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Research Article

EVALUATE THE EFFECTS OF A PESTICIDE SYNTHETIC PYRETHROID TO FRESHWATER FISH TISSUES OF *ESOMUS DANRICUS* (HAM.)

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ABSTRACT

The present investigation was designed to compare the responses in freshwater fish *Esomus danricus* exposed to a cypermethrin [(R,S)-a-cyano-3-phenoxybenzyl (1RS)-cis,tra-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate]. Cyoermethrin is a synthetic pyrethroid, in this effects may include both lethal and sublethal concentrations, which may change the growth rate of fish development, reproduction of the fish, physiology, histopathology, biochemistry and behavior on target organisms and undesirable perturbations in the environment. The effect was assessed on the basis of the comparison of results of GSI and HSI and histological examinations. The present study showed that observed alterations in all GSI, HSI and histological observations of fish treated pesticide.

Keywords: *Esomus danricus*, Cypermethrin, Toxicity, Histology.

INTRODUCTION

Cypermethrin is one of the synthetic pyrethroid which is most comprehensively used for more than three decades as possible alternative to the organophosphate, organochloride and carbomate pesticide. It is among the most effective pyrethroid preparations. The synthetic pyrethriods are reported to more toxic than other pesticides and are widely used in crop protection, home pest control, forestry and in public health (Bernet *et al.*, 1999; Velisek *et al.*, 2006; Karthigayani *et al.*, 2014; Parithabhanu and Deepak, 2014). Fish are often used as indicators of such biological impacts of pollutants as they respond to low concentrations of toxic substances, fish sensitivity to pyrethroids may be explained by their relatively slow metabolism and elimination of these compounds (Bradbury and Coats, 1989; Ayas *et al.*, 2007; Sarkar *et al.*, 2005; Velisek *et al.*, 2006; Karthigayani *et al.*, 2014).

The mechanism of its effectiveness in the case of fish is the same as that of other pyrethroids containing-cyano-3-phenoxybenzyl groups. They block the sodium channels of nerve filaments and as well as to inhibit the ion exchanging channels block, thereby augmentation of their depolarization phase and moreover, they effect the GABA receptors in the nerve filaments (Bradbury and Coats, 1989; Hayes, 1994; Velisek *et al.*, 2006). Developing countries use only 20% of the world's agrochemicals, yet they suffer 99% of deaths from pesticide poisoning (Atreya, 2008; Remor *et al.*, 2009; Patel *et al.*, 2016). Joseph and Raj (2010) have reported the application of environmental toxicology studies on non mammalian vertebrates is rapidly expanding, and for aquatic system, fish have become an indication for the evaluation of the effects of noxious compounds. Pesticides at high concentrations are known to reduce the survival, growth, and reproduction of fish and produce many visible effects of fish (Mensah *et al.*, 2014).

However, the short term effects, fish being an essential compound of the inland fisheries are particularly sensitive to a wide variety of pesticide chemicals and their productions are easily affected by such toxic polluntants (Tilak *et al.*, 2005; Karthigayani *et al.*, 2014 and Uma *et al.*, 2016). The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target aquatic organisms. Fish are among the group of non-target aquatic organisms. The present paper is a contribution to the assessment of toxicity and effects of a cypermethrin-based pesticide to Teleosts fish, *Esomus danricus.*

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MATERIALS AND METHODS

The fish *Esomus danricus* (Ham.) weighing 1-3 g, used for this research work, were collected from Mappedu, Agaram (near Tambaram, Chennai) during the month of April. The fishes were acclimatized for two weeks at lab condition before starting the experiment. The male and female fish with a ratio of 1:1 were kept in four glass tanks measuring 2'x1'x1.25'(80 lit), each. During the time of acclimatization and throughout the experiment the fishes were fed with a composition of rice barn, wheat flour, fish meal and groundnut oil cake. All the fish tanks were provided with aerators and the water changed every alternate day.

The synthetic pyrethroid, cypermethrin (commercial grade insecticide-cymbush 10EC (RS) - alpha - cyano - 3 - phenoxylbenzyl (IRS) - cis, trans - 3 - (2-2 -dimethyl - cyclopropane carboxylate) used in this experiment was obtained from local agricultural shop. The composition of the compound: Cypermethrin 10% (w/w), cresloxAE1 5%, cresloxAE2 2.4%, and cresloxAE3 0.61%. The product manufactured by the company Zeneca, Agrochemical Ltd., U K.

Experimental design

The different concentration of cypermethrin was prepared by diluting the cypermethrin. Various dilutions were prepared to find the safe concentration for the experiment. The commercial product concentration of cypermethrin is 10%, from this 1% of the stock solution of cypermethrin prepared. From 1% of stock solution, different concentrations were prepared to which fishes were exposed. At 5 ppb, 4 ppb, and 3 ppb concentration fishes showed high mortality at lesser than 24 hours. Further, it was found that the fishes were alive at the concentration of 2 ppb for 24 hours. Hence in the present study, three different concentration, 0.02 ppb 0.2 ppb and 2 ppb taken to expose the fish. (i.e., control, Exp.-I 0.02 ppb, Exp.-II 0.2 ppb and Exp.-III 2 ppb). 30 fishes were introduced in each tank with equal number of male and female fish. Cypermethrin was added in the tank at chosen concentration in 40 liters of water. The experiment was conducted for 45 days. After 45 days, the fishes were dissected, and the skin, liver, ovary, testis and brain tissues were removed, weighed and stored in -70^{o} C for further analysis.

HSI and GSI

The hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated using the following formula

$$\text{Hepato somatic index} = \frac{\text{Weight of liver}}{\text{Weight of the fish}} \times 100$$

$$\text{Gonado somatic index} = \frac{\text{Weight of gonad}}{\text{Weight of the fish}} \times 100$$

Histological observation

For the histological studies, the gonads (ovary and testis) and liver were sectioned at 5µ thickness and the routine haematoxylin and eosin staining procedure used. The tissues fixed in Bouin's fluid, dehydrated with alcoholic series, cleared with xylene, blocked with paraffin wax, sectioned with microtome, fixed in microslide, processed for dewaxing using xylene, hydrated with alcoholic series, stained with heamatoxilin, counter stained with eosin, dehydrated with alcoholic series, and mount with DPX. These slides were observed to study the differences between the control and the experimental tissues. Microphotograph taken to present the changes observed in the tissues.

RESULTS

Behaviour

Behavioural observation was carried out during the course of the experiment. The fishes exposed to cypermethrin showed signs of restlessness, convulsions, erratic swimming and breathing difficulties when compare to control fish. Although the feeding was less in the treated fish, both the control and treated fish showed grouping behaviour during feeding.

GSI

The testicular GSI decreased at 0.02 ppb and 0.2 ppb concentrations of cypermethrin, and in the 2 ppb no difference was noticed (Figure 1). The ovarian GSI decreased at all the three concentrations of cypermethrin treated fishes. The ovary showed the dose dependent decrease ($P<0.01$) of GSI. When compare the testis, ovary is more susceptible to cypermethrin toxicity.

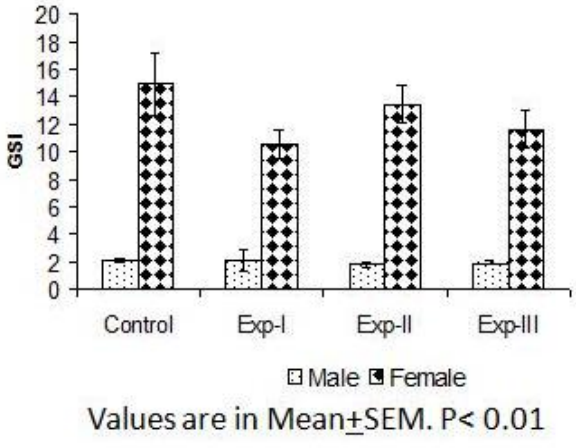

Figure 1. Effect of cypermethrin on testis and ovary (GSI) of *E. danricus.*

HIS

The HSI (Figure 2) value shows a difference in experiment fish when compared to control. The cypermethrin treated

fishes showed high level of HSI (P<0.01). In comparison between male and female shows that female HSI is more than the male.

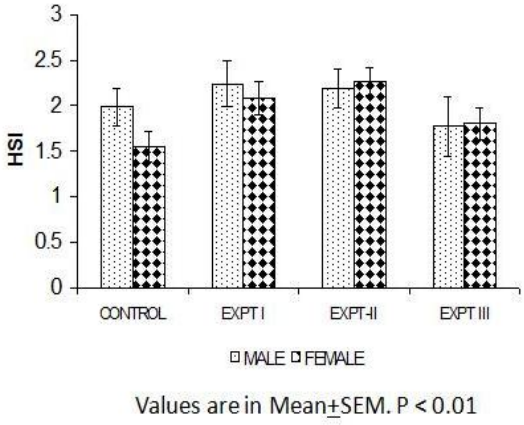

Figure 2. Effect of cypermethrin on hepatic cells (HSI) of *E. danricus.*

Histological observation

Liver

The histological analysis of liver was carried out to study the inner details of cells. The liver cell of the control fish showing awell organised hepatocytes with a prominent nucleus in the centre (Plate- I. A). The treated group liver shows that at 0.02 ppb not much difference is noticed and the cells are well developed with a nucleus (Plate-I. B). In 0.2 ppb dilated hepatocytes are noticed (Plate-I. C). While in 2 ppb, the size of the hepatocytes are reduced and the cells showing degeneration (Plate-I D).

Testis

The testis of *E. danricus* shows a typical organisation of seminiferous tubules (Plate-II. A). Each tubule is an autonomous unit with the developing germ cells. The proliferation's of seminiferous tubules taking place in this period showing the resting phase. The tubule boundary cells (germinal epithelium) are well developed. Histologically not much difference is seen in 0.02 ppb treated group (Plate-II. B) When compared to control, and the interconnective tissues are well organised. The testis of 0.2 ppb shows disintegration of seminiferous tubules (Plate-II. C). At 2 ppb, the testis showing the immature seminiferous tubules and dilated somatic cells and increased vacuolisation in spermatogonial cells (Plate-II. D).

Ovary

The control ovary has the stage-I oocyte with a densely packed cytoplasm and marginally arranged nucleolus in the nucleus (Plate-III. A). It clearly indicates that the animal is in the resting period. The ovaries of the fishes subjected to different concentration of cypermethrin showing the marked changes. The cytoplasmic agglutination was noticed at 0.02 ppb (Plate-III. B) The oocyte shows vacuole formation and disintegration of nucleus and more number of immature oocytes are noticed at 0.2 ppb (Plate-III. C). At 2 ppb ovary showing the degenerative oocyte and the presence of vacuolisation in cytoplasm. The outer thecal layer is separated from the inner content and the distance between these two layers is more (Plate-III. D).

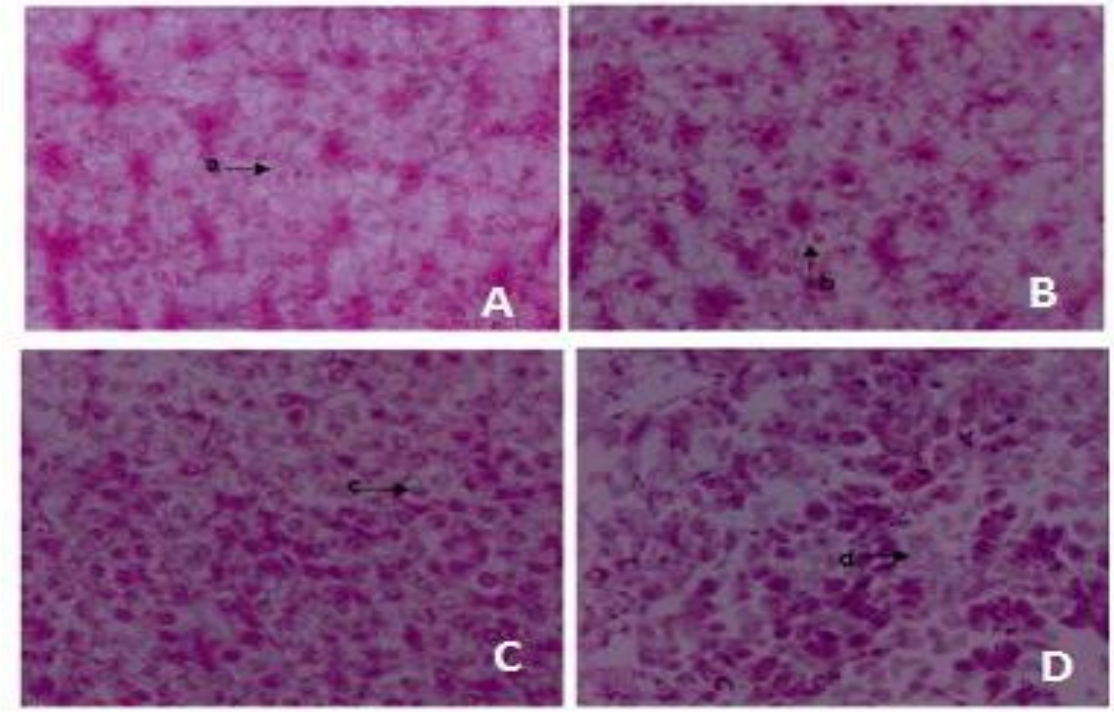

Plate I. Shows the Effect of cypermethrin on Liver histology observation of *E. danric.*

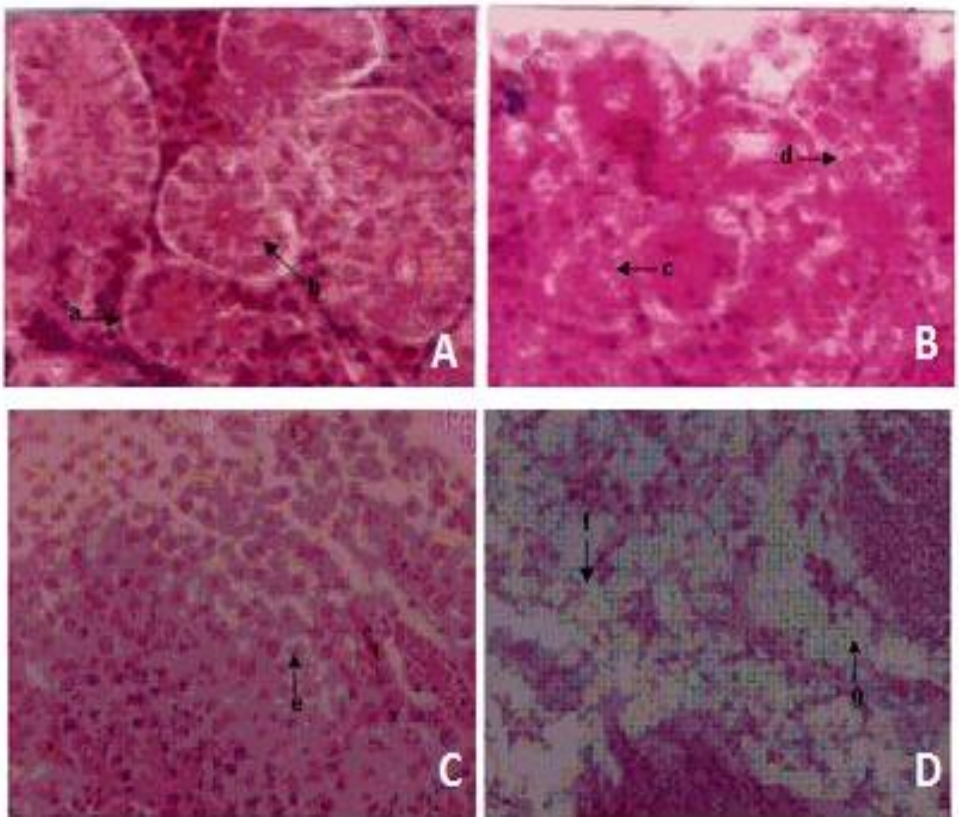

Plate II. Shows the Effect of cypermethrin on Testes histology observation of *E. danricus.*

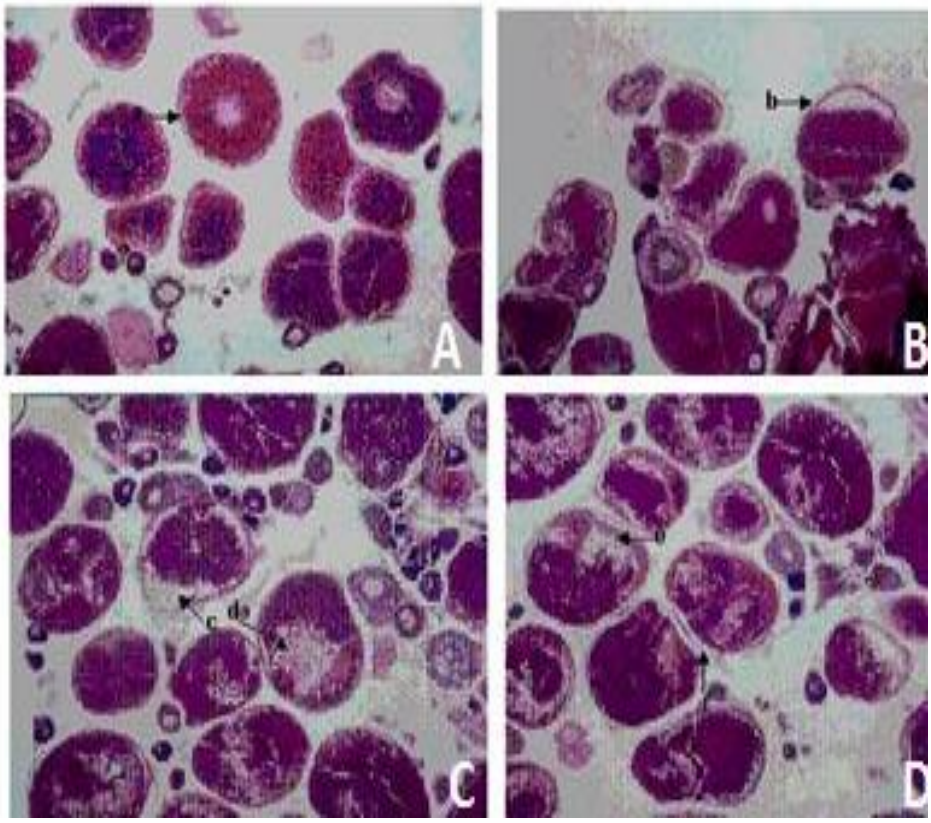

Plate III. Shows the Effect of cypermethrin on ovarian histology observations of *E. danricus.*

DISCUSSION

Insecticide effect on various species of teleosts shows histological, physiological and behavioural changes (Firat *et al.,* 2011). The acute and chronic toxicity of several insecticides to a large number of teleosts are also available (Adhikari *et al.,* 2004). However, the toxic effect of pyrethroid is not much studied. Some of the experiment conducted using pyrethroids were reported (Oortgeiesen *et al*., 1989; Willianson *et al*., 1989; Bernard and Grazyna, 1999). The present result shows that cypermethrin is more or less equally toxic as that of organochlorine compound as per the earlier finding in teleosts (Konar, 1981; Mathanna *et al*., 1986; Haider and Inbaraj, 1986). Fishes showed restlessness, rapid body movement, convulsion, and difficulty in respiration, intense opercular movement, change in colour and loss of balance when exposed to different concentration of cypermethrin. Similar changes in behaviour are also observed in various fishes exposed to different insecticides (Singh *et al.,* 1984; Das and Mukherjee, 2003).

Cypermethrin was found to exert a considerable influence in the number of melanophores. A reduction in the number of melanophores and also alteration in their shape were reported in fishes *Rasbora daniconics* treated with carbofuran (Kulshrethra and Arora, 1998). Studies on *O. mossambicus* have proved the effect of the chemical fenvalrate in changing body colour (Radhaiah and Rao, 1988). The present study has confirmed this action on *E. danricus* treated with cypermethrin. Fishes exhibited a change in body colour, reduction in number and also alteration of shape of the melanocytes. These changes in the treated fish shows that melanocytes, which are located in the basal layers of the epidermis, synthesising melatonin could have been affected due to cypermethrin. Fishes treated with cypermethrin exhibit scarce lipid droplet on the epithelial layer as compared to the control fishes with dense lipid droplets. A reduction in the density of lipids on the epithelial skin was reported by Govindan *et al.* (1994) in *Gambusia affinis* exposed to phosphamidon.

Gonadosomatic index is an indirect method of studying the spawning season in fishes. Gonads undergo regular seasonal cyclical changes in weight, particularly in females. Such cyclical changes are indicative of the spawning season (Qasim, 1973). The gonads (Testis and Ovary) are highly sensitive to toxic insecticides. A significant reduction in GSI value was reported in *Channa puntatus* exposed to fentrothion and carbanyl (Saxena and Garg, 1978). Similar results were reported in *C. carpio* treated with BHC (De Boeck *et al.,* 2001 and David *et al.,* 2004) and in *Sarotherodon mossambicus* exposed to Malathion (Shukla *et al.,* 1984). The GSI and HSI level decreased in summer flounder *Paralithus dentalis* when treated with O, P'- DDT, 17 β- Estradiol and P, P' DDE, *Oreochromis mossambicus* (Sadekarpawar and Parikh, 2014). A reduction in GSI and HSI was reported in *C. puntatus* (Ram *et al.,* 2001) and *H. fossilis* (Chatterjee *et al.,* 1997) exposed to carbofuran. This supports the present study that the GSI and HSI levels decreased with different concentration of cypermethrin. This reduction could be resulted due to the negative impact of cypermethrin on the protein production in the liver and their accumulation in the ovary.

Pesticides are known to affect the testis in vertebrates and cause significant reduction in the GSI or percent weight of the testis (Sehgal and Pandey, 1984). Similar effects were noticed in *S. mossambicus* exposed to dimecron (Lakhani and Pandey, 1985; Sadekarpawar and Parikh, 2014), and the treatment with fenitrothion and carbofuran in *C. puntatus* (Saxena and Mani, 1985). Ovaries in teleosts may be gymnoovarian or cystovarian type. In almost all freshwater fishes of India cystovarian condition is prevalent (Hoar, 1969). The germinal epithelium is projected into the ovarian lumen in the form of freely suspended folds. Histologically an ovary consists of developing oocyte stage-I oocyte are translucent with no externally visible ova with yolk nucleus of Balbiani is clearly discernible in the cytoplasm of oocytes, stage-II oocyte are visible externally during this stage. Appearance of vacuoles along periphery and initiation of yolk deposition in oocyte are the characteristics of this stage, stage-III ovaries are filled with ova and occupy maximum space in the body cavity. A vitelline membrane appears around the oocyte. Yolk deposition is completed. A clear theca now appears around the follicular epithelium, stage-IV Ovaries exhibit loose and flaccid appearance in this stage.

Partial disruption of the ovarian follicles, vacuolation in the cytoplasm of germinal cells, and secondary oocytes were reported in *H. fossilis* exposed to BHC (Hazarika and Das, 1998). Degeneration of follicular walls, connective tissues and vacuolisation in the ooplasm of stage-II and stage-III oocyte was observed in *A. testudineus* treated to carbofuran (Chatterjee *et al.,* 1997). This supports the present study that the oocyte were affected with a degeneration of connective tissue, disruption of follicles, vacuolation in the cytoplasm of germinal cells and disintegration of such oocytes were noticed in *E. danricus* exposed to cypermethrin. Pesticide effects on the testis are scarce. In *O. mossambicus* exposed to BHC caused hyperplastic changes in both primary and secondary spermatocytes (Pandey and Shukla, 1980). Likewise, hyperplasia and vacuolisation in all types of spermatogenic cells have been reported in *S. mossambicus* exposed to dimecron (Lakhani and Pandey, 1985).

Clumping of chromatin material and also cytoplasm were quite apparent in primary spermatocytes. Few sperm shows vacuolation in the head region were noticed in *H. fossilis* exposed to endosulfan. Endosulfan also causes testicular degeneration and disappearance of sperm (Singh and Sahai, 1987). Testicular deleterious changes included degeneration of spermatogenic element and necrosis of interstitial cells of Leydig was reported in *C. punctatus* exposed to carbofuran. This report supports the present study that the vacuolation, testicular degeneration was observed in *E. danricus* exposed to different concentration of cypermethrin.

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