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Impact of Synthetic Pyrethroid Fenvalerate Induced Lipid levels in certain tissues of Rana *Tigrina* (Indian Bull Frog).

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ABSTRACT

The rising use of pesticides causes chemical pollution results potential health hazards to live stock, especially to fish, frogs, birds and mammals. Since majority of pesticides are known to bio accumulate in the lipid tissues of frog and other animals. The aim of this work was to study the effect Synthetic Pyrethroid Fenvalerate Induced Lipid levels. Healthy frogs, *Rana tigrina* weighing 50 ± 3 gms were collected from the pond, acclimated to the laboratory conditions in large glass aquaria with water. The frogs were divided into groups of ten animals. They were exposed to different pesticide concentrations of fenvalerate, both commercial and technical grade, according to biomass ratio as suggested by *Doundroff et al.,* (1951). The total lipid content showed an increase over control in all the tissues after exposure to fenvalerate. The increase was consistant and significant ($P< 0.001$) in all tissues and exposures studied.The percent in change is observed to be more in muscle (48.66%) when compared with that of other tissues, after four weeks exposure. Brain of control frogs showed more lipid content and muscle showed the least among the tissues studied. Brain and liver, whose functions are actively positive for metabolism in the animal, have shown high amounts of lipid. Lipid content increased significantly in all the tissues studied after exposure to fenvalerate for I, II and IV weeks. The percent increase was more in muscle when compared with other tissues which varied between 28% to 39% after the four weeks exposure.

Key words: Fenvalerate, Lipid levels, Liver, Muscle, Intestine, Brain and Kidney, *Rana Tigrina* (Indian Bull Frog).

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INTRODUCTION

The increasing use of pesticides causes chemical pollution results potential health hazards to live stock, especially to fish, frogs, birds and mammals. Since majority of pesticides are known to bio Accumulate in the lipid tissues of fish, Frogs and other animals, when the pesticides come in contact with internal organs, irreversible changes in metabolic activities, many pesticides have been reported to produce a number of biochemical changes in fish and frogs at sub-lethal levels.
These uncontrolled uses of agrochemicals create a serious ecological problems and which causes many threats to non-target organisms like fish, prawn, frog and birds in the environment. (Das BK, Mukherjee SC, 1997). Pyrethroids (also known as synthetic pyrethroids) are insecticides chemically similar to pyrethrins found in natural pyrethrum extracted from the flowers of chrysanthemum, known for centuries for their insecticidal activity (CPCN, 2001). First developed in 1973, pyrethroids are more stable to light than natural pyrethrum and possess very good insecticidal activity. The first pyrethroid (fenvalerate) was commercialized in 1978. At present, the class of pyrethroids includes 42 active ingredients, differing in chemical structure or in relative stereoisomer composition (NPTN, 1998). Pyrethroids are historically divided into two types, (Table 1).according to their chemical structure: *type I pyrethroids*, which do not contain an alpha-cyano group in their molecule and which cause mainly tremors (T-syndrome); and *type II pyrethroids*, which do contain an alpha-cyano group (for example, fenvalerate) and which cause choreoathetosis and salivation (CS-syndrome) (Tordoir *et al.*, 1994).

Type I Pyrethroids	Type II Pyrethroids
Allethin	Cyfluthrrin
Bifenthin	Cyhalothrin
Permethin	Cypermethrin
phenothin	Deltametrin
Resmethrin	Fenvalerate
Tefluthrin	Flucythrinate
Teramethrin	Fluvalinate

Table 1. Types of Synthetic Pyrethroids

Fenvalerate is highly active contact insecticide effective against a wide range of pests. It is also used in public health and animal husbandry. When this insecticide is washed down with rain it reaches to different water bodies and brings out toxic effect on various aquatic rganisms articularly fishes. Chaudhari and Saxena (2015); Dasgupta and Panigrahi (2014); Prusty *et al*., (2015); Verma and Saxena (2010) have reported the toxic effect of synthetic pyrethroids on the biochemical constituents of different Aquatic fauna.

Rana Tigrina (Indian Bull Frog) play role as a pest controlling agents is conclusively established. Several important agricultural pests belonging to Orthoptera, Isoptera, Lepidoptera, Hemiptera and Coleoptera were found to be the major sources of frogs. These pests destroy crops and carry diseases. Thus amphibians render incalculable services to agriculture. The most important function of amphibians in nature has been grossly underestimated. While on the one hand they are active predators, on other hand they constitute a vital link in the food chain of life by serving as prey base for apex predators in the ecosystem. Being extremely voracious they are natural population regulators of the numerous invertebrate species that they feed on. *Bufo melanostictus Schneide*r (common Indian toad), the males of this species is used in pregnancy diagnosis tests of human beings (Sarkar**,** 1984).

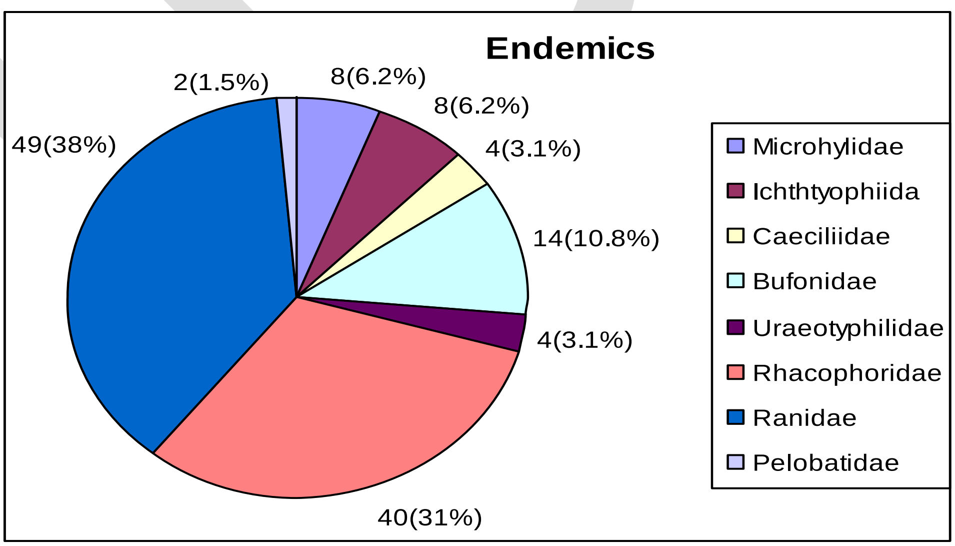


Fig: 1 The Figure illustrates the distribution of endemic Amphibians represented in India

SOURCE: Subba Reddy SV, (2007)

A total of ten families are represented among Indian Amphibians (Fig: 1) of which family Ranidae is the most represented followed by Rhacophoridae and Bufonidae. Utilization of frogs by the eradication and export sectors can make them endangered species. The consistent pesticide application in the fields may hasten the depletion of frogs. Recently, scientists found a way to make a new drug pain killer from the toxins in a frog's skin (*Epibpedobates tricolor*). Theoretical models of ill-effect caused by pesticides show that non-target organisms like frogs are ultimately eliminated instead of the pests in a given ecosystem (Rosenzweig, 1977).

Conservation of frogs has been taken up by many organizations. Convention on international trade in endangered species in West Germany has placed Indian bull frog, *Rana tigrina* in the protected list. Indian Government had banned the export of frog legs and Indian Board for wild life had recommended that Rana species should be included in Schedule - II, Part-II: of wild life (protection) Act 1972, thus providing frog special status of protection. Conservation action and recommendations made by some Education Working group (Deuti *et al*., 1998) have given by some suggestions. Populasrising Amphibians through television (wild life films depicting Indian Amphibians), cartoon films with animal of "foggy" character, quiz and radio (frog calls).Zoos to exhibit Amphibians and sell stickers, posters, leaflets, Tee-shirts promoting Amphibian protection and conservation and general knowledge of amphibians.Creating awareness among villagers of the ecological importance of Amphibians and their habitats by mass media (television, radio, newspapers) in cropping season. Usage of computer software to demonstrate dissection and minimise number of specimens dissected by students.Nature camps to be organised among students to promote "frog watching".

Material and Methods:

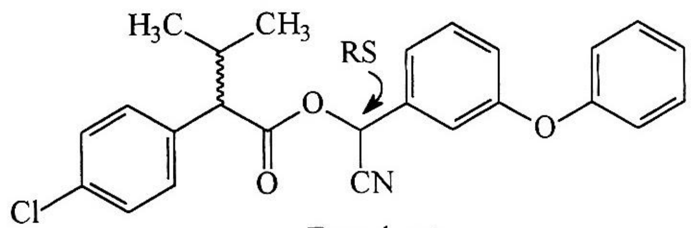
Procurement of the experimental animal:

Rana tigrina is commonly known as Indian Bull Frog. They are occurring near the tanks and ponds in and around Tirupati (A.P.). Besides experimental frogs other species of frogs were also collected and their morphological features were studied. For the present study, the locally available frog, *Rana tigrina* was selected.

Selection of the test chemical:

Fenvalerate (Sumcidin (R) (5-5602 OMS – 2000) a synthetic pyrthroid compound both commercial (Fenvalerate EC 20) and Technical grade, 93.7% (wt/vol) supplied as gratis by Rallis India Limited, Bangalore (India) was used. The following are the physico-chemical properties of fenvalerate used in the present study.

1. Common name:- Fenvalerate
2. Commercial name: - (R.S.) Cyano-3-phenoxy benzyl (IR, IS) 2-(4-Chlorophynyl) 3-methyl- Butgrate.
3. Structural formula:-



Fenvalerate

4. Synonyms:- Fenvalerate (Bis, Iso)
5. Trade names:- Sumicidin, Belmark and Pydrin
6. Empirical formula :- C_{25} H_{22} Cl No_3
7. Molecular weight :- 419.9
8. Physical weight :- Viscous liquid
9. Density :- 1.17 gm/ml. at 23°C
10. Vapour pressure:- 2.8 x 10 mm Hg . at 23°C
11. Boiling point:- 300°C /37 mm hg
12. Solubility in water :- 1 mg. in liter of water at 23°C
13. Solubility in solvents :- Acetone, Cyclohexane, Ethanol, Xylene and Chloroform

14. Odour:- Odourless

15. Colour :- Light brown

16. Courtesy:- Rallis India Agrochemical Division, Navi, Mumbai

17. Partition coefficient:- 1.03 x 10 5 (n-octyl alcohol/H2 O) at 23°C

18. Stable: - stable is most organic solvents except alcohols and inorganic mineral dilutents unstable in alkaline media.

Preparation of Stock Solution

The active ingredient of commercial grade 93.7% of fenvalerate was used for present investigation. A stock solution of fenvalerate was prepared by dissolving the fenvalerate in Acetone. Available literature indicates that low levels of acetone are harmless to the biological system (Pickering *et al*., 1962). The quantity of acetone used was found to be non-toxic to non-target animals and it was biologically safe in the preparation of stock solution of pesticides (Jagannatha Rao**,** 1981). One gram of technical grade of fenvalerate (93.7%) is dissolved in minimal quantity of acetone and this was made up to 937 ml with water to make 1000 ppm of stock solution. Fresh stock solution was prepared for experimental use.

Toxicity evaluation

Toxicity was evaluated in static waters. Animals were starved 24 hours prior to the exposure. During the exposure to pesticide, only actively moving frogs of same size were selected from the collection for testing. Preliminary screening tests were conducted to obtain the range of lethal concentrations and based on these results, the experiments were carried out.

The frogs were divided into groups of ten animals. They were exposed to different pesticide concentrations of fenvalerate, both commercial and technical grade, according to biomass ratio as suggested by Doundroff *et al*., (1951).

Experimental Design:

Healthy frogs, *Rana tigrina* weighing 50 ± 3 gms were collected from the pond, acclimated to the laboratory conditions in large glass aquaria with water (Temperature 27 ± 2°C; P^H 7.0 ± 0.2, light period – 12 hours) for 7 days. They were fed with cockroaches and earthworms *ad libitum*, with change of water daily. They were exposed for 1 week, 2 week, and 4 week in sublethal concentration (9.4 mg/lt) of fenvalerate i.e $1/5^{th}$ of LC_{50} of 48 h. After stipulated period, the selected tissues were isolated from Control and fenvalerate exposed frogs, The tissues stored at -80 ^oC for further biochemical analysis.

Biochemical investigations:

Total lipids were estimated by the method of *Folch et al*., (1957). A mixture of chloroform: methanol (2: 1. v/v) was used as the homogenizing medium. The tissues were isolated, dried, weighed, homogenized and centrifuged at 1000 xg for 15 minutes. The supernatant was taken in a small container (whose initial weight was recorded) and evaporated at 50–60^0C to dryness. Subsequently, the container was again weighed and the difference between the final and initial weights represents the total lipid content. The values were expressed as mgs total lipid/g dry weight of tissue.

Statistical Analysis:

Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance and the results were presented with the P-value.

RESULTS :

The total lipid content showed an increase over control in all the tissues after exposure to fenvalerate. The increase was consistent and significant ($P< 0.001$) in all tissues and exposures studied. The percent in change is observed to be more in muscle (48.66%) when compared with that of other tissues, after four weeks exposure (Table 2 & Fig. 2).

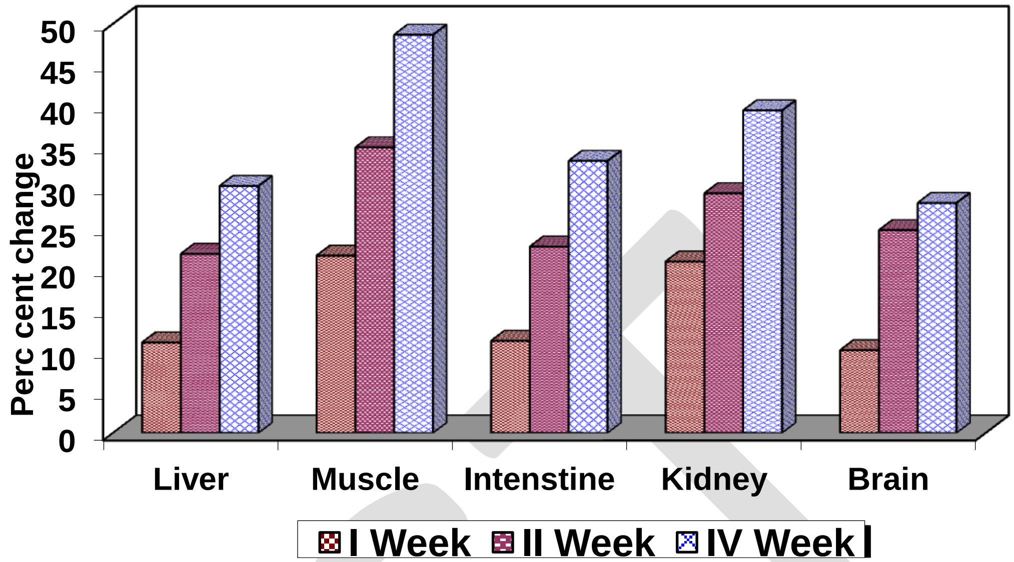
Brain of control frogs showed more lipid content (Table 2 & Fig. 2) and muscle showed the least among the tissues studied. Brain and liver, whose functions are actively positive for metabolism in the animal, have shown high amounts of lipid. Lipid content increased significantly in all the tissues studied after exposure to fenvalerate for I, II and IV weeks. The percent increase was more in muscle (48.66%) when compared with other tissues which varied between 28% to 39% after the four weeks exposure. The increased levels in fourth week tissues was as follows: **Muscle > Kidney > Intestine > Liver > Brain**

Table 2: Lipid levels in the tissues of control and fenvalerate exposed frogs (mg/gm dry wt.)

	I week		II week		IV week	
	Control	**Experiment**	**Control**	**Experiment**	**Control**	**Experiment**
Liver	213.36	236.96	214.98	262.05	215.97	281.17
S.D.	±0.409	±0.593	±0.755	±0.344	±1.064	±1.125
%Change		+11.06		+21.89		+30.18
		P<0.001		P<0.001		P<0.001
Muscle	86.69	105.48	86.15	116.21	85.84	127.44
S.D.	±0.732	±0.741	±1.008	±0.593	±0.779	±1.066
%Change		+21.67		34.89		+48.66
		P<0.001		P<0.001		P<0.001
Intestine	149.07	165.86	151.95	186.57	149.06	198.62
S.D.	±0.757	±0.926	±0.872	±0.499	±0.562	±0.578
%Change		+11.26		+22.78		+33.25
		P<0.001		P<0.001		P<0.001
Kidney	98.88	119.6	97.63	126.23	98.56	137.41
S.D.	±0.546	±1.115	±1.018	±0.613	±0.894	±0.746
%Change		+20.95		+29.29		+39.41
		P<0.001		P<0.001		P<0.001
Brain	452.37	498.13	448.81	560.01	452.86	580.14
S.D.	±1.362	±0.799	±3.49	±0.921	±2.94	±3.96
%Change		+10.12		+24.78		+28.11
		P<0.001		P<0.001		P<0.001

Values represent mean of six individual observations, ± S.D., Figures in parenthesis indicate per cent change over control. P=`t' test.

Fig. 2 : Percent change of Lipid levels in the tissues of fenvalerateexposed frogs



DISCUSSION:

Change in lipids and its derivatives are known to have profound effect on the metabolic and physiological status of an animal. Pyrethroids are lipophilic in nature (Elliott and Janes, 1978; Naumann, 1981) and have specific affinity for lipids. Hence, in the present study, levels of total lipids were estimated in the different tissues of frog during fenvalerate intoxication, in order to understand the role of these compounds during fenvalerate induced stress.

Increase in the lipid content in all the tissues after fenvalerate exposure may be due to increased lipogenesis through metabolic regulation and compensation by certain lipid fractions like phospholipids, cholesterol, free fatty acids etc., to overcome the impact of pesticide and to protect the cellular integrity and functions. Swami *et al*., (1983) stated that animal physiology favours lipid metabolism than carbohydrate metabolism through acetyl Co A leading to increase in the lipids.

A general increase in total lipid content in the tissues indicate that the tissues might have become highly activated under pyrethroid exposure, due to high sensitivity of these tissues to fenvalerate. It is also suggested that maintenance of homeostasis and tissues metabolism were carried on by the increased lipid levels in the tissues of frog exposed to fenvalerate, since metabolic compensation is bound to occur during the toxic stress, which involves synthesis and breakdown of several constituents to face the unwanted change. Kiran Rani Saxena *at el*., (2015) reported that Fenvalerate significantly increased total fat in both muscles and liver. Bhatia and Venkata Subramanian (1973), and Peter *et al*., (1973) advocated that pesticides affect the lipids, besides the carbohydrates. Siva Prasada Rao (1980) also reported that organophosphate pesticide, methyl parathion caused decrease in total lipid content of the hepatic as well as extrahepatic tissues of fish, *Tilapia mossambica*. Satyavelu Reddy (1985) observed reduction in total lipids in different tissues of fresh water muscle under methyl parathion stress. Sreekanth (2002) observed reduced cholesterol levels, tryglicerides, phospholipids and increased lipid peroxidation in rats during organophosphate toxicity. Results of several other workers (Lindvall and Jessop, 1979; Chefurka, 1980; Mahmood *et al*., 1980; Agarwal, 1981; Radhaiah *et al*., 1987; Vijaya Joseph, 1989) who worked with various animals and various pesticides showed similar increase in the total lipid content.

Lipids are heterogenous group of complex molecules and are the most important sources of energy to the organism, yielding per gram, over twice as many calories as do carbohydrates and proteins. The lipids are esters of fatty acids, or substances capable of forming esters which consists of fats, oils, phospholipids, triglycerides, glycerol, cholesterol, neutral lipids etc. The breakdown of neutral fats are closely associated with the metabolism of glucose because of the formation of common intermediate acetyl – Co A in both pathways (Harper, 1985). The initial steps in the breakdown of neutral fat involves splitting of the three fatty acids from glycerol. The fatty acids and glycerol thus produced through two separate pathways enter the krebs cycle. The lipids constitute not only the architecture of the cell but also form a co-basis for the structure of some enzymes like $Mg2^+$ ATPase. Lipids offer full complementary structure to steroid hormones. They also contribute towards energy synthesis as an alternative to the carbohydrates (Gold fine, 1968; Lehninger, 1978; Guyton, 1981). Lipids play a vital role during the biochemical adaptation of animals in stress condition (Swami *et al.*, 1983).

CONCLUSION

The growing population has increased the demand for food supply which in turn has increased the usage of insecticides and pesticides to protect the precious food source. Biodegradation is a practical approach to reduce pyrethroid toxicity in the environment. From the above observations the survey of amphibians, we believe that is it is necessary to assess amphibian fauna of this area before their natural habitate are altered or damaged beyond a true reflection of their species diversity and population abundance. Also prolonged exposure to sublethal levels of fenvalerate seems to be hazards to the aquatic life, causing drastic biochemical changes and irreparable architectural changes investigated in the present investigation.

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