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TOXICITY & HISTOPATHOLOGICAL CHANGES IN THE THREE INDIAN MAJOR CARPS, *LABEO ROHITA (HAMILTON), CATLA CATLA (HAMILTON)* AND *CIRRHINUS MRIGALA (HAMILTON)* EXPOSED TO FENVALERATE

T. Anita Susan[1], K. Sobha[2] and K.S. Tilak[3]

[1]Department of Zoology, Andhra Christian College, Guntur-522004, Andhra Pradesh, India
[2]Department of Biotechnology, RVR & JC College of Engineering, Guntur-522019, Andhra Pradesh, India
[3]Department of Zoology, Acharya Nagarjuna University, Guntur-522010, Andhra Pradesh, India

ABSTRACT: Toxicity of synthetic pyrethroid Fenvalerate (technical grade and 20% Emulsifiable concentrate) to the fry and fingerling stages of the three major carps viz., *Catla catla, Labeo rohita* and *Cirrhinus mrigala* was studied using static and continuous flow through systems. A comparison has been made to find out relative sensitivity of fry and fingerling stages of the carps exposed to the two forms of the toxicant fenvalerate. The LC_{50} values for 24h, 48h and 96h were highest in *Cirrhinus mrigala* followed by *Catla catla* and *Labeo rohita* for fry, while that of fingerling stage were highest in *Catla catla* followed by *Labeo rohita* and *Cirrhinus mrigala* in the decreasing order. On exposure to 20% EC fenvalerate, the LC_{50} values of both fry and fingerlings for 24h, 48h and 96h were highest in *Cirrhinus mrigala* followed by *Catla catla* and *Labeo rohita*. Our observations indicate that static LC_{50} values are nearly thrice (2.5 to 3 times) higher than continuous flow through LC_{50} values. For histopathological studies, fingerlings of the three carps were exposed to the sub-lethal concentration ($1/10^{th}$ of 24h LC_{50}) for ten days and the vital tissues like gill, liver and kidney were isolated and processed for histopathological examinations. All the tissues showed significant deteriorative changes as compared to control.

Key words: toxicity, pyrethroid, fenvalerate technical grade, emulsifiable concentrate, LC_{50}, histopathology

INTRODUCTION

Due to increased public awareness of the potential of persistent pesticides that cause harm to environment and public health, great stress is being laid for developing least persistent and selective pesticides. Traditionally the problem of agricultural pest control has been dealt with by formulating new and more potential pesticides. Very few pesticides kill the intended target but often lead to the death of many non target organisms. Synthetic pyrethroids are one of wide variety of pesticides contributing to this situation. Hence the thought of using plant extracts namely pyrethriods received much attention. But these insecticides also tend to affect the biology of non target species along with pests (Elliott et al., 1978; Hoyt et al., 1978; Mulla et al., 1978a; Chari, 1980). The selective toxicity of pyrethroids to vertebrates, in the order of decreasing sensitivity, is fish followed by amphibians, mammals and least to birds (Hill, 1985; Smith and Stratton, 1986; Associate Committee on Scientific Criteria for Environmental Quality, 1986). According to Casida (1980), the incorporation of α – cyano group increases the stability, lipophilicity and potency of toxic action.

Fenvalerate is one of a family of recently developed insecticides classed as synthetic pyrethroids, which exhibit very low avian and mammalian toxicity, but are very toxic to fish (Miyamoto, 1976; Casida et al., 1983). Aquaculture being intimately associated with agriculture and irrigation, the most serious threat to fishes comes from pesticides, lethal to aquatic fauna which form their food. Pesticides accumulated by pelagic organisms can be dispersed quickly and for great distances by the animals, as they swim from one area to another. Apart from causing death directly or due to starvation by destruction of food organisms, many pesticides have been shown to affect growth rate, reproduction and behaviour with the evidence of tissue damage. Fenvalerate, a synthetic pyrethroid is a potential hazard to fish, because of its use in aquatic larvicidal programmes as reported by Smith and Stratton, 1986. Hence an attempt has been made to evaluate the toxicity of two compounds of synthetic pyrethroid fenvalerate, technical grade and 20% emulsifiable concentrate.

Although toxicants impair the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence it is useful to have an insight into histological analysis. The extent of severity of tissue damage is a consequence of the concentration of toxicant and is time dependent. Also the severity of damage depends on the toxic potentiality of a particular compound or pesticide accumulated in the tissues (Jayantha Rao, 1984). Therefore an attempt has also been made to observe possible histopathological changes in certain vital tissues like gill, liver and kidney of the three Indian major carps *Labeo rohita, Catla catla* and *Cirrhinus mrigala* exposed to sublethal concentrations ($1/10^{th}$ of 24h LC_{50}) of fenvalerate technical grade for ten days.

MATERIAL AND METHODS

The test fish *Labeo rohita (Ham)*, *Catla catla (Ham)* and *Cirrhinus mrigala* (Ham) were acclimatized to the laboratory conditions in large concrete tanks, with unchlorinated ground water for two weeks at a room temperature of $28 \pm 2\ ^0C$. During the period of acclimatization fish were fed daily with fish meal on an average of 3% of their body weight. Fish were not fed for two days prior to and during experimentation. Fish used in the experiment were procured from KSN Rao Fish Farm, Nandivelugu, Guntur District., Andhra Pradesh, India. Fenvalerate technical grade, and 20% active ingredient emulsifiable concentrate (EC) were supplied by Searle (India) Ltd., Bombay 400 001. Stock solutions were prepared by dissolving it in acetone. Controls were kept for each experiment and it received the solvent acetone equal to the highest concentration used in the test.

Toxicants are present in the aquatic systems at concentration too low to cause rapid death directly, but they may impair the functioning of organisms. Though pesticides may not be present in lethal concentrations, accidental spillages may result in toxic concentrations. Hence in the present investigation, (1/10 of 24 h LC_{50} and 24 h LC_{50}) were selected as sublethal and lethal concentrations to study the effects. The ground water used for acclimatization and conducting experiments was clear and unchlorinated. The physical and chemical properties of water are Turbidity 8 Silica units, Electrical conductivity at 28 ^0C 816 Micro ohms/cm, p^H at 28 ^0C. 8.1, Alkalinity : i. phenolphthalene Nil, ii. Methyl Orange 472, Total Hardness (as $CaCo_3$) 232, Carbonate Hardness (as $CaCo_3$) 232, Non Carbonate Hardness (as $CaCo_3$) Nil, Calcium Hardness (as $CaCo_3$) 52, Nitrite Nitrogen (as N) Nil, Sulphate (as So_4^{2-}) Traces, Chloride (as Cl^-) 40, Fluoride (as F^-) 1.8, Iron (as Fe^{2+}) Nil, Dissolved Oxygen 8 – 10 ppm, Temperature $28 \pm 2\ ^0C$. Pilot experiments were conducted to choose the concentrations at which the fish were killed.

Five concentrations were taken for each test and 10 fish were introduced in containers with a capacity of 10 litres. For continuous flow-through systems, reservoirs were used of 25-litre capacity. The test water was let into test containers at a rate of four liter per hour using polyethylene drip sets with regulators, For every six hours fresh test solutions were prepared in reservoirs.

First, experiments were conducted to determine the toxicity of fenvalerate in various concentrations with technical grade and 20% EC formulation in static and continuous flow through systems. The data on the mortality rate of fish was taken into consideration at each experiment, and the dead fish were removed immediately. Data observed from these tests were recorded from time to time. Toxicity experiments were conducted to choose the mortality range from 10% to 90% for 24, 48 and 96 h in static and continuous flow through system. Finney's Probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate LC_{50} values. The respective probit values were taken from table IX of Fisher and Yates (1938), for the determination of 95% confidence limit LC_{50} values and a normal variate of 1.96 was taken into consideration.

For Histopathological studies, fresh water fish *Labeo rohita, Catla catla and Cirrihnus mrigala* (size 4-6 cm in length, 4-5 gm in weight) were acclimatized to laboratory conditions for a week. They were exposed for ten days to sublethal concentration of fenvalerate technical grade. At the end of exposure period, fish were randomly selected for histopathological examination. Tissues like gill, liver and kidney were isolated from control and experimental fish. Physiological saline solution (0.85% NaCl) was used to rinse and clean the tissues. They were fixed in aqueous Bouin's solution for 48 h, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut at 6µ thickness, stained with Ehrlich Hematoxylin/Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in canada balsam.

RESULTS

The results of the present work, i.e., the LC_{50} values and 95% confidential limits of fenvalerate technical grade and 20% active ingredient EC for 24h, 48h, and 96h in static and continuous flow through system for *Labeo rohita, Catla catla and Cirrhinus mrigala* (fry and fingerlings) are given in table 1. The regression equations of fenvalerate technical grade and 20% active ingredient EC for 24h, 48h and 96h in static and continuous flow through system for *Labeo rohita, Catla catla and Cirrhinus mrigala* for fry and fingerlings are given in tables 2 and 3. Histopathological characteristics of normal tissue are presented as Fig. A and that of toxicant exposed tissue as Fig. B in every plate numbered I to IX.

In the present study, a comparison has been made to find out relative sensitivity of fry and fingerlings of *Labeo rohita, Catla catla and Cirrhinus mrigala* exposed to fenvalerate technical grade and 20% EC formulation. When the fish were exposed to fenvalerate technical grade the LC_{50} values for 24h, 48h and 96h were highest in *Cirrhinus mrigala* followed by *Catla catla* and *Labeo rohita* for the fry, while that of fingerling stage were highest in *Catla catla,* followed by *Labeo rohita* and *Cirrhinus mrigala*. On exposure to 20% EC fenvalerate, the LC_{50} values of fry for 24h, 48h and 96h were highest in *Cirrhinus mrigala*, followed by *Catla catla* and *Labeo rohita* and for fingerlings, the highest value was for *Cirrhinus mrigala*, followed by *Catla catla* and *Labeo rohita* respectively.

Table 1 The LC50 values and their 95% confidence limits of fenvalerate technical grade and 20% active ingredient EC formulation to fish ***Labeo rohita, Catla catla*** and ***Cirrhinus mrigala*** for 24h, 48h and 96h in Static and Continuous Flow Through Systems**.**

	Size of the fish		24 Hours		48 Hours		96 Hours	
	Length (cm)	Weight (mg)	Static (mg/l)	Continuous flow (mg/l)	Static (mg/l)	Continuous flow (mg/l)	Static (mg/l)	Continuous flow (mg/l)
Labeo rohita Fenvalerate Technical Grade	1-3	200- 250	0.012 (0.0046-0.0315)	0.0047 (0.0046-0.0049)	0.012 (0.0046-0.0315)	0.0041 (0.0040-0.0043)	0.012 (0.0046-0.0315)	0.0031 (0.0030-0.0033)
	6-8	1000-1500	0.0087 (0.0078-0.0097)	0.0020 (0.0078-0.0097)	0.0085 (0.0077-0.0094)	0.0018 (0.0017-0.0019)	0.0085 (0.0077-0.0094)	0.0012 (0.0010-0.0014)
20%E.C. Fenvalerate	1-3	200- 250	0.0182 (0.014-0.022)	0.0088 (0.008-0.0098)	0.016 (0.0150-0.0169)	0.0053 (0.0048-0.0058)	0.016 (0.015-0.0169)	0.0037 (0.0019-0.0073)
	6-8	1000-1500	0.0249 (0.023-0.026)	0.0074 (0.0049-0.011)	0.0249 (0.0230-0.026)	0.0066 (0.0066-0.0067)	0.0249 (0.023-0.026)	0.0059 (0.0050-0.0070)
Catla catla Fenvalerate Technical Grade	2-4	500- 700	0.0034 (0.0031-0.0037)	0.0026 (0.0014-0.0046)	0.0033 (0.0028-0.004)	0.0018 (0.0012-0.0028)	0.0038 (0.0028-0.004)	0.0013 (0.00080-0.0018)
	6-8	4000-6000	0.00755 (0.0061-0.0092)	0.0032 (0.0025-0.003)	0.0075 (0.0061-0.0092)	0.0039 (0.0031-0.0055)	0.0075 (0.0061-0.0092)	0.0017 (0.0016-0.0017)
20% E.C. Fenvalerate	2-4	500- 700	0.018 (0.013-0.025)	0.0065 (0.019-0.054)	0.017 (0.01-0.0305)	0.0064 (0.0021-0.0052)	0.017 (0.012-0.0305)	0.006 (0.0053-0.0071)
	6-8	4000-6000	0.0203 (0.014-0.027)	0.007 (0.0059-0.0083)	0.015 (0.01-0.017)	0.0063 (0.0042-0.0094)	0.015 (0.01-0.017)	0.005 (0.004-0.005)

	Size of the fish		24 Hours		48 Hours		96 Hours	
	Length (cm)	Weight (mg)	Static (mg/l)	Continuous flow (mg/l)	Static (mg/l)	Continuous flow (mg/l)	Static (mg/l)	Continuous flow (mg/l)
Cirrhinus mrigala Fenvalerate Technical Grade	2-4	200- 250	0.0022 (0.0018-0.0025)	0.0010 (0.0082-0.0012)	0.0022 (0.0018-0.0025)	0.00088 (0.0007-0.001)	0.0022 (0.0018-0.0025)	0.00074 (0.00071-0.00078)
	6-8	1500-2000	0.0087 (0.0063-0.0120)	0.0030 (0.0025-0.0036)	0.0087 (0.0063-0.0120)	0.0026 (0.0021-0.0033)	0.0087 (0.0063-0.0120)	0.0016 (0.0014-0.0019)
20%E.C. Fenvalerate	2-4	200- 500	0.0162 (0.0018-0.029)	0.0048 (0.0042-0.0053)	0.016 (0.0094-0.0477)	0.0047 (0.0041-0.0055	0.0159 (0.014-0.057)	0.0047 (0.0039-0.005)
	6-8	1500-2000	0.0153 (0.012-0.018)	0.0061 (0.0052-0.0072)	0.0153 (0.012-0.018)	0.0059 (0.004-0.0078)	0.0153 (0.012-0.018)	0.0041 (0.0033-0.0052)

Exposure to sub-lethal concentrations of fenvalerate induced remarkable changes in vital tissues like Gill, Liver and Kidney of all the three fish as evident from histopathological observations. As gills, the most important organs of respiration, are continuously exposed to water to absorb vital oxygen, they become the first target to come in contact with external toxicant environment. Therefore the gill, in general, showed marked pathological changes such as bulging in the tips of primary gill lamellae, club shaped secondary gill lamellae, fusion of secondary gill filaments, proliferation of interlamellar cells, separation of epithelial layer from the central sinus of the filament and dilation of primary gill lamellae.

Table 2: Regression equations of fenvalerate technical grade to the fish Labeo rohita, Catla catla and Cirrhinus mrigala for 24h, 48h and 96h in static and continuous flow through systems

	Size of the fish				
	Length (cm)	Weight (mg)	Exposure Period (hours)	Static Systems	Continuous flow through system
Labeo rohita	2 - 4	200 - 250	24	Y=4.065+11.34 X	Y= -14.8943+50.24 X
			48	Y=4.065+11.34 X	Y= -16.285+20.9 X
			96	Y=4.065+11.34 X	Y=-20.3705+25.91 X
	6 – 8	1500 – 2000	24	Y= 2.6089 + 36.62 X	Y= -6.633+12.31 X
			48	Y= 2.974 + 33.54 X	Y= -6.787+12.51 X
			96	Y= 2.974 + 33.54 X	Y= -6.078+11.81 X
Catla catla	2-4	500-700	24	Y= - 5.585+10.95X	Y=3.392+2.04X
			48	Y= -5.74+11.04X	Y=3.71+1.92X
			96	Y= -3.14+8.84X	Y=0.82+5.29X
	6-8	4000-6000	24	Y=-16.0425+20.69X	Y=-13.49+18.8X
			48	Y=-16.042+20.69X	Y=-0.26+7.51X
			96	Y=-16.042+20.69X	Y=57.23+63.2X
Cirrhinus mrigala	2 - 4	200 - 250	24	Y= -7.42 + 13.24X	Y= -4.01+9.83X
			48	Y= -7.42 + 13.24X	Y= -5.09+11.04X
			96	Y= -7.42 + 13.24X	Y= -0.01+15.33X
	6 – 8	1500 – 2000	24	Y= -3.17 + 7.86 X	Y= -8.47+13.79 X
			48	Y= -3.17 + 7.86 X	Y= -4.86+10.23X
			96	Y= -3.17 + 7.86 X	Y= -6.46+10.69X

Table 3: The Regression equations of 20% active ingredient EC fenvalerate to the fish Labeo rohita, Catla catla and Cirrhinus mrigala for 24h, 48h and 96h in static and continuous flow through systems

	Size of the fish				
	Length (cm)	Weight (mg)	Exposure Period (hours)	Static Systems	Continuous flow through system
Labeo rohita	2 - 4	200 - 250	24	Y= -20.86+44.33 X	Y= -4.03+9.06X
			48	Y= 2.33+13.02 X	Y= -2.69+8.01X
			96	Y= 2.33+13.02 X	Y= -11.85+12.29X
	6-8	1500-2000	24	Y=-42.15+118.66X	Y= -42.19+9.88X
			48	Y=-32.34+94.25X	Y= -46.39+51.57X
			96	Y=-32.34+94.25X	Y= -43.22+48.27X
Catla catla	2 - 4	500-700	24	Y= 2.66+8.79X	Y=3.76+6.04X
			48	Y= 3.006+8.24X	Y=-2.40+16.31X
			96	Y=3.006+8.24X	Y=-4.26+10.03X
	6-8	4000-6000	24	Y=2.62+7.72X	Y=-42.50+47.46X
			48	Y=3.18+10.24X	Y=-18.34+23.27X
			96	Y=3.18+10.24X	Y=-20.73+26.68X
Cirrhinus mrigala	2 - 4	200 - 250	24	Y=1.83+15.05X	Y=5.006+290X
			48	Y=-0.20+23.23X	Y=-8.82+140X
			96	Y=-9.05+71.71X	Y=128.5+133.75X
	6-8	1500-2000	24	Y=-68.05+392X	Y= 2.08+32.02X
			48	Y=-68.05+392X	Y= 13.94+18.92X
			96	Y=-68.05+392X	Y= -30.82+35.95X

DISCUSSION

In general, fry stages are more sensitive to fenvalerate technical grade and 20% EC formulation than the fingerlings stages. This may be due to greater body weight of the fingerling stages. This finding is in agreement with Kumaraguru and Beamish (1981) in rainbow trout. According to Mc Kim and Heath (1983), this effect may be due to proportionately greater metabolic rate of smaller fish, which leads to greater toxicant uptake. However toxicity is not always correlated to size and age (Jolly et al., 1978). LC_{50} values among three species of Indian major carps has not varied considerably. Generally there is little difference in the lethality of pyrethroids among different families of fishes, (Clark et al., 1985; Associate Committee on Scientific Criteria for Environmental Quality, 1986). The LC_{50} values reported for fenvalerate are 3.8 ppb, 7.6 ppb for 24h for *Salmo gairdneri* (Mulla et al., 1978b; Coats and O' Donnell - Jeffery, 1979), 3 ppb for 48h in *Salmo gairdneri*, 35 and 25 ppb for 24h and 48h in *Cyprinodon macularis* (Mulla et al., 1978b) 0.14, 0.77, 0.69 mg/L for 24h, 48h and 72h in *Pimephales promelas* (Bradbury et al., 1985) 5.4 mg/L for 96h (Holcombe et al., 1982) 1.2 mg/L for 96h in *Salmo salar* (McLeese et al., 1980). The LC_{50} values for technical grade fenvalerate were found to be 5.0, 0.58, 0.31 mg/L for *Cyprinodon variegates*, *Mugil cephalus* and *Menidia menidia* respectively (Schimmel et al, 1983). The 48h LC_{50} values of fenvalerate to *Cyprinus carpio* was 0.03 ppm. (Malla Reddy and Bashamohideen, 1988). The LC_{50} value for fenvalerate was 0.85µg/L for fathead minnow larvae *Pimephales promelas* (Jarvinen et al., 1988). The 96h LC_{50} value for fenvalerate was 0.66 µg/L for Juvenile topsmelt *(Atherinops affinis*), a salt water fish (Goodman et al., 1992), 5 µg/L for sheepshead minnows (Hansen et al., 1983), 0.458 ppm for cat fish *Clarias batrachus* (Ravinder et al, 1989). The 96h LC_{50} values were reported to be 88 and 172 ng/L for Steelhead trout in intermittently and continuously exposed fenvalerate concentrations (Curtis et al., 1985). Jeba Kumar (1990) reported 24h, 48h, 72h and 96h LC_{50} of cypermethrin for *Lepidocephalichthys thermalis* as 0.96, 0.84, 0.62, 0.57 ppm respectively. The results show that 20% active ingredient EC is more toxic than technical grade fenvalerate. Findings of these studies revealed that EC formulation is 2-6 times more toxic than technical grade fenvalerate . However when toxicity is calculated to only the technical grade part (i.e. 80%) in 20% active ingredient EC formulation, there is no difference between the fenvalerate ingredient of the two formulations. For *Clarias gariepinus*, the 48h LC_{50} value of fenvalerate was reported to be 250 mg/L (Sakr and Jamal Al lail, 2005). (The 96h LC_{50} value for cypermethrin for *Heteropneustes fossilis* was found to be 0.046 ppm (Namita Joshi et al., 2007). Earlier static tests with nominal or estimated toxicant concentrations indicated that EC formulations of several synthetic pyrethroids were about 2 to 10 times more toxic to rainbow trout (Coats and O'Donnell Jeffery, 1979) and atlantic salmon (Zitko et al., 1979) than technical formulation. This is probably due to synergistic interaction between active ingredient and other components of the formulation.

Steady state static LC_{50} values for both the toxicants were reached by 48 h. There was no considerable mortality after 48 h, since pyrethroids are fast acting and produce mortality within 8-10 h. The static LC_{50} values for 24 h are same as for 48 h and 96 h. Similar observation was reported by Bradbury et al. (1985) for two fenvalerate formulations in fathead minnows. The results of present study revealed that the static LC_{50} values are nearly thrice (2.5 – 3 times) higher than continuous flow through values. This can be attributed to various reasons in static conditions, viz., depletion of oxygen content, accumulation of Co_2, NH_3 and other excretory products, which prove to be more toxic than the pesticide itself (Lincer et al., 1970) for both fenvalerate technical grade and 20% active ingredients EC formulation. Static tests are nevertheless useful when toxicants need rapid evaluation. The range of concentrations producing mortality is narrow for 20% EC formulation, compared to technical grade fenvalerate. This finding is similar with the results of Annamani (1986) in *Labeo rohita* and *Aplocheles panchax* exposed to 20% EC fenvalerate.

The environmental factors such as temperature are well known for their influence in the toxic response of biota to chemicals. Higher mortality rate, almost double was recorded in winter (mean average temp ± 18 ^{0}C) than in summer (mean average temp ± 32 ^{0}C). Some of the early synthetic pyrethroids viz., demethrin resmethrin and RU 1679 had a negative coefficient of toxicity for fish (Mauck et al., 1976; Mayer and Ellersieck,1986). The negative temperature coefficient may be due to positive coefficient of biotransformation for microsomal enzymes (Kumaraguru and Ferguson, 1982). Casida et al. (1981) described that it may be due to nerve blocking action of pyrethroids particularly marked at low temperatures. Adams & Miller (1979) reported that at higher temperature hyperexcitability increases where as mortality decreases. Kumaraguru and Beamish (1981) reported that the 96h LC_{50} for trout increased by an order of magnitude from 0.62 to 6.43 µg/L between 5 and 20 ^{0}C. Harris and Kinoshita (1978) demonstrated similar inverse relationship between temperature and toxicities of four pyrethroids to insects. Thus for pyrethroids, there appears to be an inverse relationship between temperature and toxicity. From all the results and observations, it is inferred that toxicity values were not constant for any particular group of fishes and it varies from species to species with alteration in physical and chemical factors. While assessing the toxicity and risk of pyrethroids on aquatic ecosystems, there is comparatively less harm in a tropical country like India, than in the cold temperature areas of the world. In *Labeo rohita,* fenvalerate caused atrophy and complete fusion of secondary gill filaments, obliterating the interlamellar region. Interlamellar cells proliferate in excess filling up the spaces between secondary gill lamellae and interlamellar region. Pathological lesions were found to be intensified in the gill of *Labeo rohita*, compared with the gills of other two fish (Plate I, Fig. A & B). In *Catla catla*, drastic pathological changes were induced in the gills exposed to fenvalerate. Degenerative changes in intralamellar cells and interlamellar spaces were observed. Separation of epithelial layer from the central sinus of filaments and severe proliferation of epithelial cells were noticed. Club shaped secondary lamellae are an example of progressive degeneration in the gills (Plate II, Fig. A & B). Vacuolar degeneration within the nucleus was evident. The conspicuous change observed in the gill of *Cirrhinus mrigala* is the greater dilation of primary gill lamella when compared to secondary gill lamella (Plate III, Fig. A & B). Rainbow trout administered with permethrin both directly through water and indirectly through food also evidenced similar histopathological changes such as distortion of secondary lamellae, epithelial cells separation, necrosis in gill and mucus cell hyperplasia. These changes may be the result of partially metabolized pyrethroids or their active metabolites reaching the gills through circulatory pathway inflicting damage (Kumaraguru et al.,1982). Fenvalerate exposed rainbow trout indicated lamellar aneurysms on the distal third of many filaments. Lamellae adjacent to aneurysmal lamellae often had necrotic pillar cells. The filamentous tissue between adjacent lamellae was commonly separated from its anchorage on the central sinus of the filament (Bradbury et al., 1988).

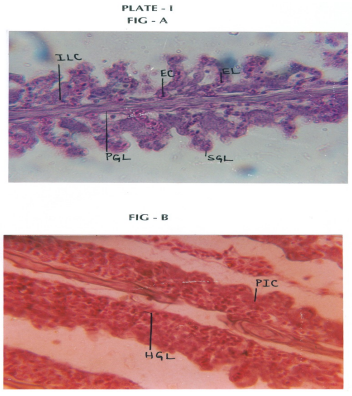

PLATE I: FIG. A Normal Gill Lamella of Labeo rohita, Haematoxylin/Eosin Stain (HE), X400 PGL: Primary Gill Lamella SGL: Secondary Gill Lamella EL: Epithelial cells EC: Erythrocyte ILC: Inter Lamellar Cells

FIG. B Gill Lamella of Labeo rohita exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 PIC: Proliferation of Inter lamellar Cells filling the spaces between secondary gill lamellae. HGL: Hyperplasia in Gill Lamella

PLATE - II
FIG - A

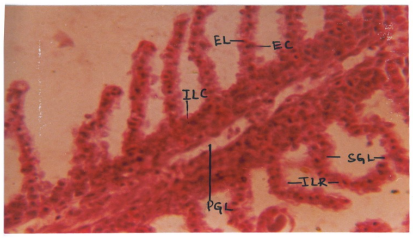

FIG - B

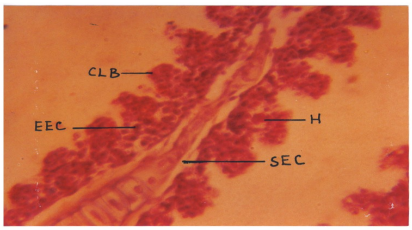

PLATE II: FIG. A Normal Gill Lamella of Catla catla, Haematoxylin/Eosin Stain (HE), X400 PGL: Primary Gill Lamella SGL: Secondary Gill Lamella ILR: Inter Lamellar Region ILC: Inter Lamellar Cells EL: Epithelial cells EC: Erythrocyte

FIG. B Gill Lamella of Catla catla exposed for 10 days to sublethal concentrations of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 CLB: Club-Shaped Secondary Lamella H: Haemorrhage EEC: Exfoliating Epithelial Cells SEC: Separation of epithelial layer from the central sinus of the filament

PLATE - III
FIG - A

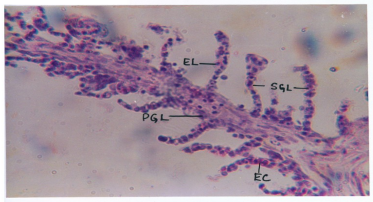

FIG - B

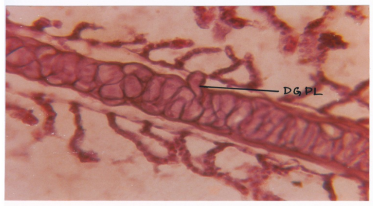

PLATE III: FIG. A Normal Gill Lamella of Cirrhinus mrigala, Haematoxylin/Eosin Stain (HE), X400 PGL: Primary Gill Lamella SGL: Secondary Gill Lamella EL: Epithelial cells EC: Erythrocyte

FIG. B Gill Lamella of Cirrhinus mrigala exposed to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 DPGL: Dilation in Primary Gill Lamella

Fenvalerate also caused profound pathological changes under chronic exposures in fish liver such as degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, necrosis, disappearance of hepatocytic wall, disintegration of lattice fibres and appearance of blood streaks among hepatocytes. All these changes indicate that fenvalerate is a highly hepatotoxic compound. In the liver of *Labeo rohita* exposed to fenvalerate, the changes observed include vacuole formation, hepatocytes became indistinguishable giving rise to a number of intercellular empty spaces with mesh like appearance. This may be probably due to disintegration of lattice fibres, which support the hepatic cells. Degenerative changes in the peripancreatic tissue and blood congestion (blood appeared as streaks) among hepatocytes were evident (Plate IV, Fig. A & B). In the fenvalerate treated liver of *Catla catla*, hepatic cell size drastically decreased compared to the normal cell size (atrophy). Degenerative changes in peripancreatic tissue was also observed (Plate V, Fig. A & B). Pathological changes observed in the liver of *Cirrhinus mrigala* include degeneration in hepatocytes, necrosis, disappearence of hepatocyte wall, decrease in size of nucleus (pycnotic) (Plate VI, Fig. A & B). These observations are in corroboration with those reported in *Clarias gariepinus* (Sakr and Jamal Al lail, 2005) and *Heteropneustes fossilis* (Namita Joshi et al., 2007). According to Jayantha Rao (1982) fenvalerate caused a number of pathological changes in the liver since it is the first organ to face any foreign molecule that is carried through portal circulation. Very few reports are available on animals other than fish exposed to different pyrethroids.

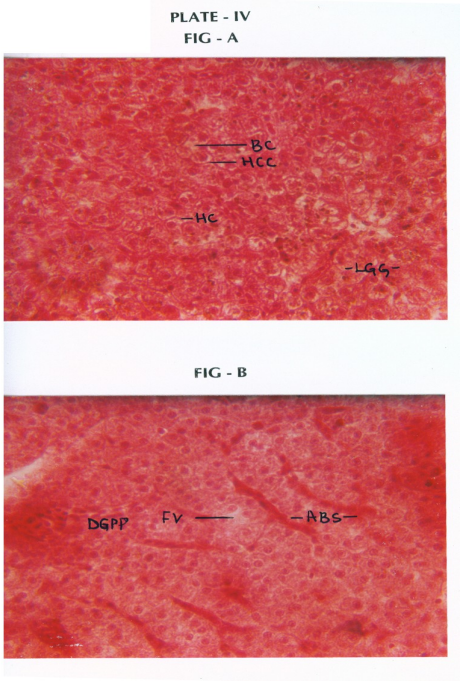

PLATE IV: FIG. A Normal Structure of Liver in Labeo rohita, Haematoxylin/Eosin Stain (HE), X400 HC: Hepatic Cells HCC: Hepatic Cell Cords BC: Bile Canaliculus LGG: Lipid and Glycogen Granules

FIG. B Liver of Labeo rohita exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 FV: Formation of Vacuoles; Intercellular empty spaces DGPP: Degenerative Changes in PeriPancreatic tissue ABS: Appearance of Blood Streaks among hepatocytes

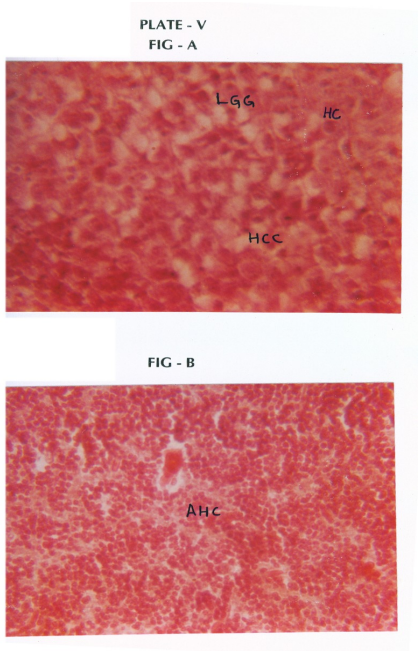

PLATE V: FIG. A Normal Structure of Liver in Catla catla, Haematoxylin/Eosin Stain (HE), X400 HC: Hepatic Cells HCC: Hepatic Cell Cords LGG: Lipid and Glycogen Granules

FIG. B Liver of Catla catla exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 AHC: Atrophy in Hepatic Cells

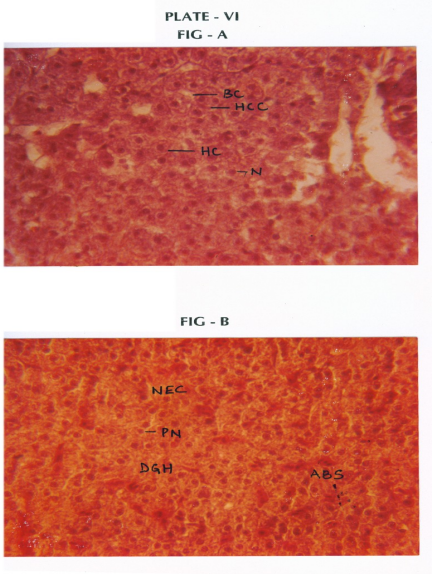

PLATE VI: FIG. A Normal Structure of Liver in Cirrhinus mrigala, Haematoxylin/Eosin Stain (HE), X400 HC: Hepatic Cells HCC: Hepatic Cell Cords BC: Bile Canaliculus N: Nucleus

FIG. B Liver of Cirrhinus mrigala exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 ABS: Appearance of Blood Streaks among hepatocytes DGH: Degeneration in Hepatocytes PN: Pycnotic nuclei NEC: Necrosis in Cytoplasm

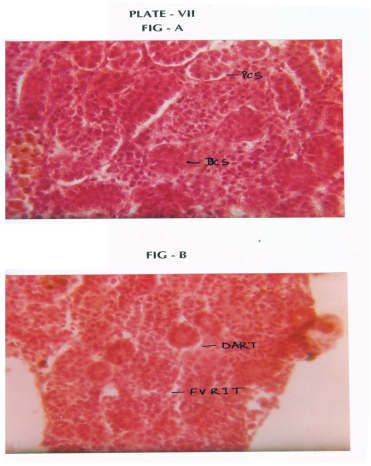

PLATE VII: FIG. A Normal Kidney Structure in Labeo rohita, Haematoxylin/Eosin Stain (HE), X400 PCS: Proximal Convoluted Segment DCS: Distal Convoluted Segment

FIG. B Kidney of Labeo rohita exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 FVRIT: Formation of Vacuoles in the Renal Interstitial Tissue DART: Degeneration and Atrophy in Renal Tubules

Renal excretion is one of the ways of eliminating the undetoxified toxicant molecule resulting in severe pathological changes in haemopoietic tissue, severe necrosis, cloudy swelling of renal tubules, disintegration of interstitial tissue, pycnotic nuclei etc.. Renal tissue in all the three major carps under fenvalerate toxicity evidenced marked pathological changes. Highly degenerative changes in haemopoietic tissue which include severe necrosis, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm were evident. In *Labeo rohita*, proximal convoluted and secondary convoluted tubule were moderately degenerated. Renal interstitial tissue showed formation of vacuoles and cellular contours were not clearly distinguished (Plate VII, Fig. A & B). The renal interstitial tissue was less affected in *Cirrihinus mrigala* than in *Catla catla* (Plate VIII, Fig. A & B) and *Labeo rohita* (Plate VII, Fig. A & B). However large vacuole was noticed around the renal tubule in *Cirrihinus mrigala*. The epithelial cells of the distal convoluted tubule decreased in size. Renal tubules were hypotrophied and hemorrhage was also observed (Plate IX, Fig. A & B).

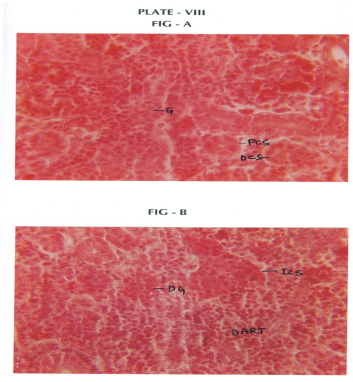

PLATE VIII: FIG. A Normal Kidney Structure in Catla catla, Haematoxylin/Eosin Stain (HE), X400 PCS: Proximal Convoluted Segment DCS: Distal Convoluted Segment G: Glomerulus

FIG. B Kidney of Catla catla exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 DART: Degeneration and Atrophy in Renal Tubules DG: Degeneration in Glomerulus ICS: Intercellular Spaces formation giving mesh like appearence

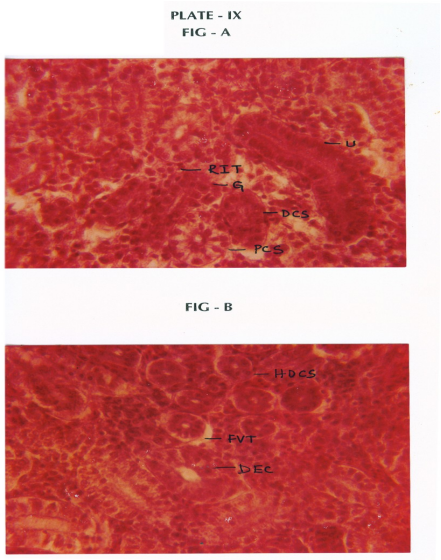

PLATE IX: FIG. A Normal Kidney Structure in Cirrhinus mrigala, Haematoxylin/Eosin Stain (HE), X400 PCS: Proximal Convoluted Segment DCS: Distal Convoluted Segment G: Glomerulus U: Ureter RIT: Renal Interstitial Tissue

FIG. B Kidney of Cirrhinus mrigala exposed for 10 days to sublethal concentration of fenvalerate technical grade, Haematoxylin/Eosin Stain (HE), X400 HDCS: Hypotrophied Distal Convoluted Segment FVT: Formation of Vacuoles around the tubules DEC: Decrease in Size of Epithelial Cells

Many reports on the pathological changes in the renal tubules of fish exposed to various pesticides have been published (Konar, 1970; Sastry and Sarma,1979; Radhaiah, 1985 and Ramamurthy, 1988). Administration of methothrin and pyrethrin caused mitochondrial swelling and necrosis in the renal tubules of rats (El-zalabani and Soliman, 1981). All the observed changes in the present investigation indicate the irreparable damage to vital organs of the fish exposed to sublethal concentrations of technical grade fenvalerate making them less fit for better survival.

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