market.

Experimental setup

The animal model selected was common molly fish of length 5±1 cm.Allthe experimental aquaria were cleaned and filled with 5 L of de-chlorinated tap water (temperature: 26±30ºC, pH: 7.5-8.5. Natural light: Dark photoperiod). Fishes were obtained and kept in the laboratory in sufficiently aerated aquarium tanks for a period of 3 days to acclimatize them to the laboratory conditions. Fishes were fed artificial food once in a day.

LC_{50} value

The fishes were not fed 24 h prior to the experiments and throughout the acute toxicity tests. The experiments consisted of a control group and five experimental groups. Acute test was conducted to determine the appropriate toxicity range. Assuming acute toxicity, 8 fishes of equal length and weight per group were exposed to different concentrations (1.5, 2.0, 2.5, 3.0 and 4.0 ml/5L) and a control group in the same capacity aquariums. During the 48 h acute toxicity experiment, the water in each aquarium was aerated continuously and had the same conditions as the acclimation period. Every 6 h the dead fishes were removed and the numbers of survivals were recorded. The LC_{50} values were calculated by the Probit Analysis test done by GraphPad Prism software. With the calculation of LC_{50} values, the dosage was decided for the experiment with 4 different groups.3 aquaria and 1 control aquaria groups with the fishes were kept in the de-chlorinated, properly aerated and well-conditioned water for the next study of analysing the changes in behaviour after the introduction of different sets of dose of deltamethrin.

Table 1. Experimental dose after estimating the LC_{50} value.

Sr. No.	Group	Dose
1	Control	-
2	Low	0.05 μ/L
3	Medium	0.1 μ/L
4	High	0.2μ/L

Behavioural study

Regular observations of changes in the behaviour of the fishes were recorded. Recordings were done with intervals of 10 minutes for 2-3 hours. Abnormal swimming behaviour like hitting aquarium walls, jumping, vertical and downward swimming pattern, operculum movement, sluggish behaviour and loss of balance were recorded at different time of interval.

Histopathology

Following the exposure of deltamethrin in the three groups, random samples of fishes were collected from each of the group, some of which had already died due to the different concentrations of deltamethrin or if alive were sacrificed by rapid chilling hypothermia. The gills and muscles were dissected out from the exposed fishes and fixed in 10% formalin for 24 hours. Further, they were processed for preparation of histo-pathological slides.

3 Results

The designed 48-h acute LC_{50} value (95% confidence limits) of Deltamethrin (Fig.1), dissolved in water, using a static bioassay method to adult Mollies (*Poecilia shenops*) was 0.4 μ/L. The control mortality was found to be zero. The results show that deltamethrin is highly toxic tofish.

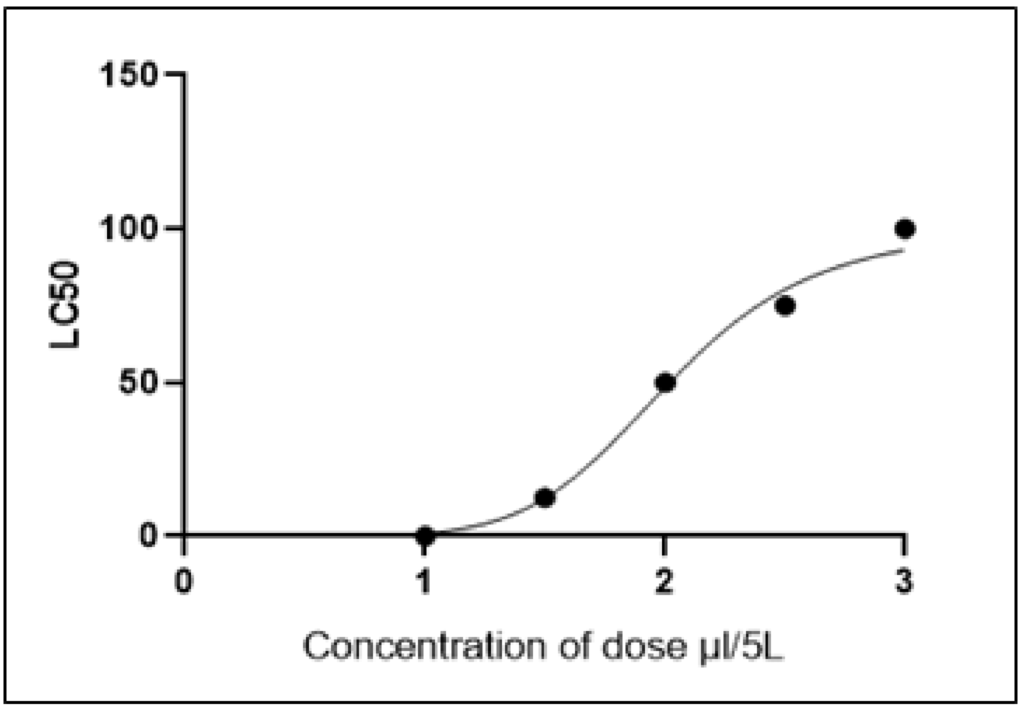

Figure 1. Dose concentration Vs LC_{50} value of Deltamethrin.

Behavioural observation

Observations of the behavioural responses of the Molly were recorded in every 10 minutes with and interval for 2-3 hours during the acute toxicity tests.

Table 1. Experimental dose after estimating the LC_{50} value.

Sr. No.	Dosage	Behavioural changes
1	Control	Very attentive to any kind of disturbance in or near the tank and no changes were observed.
2	0.05 μ/L	Not many changes were observed.
3	0.1 μ/L	Lessened general activity was recorded when compared with the control group and loss of equilibrium, jumping, and irregular swimming was observed.
4	0.2μ/L	Rapid opercular movement, inconsistent swimming more at the water surface and continuous gulping for air and prolonged and motionless behaviour by lying down on the aquarium bottom was observed after 20-30 minutes of sluggish movement.

Histopathology

The individuals in the control group did not exhibit any histological changes in any of the examined tissues. Morphological lesions observed in gills and muscles of Molly fish, *Poecilia sphenops* revealed some important alterations throughout the experiment. Tissue damages and injuries after 4 days (96hrs) of deltamethrin exposure are discussed.

Gills

The gill arches of *Poecilia sphenops* in the control group shows regular arrangement pattern. The arch contains primary lamellae (Figure 1) and projecting on the lateral sides of primary lamellae are the secondary lamellae (respiratory lamellae). The entire mass of primary lamellae is enclosed by stratified squamous epithelium. The surface of the secondary lamellae is covered with a delicate layer of a simple squamous epithelium that is the active exchange pillar cells. In the core of the primary lamellae, lies an inflexible mass of cartilaginous tissues around which are traces of vascular channels. The chloride cells are more frequent at the base of the secondary lamellae. Fish exposed to deltamethrin showed curling of secondary lamellae, hyperplasia of gill lamellae and augmented gill lamellae thickness resulting in fusion and cell death. Hyperplasia of the epithelial lining of the secondary lamellae, necrosis and decrease of the secondary lamella, uncharacteristic raising or inflammation of the epithelium, as well as fusion of the secondary lamellae and edematous changes were observed in fish exposed to deltamethrin (Plate1). These changes intensify with the enhancing of deltamethrin concentration.

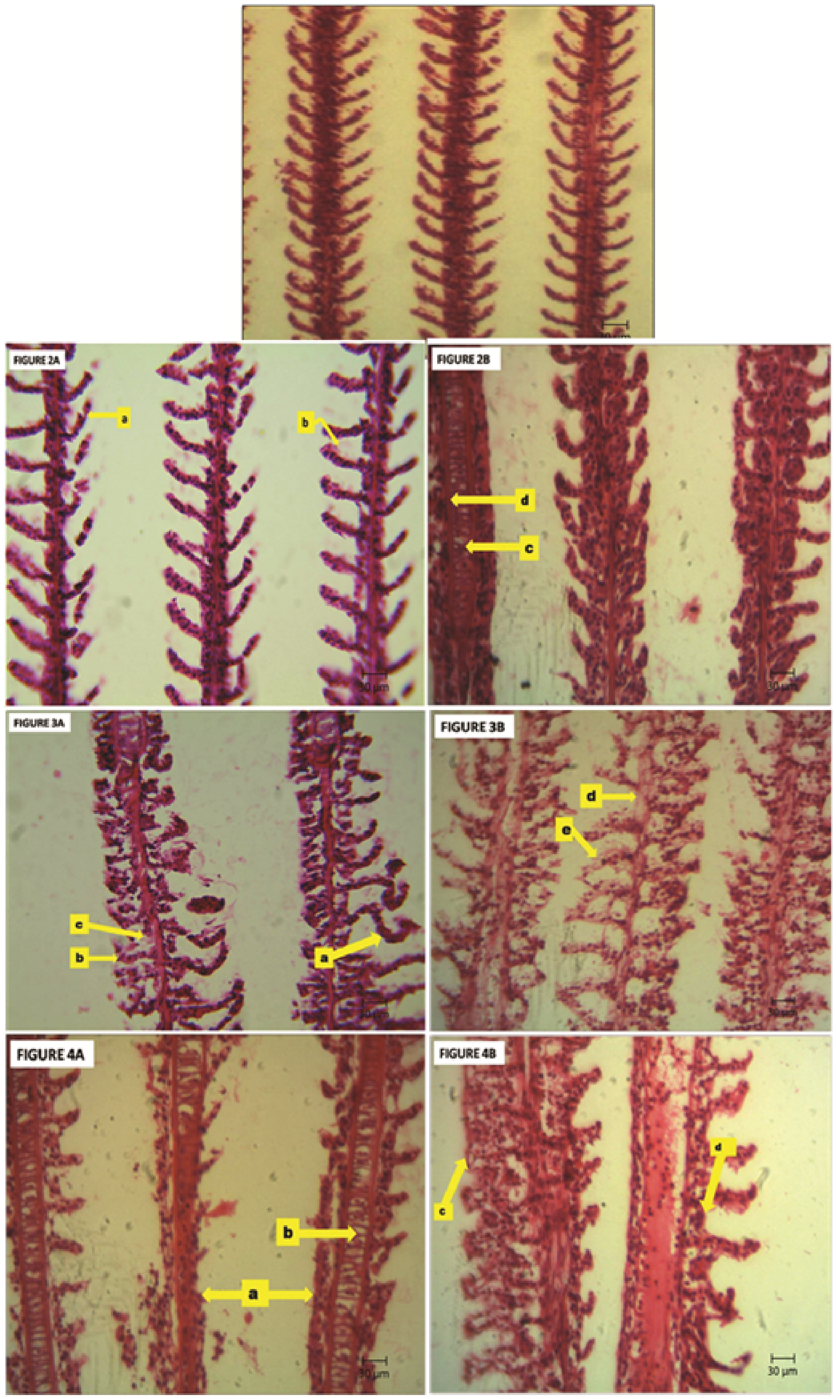

Figure 2. Control group of gill tissue of Poecilia sphenops (40x) showing primary lamellae and secondary lamellae. Figure 2A and 2B. 0.05 μ/L (low dose) deltamethrin treated fish gills. (40x) (a) Breakage of secondary lamellae, (b) Structural abnormalities in epithelium of secondary lamellae, (c) Enlargement of secondary lamellae,(d) Depicting proliferation epithelium of primary lamellae. Figure 3A & 3B. 0.1 μ/L (medium dose) deltamethrin treated fish gills (40x). (a) Curling of secondary lamellae, (b) Degeneration of secondary lamellae, (c) Haemorrhage in primary lamellae, (d) Degeneration of epithelial cells of primary lamellae, (e) Degeneration of primary lamellae. Figure 4A & 4B. 0.2 μ/L (High dose) deltamethrin treated fish gills (40x). (a) Complete erosion of secondary lamellae, (b) Enlargement of primary lamellae, (c) Epithelial necrosis and fusion of adjacent lamellae, (d) Lost of lamellar basement..

Muscles

Muscle is the tissue related to motion and is extensively distributed in various organs of the body. It is composed of elongated muscle fibres, each an individual muscle cell, connected together by connective tissues. Individual muscles show an arrangement of fibres into bundles separated from each other by connective tissue portions. Segmentation or metamerism of vertebrate musculature is clearly observed in the lateral muscles of the fishes. They are divided into myotomes or muscle segments, each of which is bent into a single V with the angle directed anteriorly (Ramesh and Nagarajan et al., 2013). The function of these lateral muscles is for movement. Basically, alternating contractions passing posterity along the muscles on the two sides of the body result in the well-known undulate movements of trunk and tail. Each myotome is divided into an upper (epaxial) and a lower (hypaxial) half by a groove running along the side of the fish (Ramesh and Nagarajan et al., 2013). Removal of the skin reveals the zigzag arrangement of the myotomes with the myosepta. Several histo-pathological alterations were seen in the muscles of Poecilia sphenops. The histological findings include deterioration in muscle bundles with aggregation of swollen cells between them and focal areas of necrosis. Also, fibrilae integrity lost, fasicular membrane damage, fasicular connective tissue damage, vacuolar degeneration in muscle bundles and shrinking of muscle bundles were observed. Edema between muscle bundles and spitting of muscle fibers were also seen.

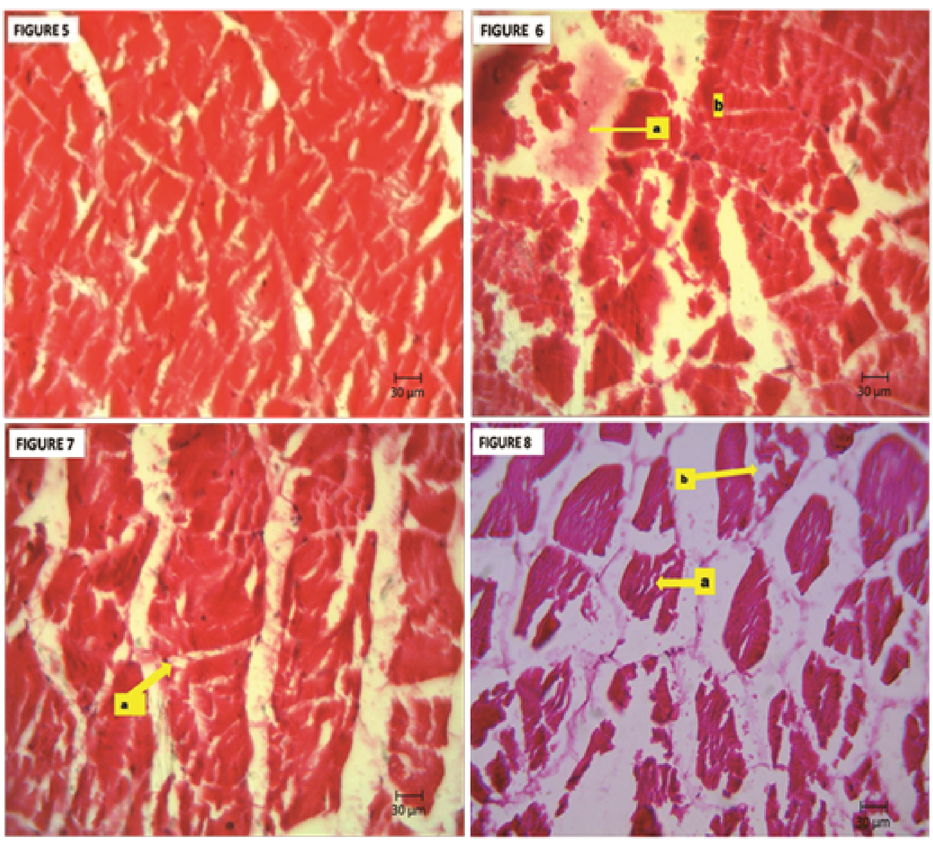

Figure 5. Control, Muscle tissue of Poecilia sphenops (40x). Figure 6. 0.05μ/L (low dose) deltamethrin treated fish muscle (40x). (a) Necrosis, (b) Weak integrity between muscles fiber bundles. Figure7. 0.1μ/L (medium dose) deltamethrin treated fish muscle (40x). (a) Connective tissue degenerated and fasicular integrity lost. Figure 8; 0.2 μ/L (High dose) deltamethrin treated fish gills (40x). (a) Degeneration in muscle bundles and muscle fibre bundles, (b) Connective tissue completely damaged, hence the vacuity is developed.

4 Discussion

The present study deals with the toxic effect of deltamethrin on teleost fish (Poecilia sphenops). Based on the findings of this study 48hr LC_{50} values for deltamethrin in the Molly fish (Poecilia sphenops) were $0.4\mu/L$ in present work. The USDA National Agricultural Pesticide Impact Assessment Program's EXTOXNET has reported deltamethrin acute toxicity to fish during laboratory testing to be in the average range LC_{50} value of 1–10 mg/L. (Mittal et. al., 1994) reported deltamethrin toxicity to Mollies (Poecilia reticulate) as the most toxic of the pyrethroids studied. The sensitivity of different species and probably diverse environmental conditions can be the most important factor for such a significant difference in the LC_{50}values for deltamethrin among various species of fish.

The study shows that there is change in swimming pattern, loss of equilibrium, jumping, gulping of air and rapid gills movement of fish exposed to deltamethrin. Previous studies have shown that behaviour changes like loss of equilibrium, rapid surfacing and gulping of air noticed (Barot and Bahadur, 2012). There could be attributed due to the disruption of nervous system in fish (Desai and Parikh, 2013).

In the present study, the histopathology of muscle showed progressive damage in the arrangement of muscle with escalating concentrations of the deltamethrin insecticide. Muscle tissue from P. sphenops on low dose exposure of deltamethrin (Figure 6) shown cell necrosis and weak integrity between muscles fibre bundles. However, at medium dose of deltamethrin (Figure 7) exposure, there were different faciculae are usually packed with connective tissue, here ass toxicological impact that this connective tissue degenerated and hence fasicular integrity lost. At high dose of deltamethrin (Figure 8) exposure, there were deterioration in muscle bundles and muscle fiber bundles are highly disintegrated, and surrounding connective tissue completely damaged hence the vacuity are developed. The fish showed noticeable thickening and separation of muscle bundles with severe intracellular swelling. Such similar observation has also been made by (Das and Mukharjee, 2000). Previous studies observed the degeneration of muscle bundles with assemblage of inflammatory cells between them and primary areas of necrosis. Also, vacuolar degeneration in different muscle bundles and atrophy of muscle bundles in fish exposed to different pollutants has been performed(Mohamed, 2009).

The environment and particularly susceptible to changes in the physic-chemical quality of the water are considered as the primary target of the contaminants. These histo-pathological changes may be a response to the toxicant intake or an adaptive reaction to prevent the ingress of the toxicant through the gill surface of fishes. Besides, changes like increase of epithelial cells, partial and total combination of secondary lamellae as well as lifting of epithelium are defense mechanisms as this would resulting in the augmentation of the distance connecting the external environment and the blood thereby serving as a barrier to the entrance of the pesticides (Ortiz-Hernández et al., 2011).

5 Conclusion

The present study shows that the insecticide deltamethrin on acute exposure shows adverse effects on molly fish, *Poecilia sphenops*. Thus, it has the potency to cause varied effects on the behaviour by rapid movement of the operculum, sluggish movement at the bottom of the tankby the fish in $0.2\mu/L$ of deltamethrin. The insecticide thus, can cause damage to aquatic organisms being exposed to it. As seen in molly, deltamethrin has a potential to cause damage even at low concentrations.

The alterations in gills and muscle structure are evidence to it showing degeneration of the fibers and necrosis of cells.

Conflict of interests

The authors declare that there are no competing interests.

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