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Effect of Ethofenprox (98%) - A synthetic Pyrethroid on *Oreochromis mossambicus*

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ABSTRACT

The use of wide variety of chemicals by man has been greatly increased. To cope up with the increasing human population in the world every year, the problem of producing more food annually is apparent. Increased agricultural population has been achieved by use of wide variety of fertilizers and pesticides. The necessity of the proteins, for the increasing human population is obtained from fishes cheaply. Large scale fish culture practice is hampered mainly due to pollution of rivers and reservoirs by agricultural waste release. Insecticides are widely used type of pesticides, which are used to prevent substances from attack of insect. An attempt was made to assess the toxic effect of an insecticide Ethofenprox Technical (98%) on the fish Oreochromis mossambicus. In the acute study, LC_{50} of the Ethofenprox was assessed to be 5.2ppm. The toxic symptoms elicited were erratic movement and skin erosion. Based on LC_{50} value, doses of Ethofenprox (98%) selected for the subacute study was 0.13ppm, 0.26ppm and 0.52ppm for a period of 10 days.

Key words: *Ethofenprox, Oreochromis mossambicus, Toxic effects.*

INTRODUCTION

The use of wide variety of chemicals by man has been greatly increased. Chemicals find their application as pesticides, fertilizers, drug, food additives and cosmetics. Man, knowingly or unknowingly interferes with the ecosystem and upsets the balance in the natural environment. With increasing human population in the world every year, the problem of producing more food annually is apparent. This has resulted in increased use of pesticides and fertilizers. According to the "Environmental Protection Agency United Nation", the total annual pesticide use is estimated as 2.7 billion pounds. Pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic quality and water quality. While increasing human population need increased cheap protein. Aquatic systems are good source of protein with fish as the main source of protein.

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Since the study of Ethofenprox a synthetic pyrethroid in aquatic organisms were very limited, an attempt was made to study the effect of Ethofenprox on aquatic organism and the present study was designed to assess the effect on a fresh water fish *Oreochromis mossambicus*.

MATERIALS AND METHOD

The specimen employed here for the present study is commonly called Tilapia. The nomenclature was revised as *Oreochromis mossambicus* belonging to the family Cichlidae. The healthy and active fishes were sorted into groups and were acclimatized to the laboratory conditions for 10 days.

Acute Study (Assessment of LC_{50})

In the bioassay study, the 96 hours LC_{50} determination is the standard laboratory test for evaluating the acute lethal toxicity. The LC_{50} was determined following the procedure of Finney[4]. The LC_{50} bioassay method was carried out placing groups of organisms (10 fishes per group) in a range of 4 different concentration of pesticides. Ethofenprox (98%) was diluted with 1 ml acetone and mixed with water and their survival rate was observed throughout the 96hrs test period.1 ml acetone was added even to the control group of fishes. The mortality rates were observed and recorded at the time intervals of 24 hrs, 48hrs, 72 hrs and 96 hrs. Each experiment was repeated 3 times at the selected pesticide concentration up to 96 hrs, every time noting the number of fishes' dead. The average mortality in each concentration was taken to determine the LC_{50} by graphical method of Finney[4] in which the probit mortality was plotted against log concentration of pesticide. The results were tabulated in (table 1, fig 1).

Subacute Studies

Based on the results of the acute toxicity study, doses of 1/10, 1/20, 1/40 the LC_{50} values of Ethofenprox (98%) were selected following the procedure of Desi *et al*[3]. The experimental setup was kept for 10 days. The fishes were grouped into 4 batches of 6 each. The first group served as control, and were maintained along with 3 other groups, which were subjected to treatment. The chemicals of the required doses were weighed, dissolved in 1 ml of Acetone and mixed directly into the water into which fishes were introduced and maintained. A daily change of water with respective concentration were made in order to maintain the fishes in same concentration of chemicals throughout the treatment period.

General Observation

General observation was seen, Mortality of fishes under normal (control) conditions and during the time of treatment with the chemicals and all signs of ill health of reaction to treatment were recorded in both acute toxicity and subacute studies.

Statistical Analysis

The data collected by acute toxicity study was analysed using Finney's profit analysis[4]. For subacute toxicity study, the data were subjected to statistical analysis such as mean, standard deviation, standard error and students *"t"* test.

Histopathology

Necropsy was conducted after treatment period and organs like Gill, Intestine, Liver, Brain and Skin of both control and treated animals were processed by routine histopathological technique. The body parts were preserved in freshly prepared buffered Formalin and embedded in paraffin wax after standard dehydration procedure. Histological sections were obtained to a thickness of 5µm using a microtome and were stained with Haematoxylin and Eosine following standard staining procedure, Abnormalities of these tissue sections were examined under high power light microscope and compared with healthy tissue sections of the control fish to assess the degree of histopathological changes caused by Ethofenprox.

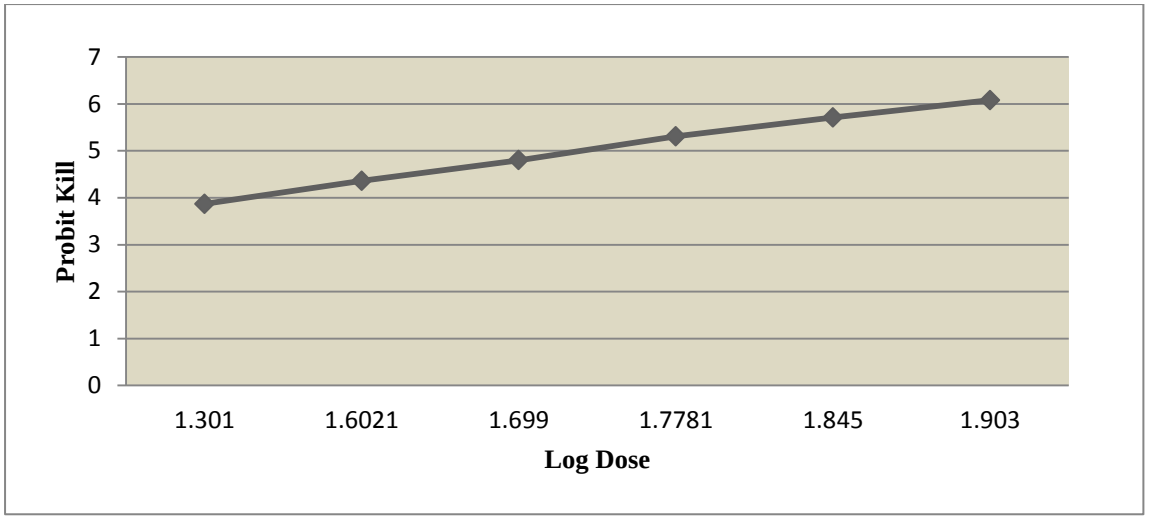
RESULTS AND DISCUSSION

Acute Study

S.No	No	ppm	log Dose	Empirical probit	Expected probit(y)	working probit(Y)	Working Coefficient	Weight (W)	Wx	Wy	Wx2	WY2	Wxy
1	10	control	0	0	0	0	0	0	0	0	0	0	0
2	10	2	1.301	3.87	3.8	3.87	0.37	3.7	4.81	5.034	6.262	55.41	18.62
3	10	4	1.6021	4.36	4.3	4.36	0.532	5.32	8.52	6.98	13.65	101.08	37.16
4	10	5	1.699	4.8	4.8	4.82	0.627	6.27	10.65	8.18	18.09	145.65	51.34
5	10	6	1.7781	5.31	5.3	5.33	0.61	6.1	10.84	9.477	19.28	173.24	56.05
6	10	7	1.845	5.71	5.7	5.7	0.532	5.32	9.815	10.516	18.1	172.84	55.94
7	10	8	1.903	6.08	6	6.07	0.439	4.39	8.37	11.55	15.89	161.72	50.81
								SW	SWX	SWY	SWx2	SWy2	Swzy
								31.1	53	51.7	91.27	809.94	269.92

The acute toxicity is expressed in terms of LC_{50} value obtained from probit analysis following the method of Finney[4]. LC_{50} value of Ethofenprox Technical (98%) for 96 hours in *Oreochromis mossambicus* in the present study was assessed to be 5.2ppm (Table 1)

Relationship between log dose of Ethofenprox (98%) and Probit kill of *Oreochromis mossambicus*



The acute sign of poisoning appeared rapidly. The toxic symptoms were characterized by hyperactive movements. They exhibited chasing movements (restlessness) and skin erosion was also noted. According to PAN pesticide database[12], the LC_{50} value of Ethofenprox for 48 hours in Japanese eel was reported as 14ppm, and for 24 hours as 18ppm. According to PAN pesticide database[13] the LC_{50} value of Ethofenprox for 3 hours, in Tilapia was reported as 2.03ppm, for 6 hours as 1.95ppm, for 12 hours as 1.90ppm, for 24 hours as 1.85ppm, for 48 hours as 1.79ppm, for 72 hours as 1.76ppm and for 96 hours asn1.74ppm.

The difference observed may be due to the difference in the capacity of the fish to tolerate or may be due to purity of the Insecticide as suggested by Hayes[7]. The difference in the LC_{50} value may also due to body weight, size, time of exposure with the chemical, age ,sex, climatic conditions.

Subacute Studies

Under general observation, darkening of the body colour from grey to black was observed in the present study. This may be due to skin sensitivity or due to the physiological stress induced by the chemicals induced in the medium. Muniyan and Veeraragavan[11] have also reported chromatic changes on the skin of fresh water fish (*Oreochromis mossambicus*) when exposed to Ethofenprox, similar to the present findings. In the present study also, the

histopathological examinations of skin revealed damage of Epidermis and Dermis.

HISTOPATHOLOGICAL STUDIES

Gill Extensive damage of gill lamella and dissociation of acidophilic cells was observed (Fig 1). According to Mc.Kim, *et al*.,[10] a similar effect was observed when rainbow Trout was exposed to a synthetic pyrethroid fenvalerate. Changes may be due to the result of the chemicals reaching the gills through the circulatory pathway and inflicting damage. The tissue structure of the Intestine showed damage of the columnar epithelial cells.

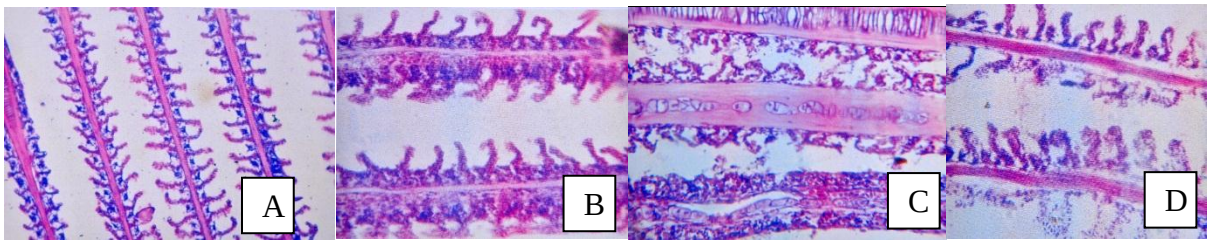
Fig. 1: Histopathological changes of Tilapia gill tissues (45x) upon exposure to Ethofenprox

(A) Normal gill filament showing primary Lamella (PL) and secondary lamella (SL) of the control

(B) Low Dose showing deformed secondary lamellae

(C) Intermediate Dose showing mild hyperplasia

(D) High Dose Mucosal proliferation and Lamella Disorganization.



Extensive damage to blood cells and vacuolar formation was also observed (Fig 2). Changes may be due to the result of the chemical reaching the intestine through the food pathway.

Fig. 2: Histopathological changes (45X) of the Liver Tissue of Tilapia upon exposure to Ethofenprox

(A)Normal liver tissue of the control fish containing Normal architecture,

(B) Low Dose showing Sloughing of cells and Blood clots,

(C) Intermediate Dose showing Melanomacrophage and vacuolated cells

(D) High Dose showing Inflammated cells and Vacuolated Hepatic necrosis.

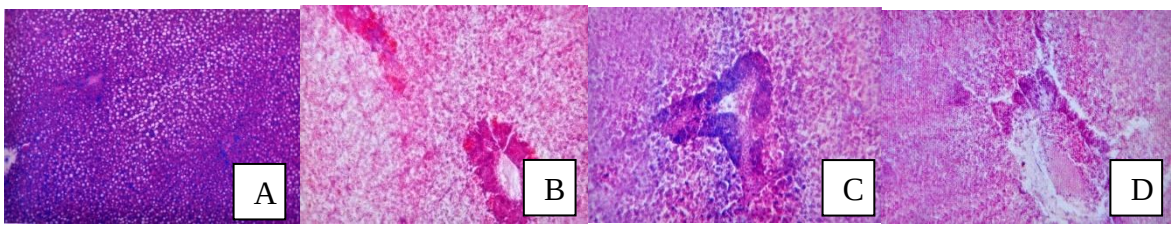


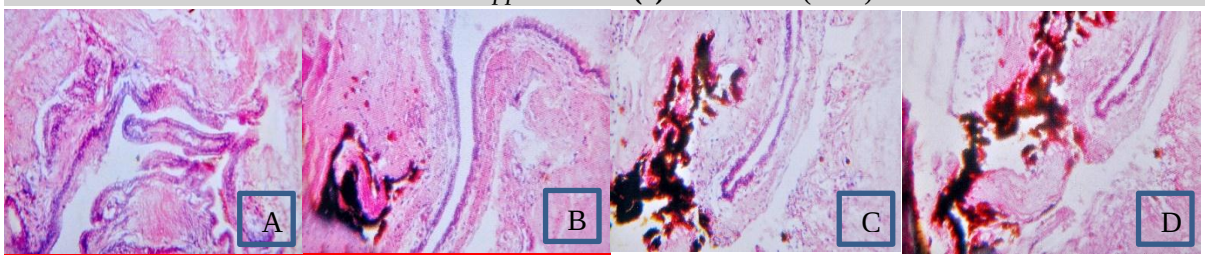
Fig. 3: Histopathological changes (45X) of the intestine of Tilapia upon exposure to Ethofenprox

(A) Normal structure of Intestinal Wall

(B) Low Dose showing damage of columnar epithelial cells with Blood clots

(C) Intermediate Dose showing Moderate damage of columnar epithelial cells with Blood clots.

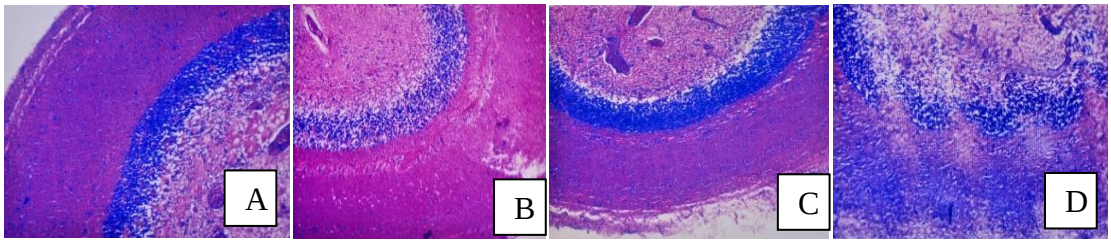
(D) High Dose showing High damage of columnar epithelial cells with Blood clots and Vacuolar formation



The Liver cells showed vacuolated hepatic necrosis. Inflammation of cells and sloughing of hepatocytes were also observed (Fig 3) The liver damage can be correlated with enzymatic activity (ie), Increase in ALT activity. Incidence of Liver damage under toxic exposure elevates the level of enzymes as reported by Lynch *et al*.[9], Hanke and Pitorowski[6] . Coombs, *et al*[1]., conducted a 90 day inhalation study in rats and reported a related increase in mean liver weight. Green *et al*.[5], experimented with Ethofenprox in rats and have reported centrilobular hepatocyte enlargement. Green, *et al*.[5], experimented with Ethofenprox in mice and have reported relative increase in the weight of the liver. The severity increased with the increase in the concentration Of Ethofenprox.

Fig. 4: Histopathological changes (10X) of the Brain Tissue of Tilapia upon exposure to Ethofenprox

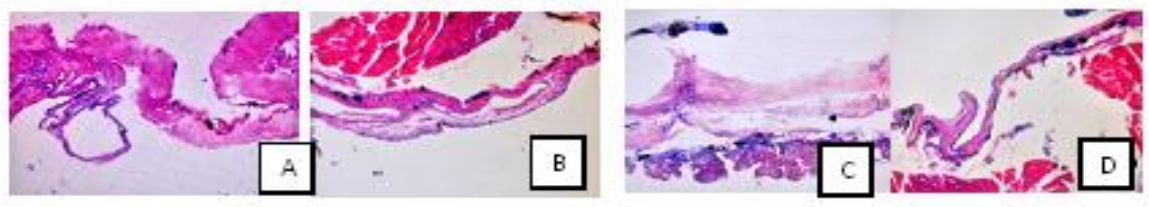
(A) Normal Brain tissue of the control fish containing Normal architecture,
(B) Low Dose showing congestion of cappillaries.
(C) Intermediate Dose showing Delamination of the outer layer
(D) High Dose showing (45X) showing alteration of layer of Brain tissue.



The brain section revealed congested capillaries, delamination and alteration in the layer of Brain tissue (Fig 4) Cummins and Gardner[2] administered MTI 500 (99.6%) to rats and have reported decreased motar activity. The results obtained from the present study revealed that, the changes in the brain may be due to effect of Ethofenprox. The loss of equilibrium observed during general observation in the subacute study also supports this report.

Fig. 5: Histopathological changes (10X) of the skin of Tilapia upon exposure to Ethofenprox

(A) Normal section of skin of the control fish containing Normal architecture,
(B) Low Dose showing slight damage of dermis.
(C) Intermediate Dose showing damage of dermis and Epidermis.
(D) High Dose (10X) showing highly damaged dermis with blood clots



A complete damage of Epidermis and Dermis was observed .Blood clots (Haemorrhage) were also observed (Fig 5) These conditions may be due to potent effect of Ethofenprox technical (98%). Based on the report of Munniyan and Veeraghavan[11] Ethofenprox caused chromatic changes on the skin of the fish *Oreochromis mossambicus* .Skin damage in Rabbits have also been reported by Killeen[8]. When Technical MTI-500 (99.18%) was applied to the dorsal skin of Rabbits, microscopic examination of treated skin exhibited related diffuse hyperplasia.

CONCLUSION

The various doses of Ethofenprox technical (98%) tested in *Oreochromis mossambicus* seems to have Behavioural Changes. Histopathological study revealed changes in the organs like Gill, Intestine, Liver, Brain and Skin which reveals that use of Ethofenprox is not safe and it could be hazardous to the non-targeted organisms like fish and man. Thus, awareness should be created among the agricultural public and indiscriminate use of the insecticide should be avoided.

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