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Residue analysis of fenvalerate in fish tissues by gas chromatography

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Abstract
The present study was undertaken to evaluate the effect of Fenvalerate in freshwater fish *Labeo rohita*. Fish were exposed to lethal concentrations for 24 h, the LC_{50} values for Fenvalerate at 24 h was 0.4749µgL^{-1} respectively. The 1/10th of 24 h LC_{50} was selected as sublethal concentrations. The present study was undertaken to determine the bio-accumulation of Fenvalerate in tissues of *Labeo rohita* exposed to sub-lethal concentrations 0.04749 µgL^{-1} for 15 days.

Keywords: *Labeorohita***,** fenvalerate, gas chromatography, FID

Introduction
The economy of developing countries like India is agricultural based, but pests act as main challenge in maintaining the economy. So, different pesticides are being used by the farmers for so many years all over the world. The various classes of pesticides include the organochlorines, organophosphates, carbamates, pyrethroids etc [1].
Pyrethroid insecticides have been used in agriculture for more than 30 years to control insect pests in a range of crops. Pyrethroids are synthetic insecticides derived from naturally occurring pyrethrin compounds.
Fenvalerate is one of the pyrethroid insecticide and most widely used in agricultural crops such as cotton, paddy, jowar, maize, soyabean, tomato, lady's finger, cauliflower, tobacco and tea. These pyrethroids account for approximately one fourth of the worldwide insecticide market [2].
The primary transport pathway for pyrethroids entering receiving waters from agricultural and urban applications is through runoff. Pyrethroids are persistent compounds, are not very soluble in water and have a tendency to concentrate in aquatic organisms [3]. Fish samples can be considered as one of the most significant indicators in fresh water systems for the estimation of pesticide contamination [4].
Many pyrethroids possess one or more halogenated atoms, which makes them sensitive to gas chromatography (GC). GC is an analytical method for measuring synthetic pyrethroids in water, sediment and tissue samples at environmentally relevant concentrations [5]. GC is still the method of first choice for the determination of pyrethroid residues [6].
The aim of this study is to determine the bio accumulation of Fenvalerate in tissues of *Labeo rohita* exposed to sublethal concentrations of fenvalerate for 15 days.
In the present investigation, two kinds of chromatographic methods were employed, Column Chromatography (CC) and Gas Liquid Chromatography (GLC). CC is for clean up where by the residue is made free from co-extractives by differential adsorption running through a column packed with adsorbents and the GLC for estimation of residues of the pesticide from tissues of fish[7].

Material and Methods
The freshwater fish *Labeo rohita* (Hamilton) is an edible and commercially valuable fish. Live fish of size 6-7 ±1cm and 6-8 g weight were brought from a local fish farm and acclimatized at 28 ± 2 ^{0}C in the laboratory for one week. The stock solutions for Fenvalerate 20% Emulsifiable Concentrate (EC) were prepared in 95% acetone to yield a concentration of 100mg/100ml which were further diluted with distilled water to get a working solution.

The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a capacity of ten liters. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from ten percent to ninety percent for 24 hrs in static tests. The concentration that produced fifty percent mortality in test species noted. LC_{50} values were calculated by Finney's Probit analysis (1971) [8].

Residue Analysis

After exposure to sublethal concentrations of Fenvalerate for 15 days, the fish were sacrificed and analyzed for residues.

Tissue Extraction

The residues from the fish tissues of brain, liver, kidney, muscle and gill were extracted by the modified Mills and Olney (1977)[9] method incorporated in the pesticide analytical manual (PAM). To 1 g of tissue 0.5 g of anhydrous sodium sulfate was added and extracted into 20 ml of Hexane: Acetone (1:2), where as the brain was extracted into 20 ml of acetonitrile. The extract was filtered and evaporated to 1 ml on boiling water bath.

Cleanup and Removal of the co-extractives:

After extraction, acetone was washed out and the hexane was dried out over sodium sulfate (AR grade). The extract was stored in stoppered glass vials and kept in the refrigerator for further processing.

The hexane extract was concentrated to about 1 ml (when the volume of extract was 4 ml or more) and transferred directly on to a florisil column prepared according to the pesticide analytical manual (PAM) method. Florisil was obtained from Sigma Chemical Company (60-80 mesh PR grade) and was heated overnight at 130^0C and after cooling it was deactivated with water 2% (v/w). The column was eluted with 100 ml of hexane followed by successive grades of increasing polarity using hexane and acetone.

Gas-Liquid Chromatography Analysis:

The quantitative analysis of test toxicants was carried out with a gas chromatography (Shumatzu) in combination with Flame Ionization Detector (FID). The temperature of FID detector was maintained at 295 ºC. Nitrogen was used as the carrier gas; the flow rate was maintained at 50ml/min. 0.5 µl of sample was injected through the injection port by using Hamilton syringe. The temperature of the column oven was maintained at 120 ºC. Quantitation of pesticides in different tissues was calculated based on comparsion with the standard calibration curve.

Results and Discussion

The results of the gas liquid chromatographic analysis in the tissues of brain, muscle, gill, kidney and liver of the fish, *Labeo rohita* were given in Table (1) and Figures (1-6).

Table 1: Residue level of Fenvalerate in different tissues of *Labeo rohita* exposed to sublethal concentration of Fenvalerate for 15 days.

S.NO	Organs	Area	Amount of Residue mg g^{-1}
1.	Standard	77.5	0.1
2.	Muscle	16.27	0.0209
3.	Kidney	21.6	0.0278
4.	Brain	23.871	0.0308
5.	Gill	29.359	0.0378
6.	Liver	51.3	0.0661

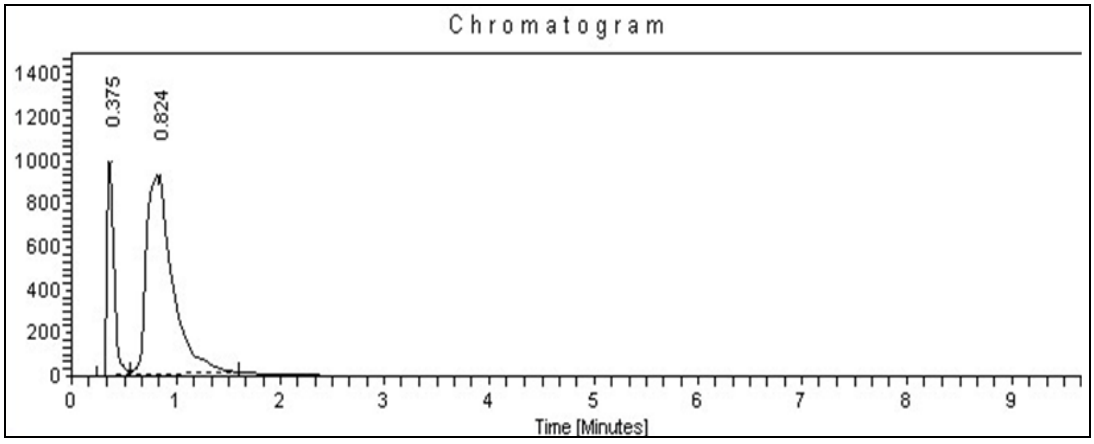

Fig 1: Chromatogram of fenvalerate Standard

Table 2

Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%
1	0.375	4234.843	996.015	21.172	51.902	0.084
2	0.824	15767.317	923.026	78.828	48.098	0.266
Total		20002.16	1919.041	100	100	

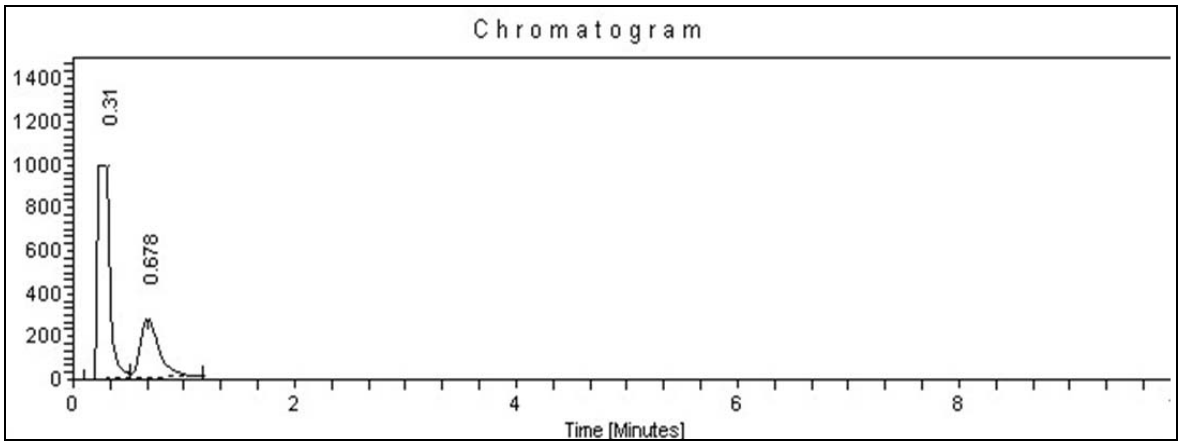

Fig 2: Chromatogram of *Labeo rohita* gill tissue on exposure to fenvalerate for 15 days

Table 3

Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%
1	0.31	7904.918	994.343	71.056	78.193	0.134
2	0.678	3219.931	277.308	28.944	21.807	0.184
Total		11124.849	1271.651	100	100	

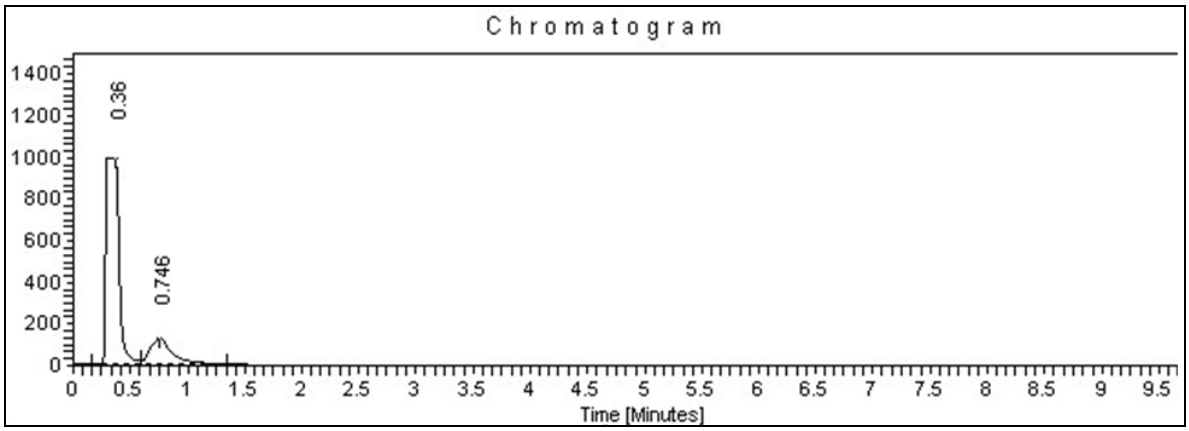

Fig 3: Chromatogram of *Labeo rohita* brain tissue on exposure to fenvalerate for 15 days

Table 4

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	0.36	7181.027	994.487	81.624	88.872	0.134
2	0.746	1616.626	124.526	18.376	11.128	0.184
Total		8797.653	1119.013	100	100	

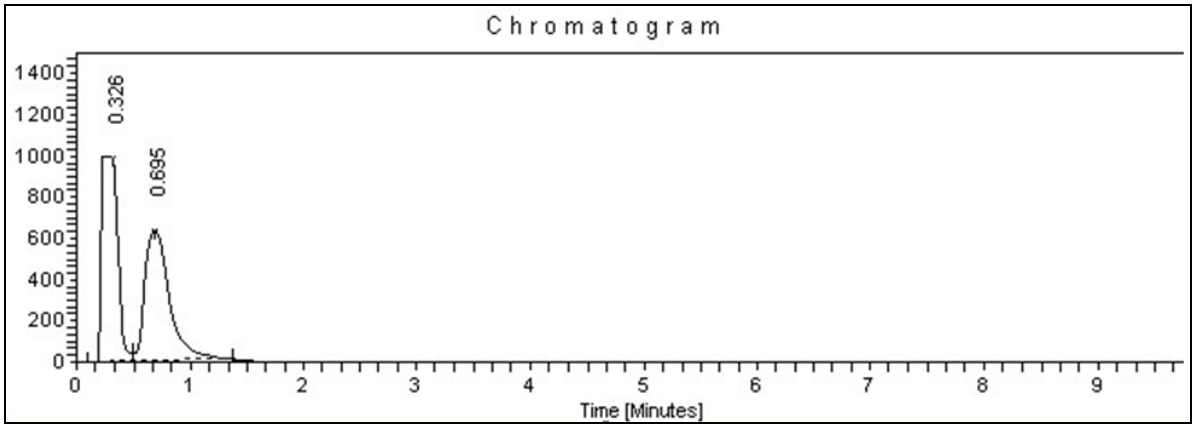

Fig 4: Chromatogram of *Labeo rohita* liver tissue on exposure to fenvalerate for 15 days

Table 5

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	0.326	9693.799	994.473	50.844	61.033	0.168
2	0.695	9371.872	634.917	49.156	38.967	0.234
Total		19065.671	1629.39	100	100	

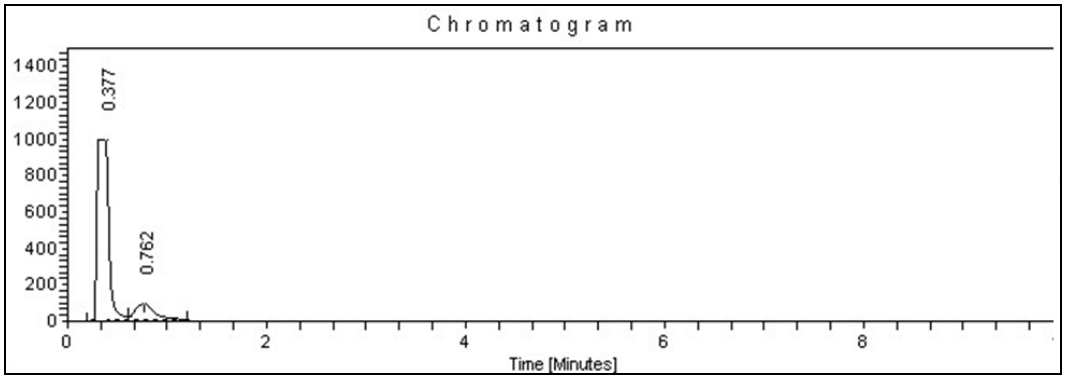

Fig 5: Chromatogram of *Labeo rohita* kidney tissue on exposure to fenvalerate for 15 days

Table 6

Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%
1	0.377	7366.156	994.661	86.556	91.563	0.117
2	0.762	1144.083	91.657	13.444	8.437	0.201
Total		8510.239	1086.318	100	100	

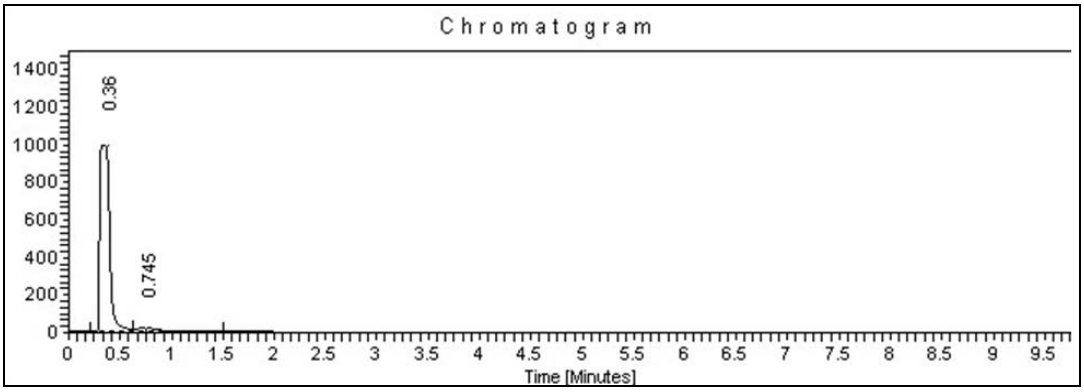

Fig 6: Chromatogram of *Labeo rohita* muscle tissue on exposure to fenvalerate for 15 days

Table 7

Peak No	Retn. Time	Area	Height	Area %	Height %	Width@50%
1	0.36	5883.779	994.445	94.682	97.902	0.1
2	0.745	330.47	21.314	5.318	2.098	0.251
Total		6214.249	1015.759	100	100	

The variations in the residue analysis are attributed to factors like difference in uptake rate and lipid content of respective animal tissue. The chemical structure, solubility, fish interaction and metabolic pattern are responsible for pesticide uptake.

Under sublethal exposure to Fenvalerate 15 days, it was observed that *Labeo rohita* tissues bioaccumulated the Fenvalerate.

In the tissues of test fish, residue levels were in the order

Liver > Gill> Brain > Kidney > Muscle.

Pesticide residues have been recorded in water and fish samples from various authors showing that level of exposure of pesticide could be harmful to human. Assessment of Organochlorine residues in the surface sediments of River Yamuna in Delhi, India has been done [10]. Positive detections of pesticides in fish tissues have also been made from river Gomti, Uttar Pradesh [11] and Lake Kolleru, Andhra Pradesh [12]. The levels of organochlorine pesticide residues have been determined in five freshwater fish species in Punjab [13].

The higher levels of pesticides found in the fish might be due to the fact that fishes have a greater tendency to accumulate the pesticides in their body due to bioaccumulation [14]. Endosulfan and its metabolites were determined in human urine by using gas chromatography [15]. The levels of organochlorine pesticides in fish from Zareb area were determined by using Gas chromatography [16]. Under sublethal exposure to Chlorpyrifos for a period of eight days, it was

observed that *Labeo rohita* tissues accumulated more amount of residue when compared to other two fish, *Catla Catla* and *Cirrhinus mrigala* by GC/FID [17].

The levels of organochlorine (OC) pesticide residues in Lake Parishan have been investigated in water, sediment and fish (*Barbus brachycephalus caspius*) samples by using Gas chromatography. The levels of most of the residues in fish were higher than those found in water [18]. Residue analysis of endosulfan from tissues of fresh water fish (*Labeo rohita*) was done to determine its bio-accumulation in tissues of *Labeo rohita*, exposed to sub-lethal concentrations 0.06876 µg/L [19].

Conclusion

The results of the present study revealed that, prolonged exposure to sublethal concentrations of Fenvalerate in *Labeo rohita* leads to increased accumulation of residues than in short term exposures to lethal concentrations [20]. This is in corroboration with the earlier reports of synthetic pyrethroid residues. A thorough literature search revealed that repeated or continuous exposure to low concentrations of pesticides can lead to high residue concentrations without mortalities. Thus the uptake and persistence of Fenvalerate depends not only on a number of physical and chemical properties, but also varies according to the biological factors.

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