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Significance of Chronic Synthetic Pyrethroid Fenvalerate Administration on Carbohydrate metabolism in the Muscle, Brain and Intestine of *Rana tigrina* (Indian Bull frog)

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ABSTRACT

The natural pyrethroids are not so much effective against agricultural pests, thus artificial synthesis of synthetic pyrethroids like fenvalerate was carried out by scientists. Fenvalerate is a insecticide has been proved to be highly toxic to non-target species like amphibians. In our study, 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 carbohydrate content in the present study Control Muscle tissue has shown higher amount of carbohydrates than compare with Brain and Intestine tissue. Under fenvalerate intoxication, the frog tissues showed consistent decrement in all the periods and it may be due to rapid utilization to withstand pesticide of stress. Total protein contents in control frog tissues the Muscle showed higher concentration of proteins than Intestine and Brain. The protein content in experimental frog tissues showed depleted levels when compared to controls. The significant decrease in protein and it may be due rapid utilization to withstand pesticide of stress. Total free amino acid (FAA) showed enhanced Intestine FAA levels, the levels in fenvalerate exposed frog tissues were increased significantly when compared to control. These elevated levels may be due to improved protease activity or enhanced transamination. From the above observations fenvalerate seems to be hazards to the aquatic life, causing severe biochemical changes and permanent architectural changes investigated in the present investigation.

Key words: Fenvalerate, total Ccarbohydrate content, Total Protein content, Total free amino acid (FAA) levels, Brain, Intestine and Muscle, *Rana Tigrina* (Indian Bull Frog).

Introduction

Synthetic pyrethroid insecticides have been used for more than 20 years to control insect pests in a variety of crops (Maund *et al*., 2001), but they have become increasingly popular following outright bans or limitations on the use of cholinesterase-inhibiting insecticides (Luo & Zhang, 2011; Feo *et al*., 2010). Fenvalerate is the most widely used compound of the cyanophenoxy-benzyl group of the synthetic pyrethroid pesticides and it is used in agriculture to protect a wide variety of crops including

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cotton, soybeans, corn, vegetables, apples, peaches, pears and nuts from insect pests .In India, it is used primarily to control pests of cotton and vegetables (Madan VK *et al.,* 2000). Fenvalerate is highly toxic for fish (Madhuban Datta Bhattacharya, Anilava kaviraj, 2006) and bees, while for birds and mammals its toxicity is low. However, the current information is not sufficient to adequately assess the risk posed by fenvalerate to non-target organisms, though some work has been done to assess its toxicity to non-target species (Sanchez-Fortun S and Barahona MV. 2005). Pyrethroids and substantially lower oxidative activity than warm-blooded animals (Bradbury and Coats 1989a, 1989b); efficiently accumulate fenvalerate from the medium (Gray and Soderlund 1985); and show greater intrinsic sensitivity of the central nervous system when compared with birds and mammals (Gray and Soderlund 1985; Bradbury and Coats 1989a).

Types of Pesticides	Consumption percentage	
	Global	India
Insecticides	29	63
Herbicides	44	14
Fungicides	21	21
Others	6	2

Source: Consumption levels of various pesticides globally and India.
(S.V.Subbareddy, 2007)

Fenvalerate is one of the more persistent synthetic pyrethroids in soils (Klaassen *et al*., 1986). Liver is the predominant site of fenvalerate metabolism through hydrolysis by one or more hepatic microsomal esterases; inhibition of these enzymes results in enhanced toxicity (Ghiasuddin and Soderlund 1984). Hydrolysis has also been demonstrated in plasma, kidney, stomach, and brain tissues. Except for brain, however, these tissues were relatively unimportant in the detoxification process (Ghiasuddin and Soderlund 1984; Gray and Soderlund 1985). Fenvalerate inhibits intercellular communication between fibroblast cells and enhances the development of hepatocyte loci in rat liver at nonheptatotoxic dose levels. Chemicals that possess these properties are likely to be tumor promoters (Flodstrom *et al*. 1988). High doses of Fenvalerate has been reported to be associated with reduction of body mass, increase in liver mass, and proliferation of the smooth endoplasmic reticulum in hepatic cells, and induction of the activity of microsomal enzymes (El-Sewedy SM *et al*.,1982; WHO,1991).

Taxonomic position of *Rana tigrina* (Indian Bull Frog)

Phylum: Chordata
Class : Amphibia
Order : Anura
Family : Ranidae

Amphibians are more threatened and are declining more rapidly than either birds or mammals distribution, tolerance of a broad range of habitats, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category. (Stuart, S. N. *et al*., 2005). Frogs have been a major source of food for many countries. India, Indonesia and Bangladesh were the main exporters of frog legs to European countries. Their flesh taste somewhat like a real calf fish, chicken and is regarded as a great delicacy. Most of the frog legs served as gastronomic delicacies in Europe is made from Asian Bull Frogs. The Indian Bullfrog Hoplobatrachus tigerinus is the largest frog in India, which grows up to 15 cm in length. They are found in various colours ranging from yellow to olive green, with dark irregular markings. They have a pointed snout and long hind limbs. Their toes are nearly entirely webbed. The Indian Bullfrog is a lone forager and nocturnal. Their diet includes invertebrates, small mammals and birds. Breeding takes place during the monsoon season, when adults congregate at ephemeral

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rainwater pools. They produce a large number of eggs, but the mortality rates among tadpoles, froglets and adult frogs **(***Rana tigrina***)** are high.(Fugler,1983).Distribution of frogs disrupts the ecological balance and increases incidence of pest out breaks, necessitating the use of poisonous pesticides. The millions of frog legs that are slaughtered every year would have consumed several hundred thousand tonnes of insects and saved a great deal more than Rs. 10 crores in pesticides use and several times more in available ecological disruption where the costs are incalculably higher **(***Vijaya Joseph and Jayantha Rao*, 1987**).**

Hence, the toxicity of fenvalerate in terms of sub-lethal concentration was studied with some biochemical aspects of different tissues like Muscle, Brain and Intestine of frog. The present study reveals significant variation in Carbohydrate metabolism and associated enzymes systems after exposure of fenvalerate in different tissues of *Rana tigrina*.

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.

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 upto 937 ml with water to make 1000 ppm of stock solution. Fresh stock solution was prepared for experimental use.

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 liver and kidney tissue was isolated from Control and fenvalerate exposed frogs, The tissues stored at -80 ^oC for further biochemical analysis.

Biochemical Analysis:

The total carbohydrate content was estimated in the control and experimental tissues by the method of Carrol *et al*., (1956). The tissues were isolated and 2% homogenates in 10% trichloroacetic acid were prepared. The homogenates were centrifuged at 2500 rpm for 15 minutes. 0.5 ml of the clear supernatant was taken, followed by 5 ml of anthrone reagent. The contents were boiled for 15 minutes. The tubes were cooled and the color developed was read at 620 nm in a spectrophotometer using blank, containing trichloro acetic acid and anthrone reagents in the same proportion. The OD of the sample was compared with that of the standard and the total carbohydrates with that of the standard and the total carbohydrates content was expressed as mg/g wet weight of the tissues.

Total protein content was estimated by the method of Lowery *et al.,* (1951). Different tissues were isolated and 2% homogenates were prepared in 10% trichloroacetic acid. 1 ml of the crude homogenate was taken and centrifuged at 2500 rpm for 10 minutes. The sediment was dissolved in 5 ml of 1N sodium hydroxide by thorough shaking. From this 0.1 ml of solutions were taken and 4 ml of alkaline

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copper reagent was added followed by 4 ml of folin phenol reagent (1:1, Folin:H_2O). The color (Light blue) was read at 600 nm against the blank in spectrophotometer. The standard graph was prepared with bovine serum albumin. The protein content was expressed in mg/g wet weight of the tissue.

Total free amino acid content in control and experimental animal tissues was estimated by the method of Moore and Stein (1954) as described by Colowick and Kaplan (1951). 2% homogenate of tissues were prepared in 10% trichloro acetic acid. The contents were centrifuged at 2500 rpm for 15 minutes. To 0.05 ml of the supernatant 2 ml of ninhydrin reagent was added and kept in boiling waterbath for exactly 12 ½ minutes and cooled immediately to room temperature. The solution was then made upto 10 ml with distilled water and the bluish pink color developed was read at 570 nm in spectrophotometer against blank. The free amino acid content was expressed as "μ" moles of tyrosine equivalent/g wet weight of the 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 and Discussion

The Total carbohydrate:

The total carbohydrate levels in control and fenvalerate exposed frogs of different tissue are presented in **Table.1**. Control Muscle has

Table-1. Carbodydrate levels in the tissues of control and fenvalerate exposed frogs (mg/gm wet wt.)

	I week		II week		IV week	
	Control	Experiment	Control	Experiment	Control	Experiment
Muscle						
S.D.	72.75	60.98 ± 0.809	76.08±1.301	50.00 ± 0.589	76.49±0.961	38.63±1.061
% Change	±1.225	-16.17		-34.28		-49.48
		P<0.001		P<0.001		P<0.001
Intestine						
S.D.	70.39±1.061	59.41±1.131	71.53±1.250	57.57±0.808	71.77±0.679	42.94±0.588
%Change		-15.59		-19.52		-40.17
		P<0.001		P<0.001		P<0.001
Brain						
S.D.	66.86±1.720	56.08±0.877	67.65±0.588	45.80±1.645	66.37±0.713	38.82±0.961
%Change		-16.12		-32.30		-41.51
		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.

Table-2. Protein levels in the tissues of control and fenvalerate exposed frogs (mg/gm wet wt.)

	I week		II week		IV week	
	Control	Experiment	Control	Experiment	Control	Experiment
Muscle						
S.D.	105.76±3.19	84.87±2.18	104.94±3.45	71.76±2.36	99.54±2.32	57.87±2.31
%Change		-19.75		-31.62		-41.86
		P<0.001		P<0.001		P<0.001
Intestine						
S.D.	101.08±3.18	79.47±4.94	103.99±4.18	57.87±2.31	96.45±4.94	48.61±2.31
%Change		-21.38		-44.35		-49.61
		P<0.001		P<0.001		P<0.001
Brain						
S.D.	99.54±2.32	83.33±3.78	101.08±3.18	59.41±3.71	94.13±3.45	42.44±3.18
%Change		-16.28		-41.22		-54.91
		P<0.001		P<0.001		P<0.001

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

shown the higher amount of carbohydrates than other tissues, followed by, intestine and Brain. Exposure to fenvalerate resulted in the decrease of the carbohydrate in all the tissues (Table.1). The decrease was consistently progressive and significant in all the three exposures of I, II and IV weeks. Muscle recorded 49.48% decrease of carbohydrate after four weeks of fenvalerate exposure, which is the highest percent change among the other tissues. This change is nearly threefold when compared to first week exposure. Brain carbohydrate levels decreased to 41.51% after four weeks which is the lowest percentage and it is two and half fold more compared to first week. The decrease was statistically significant (P> 0.001) over control. The decreased trend in the fourth week exposed tissues of frog is as follows: (**Muscle > Brain > Intestine).**

Carbohydrate metabolism takes place both in aerobic and anaerobic conditions. In anaerobic condition glycogen is broken down to release energy after going on a series of reactions. Aerobic condition consists of pyruvate oxidation to acetyl Co A to be utilized through another cycle viz., citric acid cycle. Utilization of reduced co-enzymes leads to ATP synthesis through oxidative phosphorylation (Lehninger**,** 1983). There is an alternative respiratory parthway, which does not require glycolysis. This pathway and operation of glycogenesis and gluconeogenesis from amino acids impart a great importance to the carbohydrate metabolism, especially under stress condition which include pesticidal stress also. Disturbances in carbohydrate metabolism are among the most understanding biochemical lesions arising by the action of toxic compounds and the compensatory shift form aerobic towards anaerobic metabolism in the presence of toxic substances seems to be inevitable in tissue cells for survivability (Bhatia *et al*., 1973). This may prove to be of negative survival value for the affected organisms.

In general, the monosaccharides and disaccharides, which are smaller (lower molecular weight) carbohydrates, are commonly referred to as sugars. Carbohydrates perform numerous roles in living organisms. Polysaccharides serve for the storage of energy (e.g., starch and glycogen) and as structural components (eg: cellulose in plants and chitin in arthropods). Saccharides and their derivatives include many other important biomolecules that play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting, and development.

Table-3. Free Amino Acid levels in the tissues of control and fenvalerate exposed frogs (mg/gm wet wt.)

	I week		II week		IV week	
	Control	Experiment	Control	Experiment	Control	Experiment
Muscle						
S.D.	12.40±0.409	13.98±0.569	12.31±0.280	15.08±0.453	12.51±0.494	16.58±0.598
%Change		+12.74		+22.58		+32.53
		P<0.001		P<0.001		P<0.001
Intestine						
S.D.	17.02±0.607	19.44±0.444	71.55±0.664	21.03±0.282	16.17±0.534	22.69±0.292
%Change		+14.20		+19.83		+40.32
		P<0.001		P<0.001		P<0.001
Brain						
S.D.	20.53±0.569	22.72±0.409	21.84±0.499	24.51±1.209	21.54±0.424	25.74±0.799
%Change		+10.67		+12.23		+17.13
		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.

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To some extent every cell depends on glucose. The cells of the nervous system and the brain almost exclusively use glucose for energy. The major function of carbohydrates in metabolism is as a fuel to be oxidized and provide energy for other metabolic processes (*Martin et al.,* 1983).

Total protein content:

Total protein content levels in control and fenvaleate exposed frogs of different tissues are presented in the **Table 2:** In control frog tissues the Muscle showed higher concentration of proteins followed by Intestine and Brain, being the seat of metabolic regulation has more proteins. As muscles are in constant need of energy, have high amounts of total proteins, apart from their involvement in structural aspect. However, fenvalerate has brought about negative changes in the protein levels in all tissue studied **Table 2**. Exposure to sub lethal concentrations of fenvalerate for I, II and IV weeks reduced the protein levels in the tissues. Brain showed highest percent decrease after four weeks exposure (54.91%) and muscle showed least (41.86%) among the tissues studied. In brain the decrease is three folds in second week exposed and three and half fold in fourth week when compared to first week. Muscle tissue showed two fold decreases in fourth week. The decrease in all the tissues of exposure periods was statistically significant ($P< 0.001$). The decreased trend in the fourth week exposed tissues is as follows: (**Brain > Intestine > Muscle**).

Proteins are the important biomolecules in a wide spectrum of cellular and metabolic functions. They serve indispensible functions in cellular architecture, catalysis, metabolic regulation and contractile processes and are weapons in the defense arsenal of many higher organisms. They are highly complex macromolecular compounds of a large number of different aminoacids. They also serve as precursors for several other important biomolecules such as hormones, purines, pyrimidines, porphyrins and some vitamins. Moreover, they also serve as a source of energy, particularly when they are ingested in excess, and their consumption was accomplished either through gluconeogensis or oxidation to CO_2 via tricarboxylic acid cycle. Protein profiles of the cell are indicative of the physiological status of the animal (Harper, 1985) and there exists a dynamic equilibrium between the synthetic and degenerative pathways associated with these molecules. The protein metabolism constitutes as one of the physiological events involved in the compensatory mechanism interms of homeostasis during any stress condition (Krishnamurthy**,** 1981). In view of this, an attempt has been made in the present investigation to study the effects of fenvalerate on total protein levels in different tissues of *Rana tigrina.*

The present study reveals significant variation in protein metabolism and associated enzymes systems after exposure of fenvalerate in Muscle, Brain and intestine tissues of *Rana tigrina*. The physiological and biochemical activities in the frog were disturbed after sublethal concentration of fenvalerate change indicates stress. The reason for decrement of protein is that tissue protein might be metabolized to produce glucose by the process of gluconeogenesis and glucose is utilized for energy production during stress condition. The significant decrease in protein of experimental animals could be due to increased proteolysis under fenvalerate toxicity.

Free amino acid content:

Free amino acid content estimated in different tissues of control and pesticide exposed of frogs are presented in the Table 3. Among control tissues Brain has recorded high free amino acid content than other tissues. It is followed by Brain, intestine and muscle. Thus the Muscle showed the least amino acid content.

The fenvelarate intoxication resulted in elevted levels of FAA in all the tissues studied in experimental animals. The maximum increase observed was in Intestine (40.32%) after four weeks of fenvalerate exposure and it is three fold when compared to first week and two fold increase was observed in second week. The increase observed in brain was 17.13% after four weeks of fenvalerate exposure which was least among all the tissues investigated. The consistant increase in all experimental tissues was statistically significant ($P< 0.001$). However the increased levels in fourth week tissues were as follows: (**Intestine > Muscle > Brain**.).

The free amino acid (FAA) levels in fenvalerate exposed frog tissues were increased significantly when compared to control. This elevated levels may be due to enhanced protease activity or enhanced transamination.

The physiological charactersitcs and enzyme functions of the animals were known to alter as a consequence to pesticide toxicity (Knox and Green gard, 1965; Mayers *et al.,* 1985). The free aminoacids play a vital role in maintaining the intracellular osmotic balance, during physiological stress conditions. The rate of synthesis of proteins also depends on the levels of elimination of nitrogen from various amino acids is the result of such catalysis. These keto acids are the source for the TCA cycle and gluconegenesis, thus regulating the protein ad carbohydrate metabolism (Knox and Green gard, 1965). The physiological state of the cell can be understood by means of quality and quantity of FAA pool which can be considered as the best diagnostic tool (Adibi, 1980). The amino acids released during protein degradation due to activation of proteolysis will once again return to the amino acid pool and thus the FAAs are the currency through which the protein metabolism operates (Munro, 1970) showing the interdependence of both amino acids and proteins (Mahle and cordes, 1970 and Sivasankar et al, 2014). An abnormality in the protein or amino acid metabolism will have its own consequences in the tissues due to increased flow of the protein catabolic products. In view of this, the levels of FAA in Brain, Muscle and intestine tissues of control and fenvalerate exposure of frogs were studied to gain an insight into the pattern of amino acid metabolism.

Conclusion

This investigation draw a conclusion stating that, In the present investigation studies The severity of damage was more in four weeks exposed animals when compared to first and second weeks. We believe that is it is necessary to assess amphibian fauna of this area before their natural habitat are altered or damaged beyond a true reflection of their species diversity and population abundance.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. **Adibi, S.A** (1980): Role of branched – Chain amino acids in metabolic regulation *J. Lan. Clin. Med.,* **95:** 475.
2. **Bhatia, S.C., Sharma, S.C and T.A. Venkitasubramanian** (1973): Effects of dieldrin on hepatic carbohydrate metabolism and protein biosynthesis in vivo. *Toxicol. Appl. Pharmocol.,* **24:** 216-221.
3. **Bradbury, S. P., and J. R. Coats.** (1989a). Comparative toxicology of the pyrethroid insecticides. *Reviews of Environmental Contamination and Toxicology* .,**108**:133-177.
4. **Bradbury, S. P., and J. R. Coats**. (1989b). Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. *Environmental Toxicology and Chemistry*., **8:373**-380.
5. **Carrol, N.V., Longley, R.W and J.M. Row** (1956): Glycogen determination in liver and muscle by use of anthrone reagent. *J. Biol. Chem.***22:** 583-593.
6. **Colowick, S.P and N.C. Kaplan** (1951): In methods in enzymology. Acad. Press, New York, **3:** 501.
7. **El-Sewedy SM, Mostafa MH, El-Bassiouni EA, Abdel-Rafee A, El-Sebae AH** (1982) Effect of fenvalerate on kynurenine metabolizing enzymes and acid ribonuclease of mouse liver. *J Environ Sci Health B*.,**17:** 571-579.
8. **Feo, M.L., Eljarrat, E., Barcelo', D.** (2010). Determination of pyrethroid insecticides in environmental samples. *Trends in Analytical Chemistry.,* **29(7)**:692-705.
9. **Flodstrom, S., L. Warngard, S. Ljungquist, and U. G. Ahlborg**. (1988). Inhibition of metabolic cooperation in vitro and enhancement of enzyme altered foci incidence in rat liver by the pyrethroid insecticide fenvalerate. *Archives of Toxicology.,* **61**:218-223.
10. **Ghiasuddin, S. M., and D. M. Soderlund**. (1984). Hydrolysis of pyrethroid insecticides by soluble mouse brain esterases. *Toxicology and Applied Pharmacology***., 74**:390-396.
11. **Gray, A. J., and D. M. Soderlund**. (1985). Mammalian toxicology of pyrethroids. Pages 193-248 in D. H. Hutson and T. R. Roberts, editors. *Progress in pesticide biochemistry and toxicology*. **Vol. 5**. Insecticides. John Wiley, New York.
12. **Gray, A. J., and D. M. Soderlund.** 1985. Mammalian toxicology of pyrethroids.*Neurotoxicology* Pages 193-248.
13. **Harper, A** (1985): In 'Harper's Review of Biochemistry'. (ed: D.W.Martin, P.A. Mayes and

V.W. Rodwell), 2oth edition, Lange Medical Publications, Maruzen. Asia (Singapore).
14. **Jagannatha Rao, K.S** (1981): Solvent validity test models, Action Committee report FRAME, Royal society buildings, London. U.K. 120.
15. **Klaassen, C. D., M. O. Amdur, and J. Doull, editors**. (1986). Casarett and Doull's toxicology. *Third edition*. Macmillan, New York. 974 pp.
16. **Knox, W.E and O. Greengard** (1965): In: An introduction to enzyme physiology. Advan. Enzyme Regul. Ed weber, G. Pergman Press, New York, London, Vol III, 247 – 248.
17. **Krishna Murthy, C.R** (1981): Biological effects of environmental pollution. *Indian J. biophys. Biochem.,* **8:** 245.
18. **Lehninger, A.L** (1983): In: Biochemistry, Kalyani publishers. Ludhiana, New Delhi.
19. **Lowry, O.H., Rosenbrough, N.J., Farr, A.L and R.J. Randall** (1951): Protein measurement with Folin phenol reagent: *J. Biol. Chem.* **193:** 265 – 275.
20. **Luo, Y., and Zhang, M.** (2011). Environmental modeling and exposure assessment of sediment-associated pyrethroids in an agricultural watershed. PLoS ONE 6(1):e15794. Doi:10.1371/journal.pone.0015794.
21. **Madan VK, Singh R, Kumari B, Naresh JS and Kathpal TS.** (2000). Dissipation of lindane and fenvalerate residues in chickpea (Cicer arietinum L.) under Indian climatic conditions. Ecotoxicology and Environmental Safety 46, 163-166.
22. **Madhuban Datta Bhattacharya, Anilava kaviraj** (2006). dietary ascorbic acid supplementation to ameliorate chronic toxicity of fenvalerate to clarias gariepinus. *Australasian Journal of Ecotoxicology*, **vol. 12**, pp. 129-134.
23. **Mahle, H.R and Cordes, E.H.** (1970): In: "Biological Chemistry". (eds: Harper and Row). Academic Press, New York.
24. **Martin, D.W., Mayes, A and W.V. Rodwell** (1983). In: Harper's review of Biochemistry, Lange medical publications, Maruzan, Asia.
25. **Maund, S. J., Travis K Z, Hendley P, Giddings J M and Solomon K R.** (2001). Probabilistic risk assessment of cotton pyrethroids: V. Combining landscape-level exposures and ecotoxicological effects data to characterize risks. *Environmental Toxicology and Chemistry.,* **20:** 687-692.
26. **Mayers, A** (1985): The citric acid cycle In. Harper's Review of Biochemistry. (eds. Martin, D.W., Mayars, P.A., Rodwe;;, V.W. and Granner, D.K). Lamge Medical Publications, California. USA. **20:** 158-166.
27. **Moore, S and W.H. Stein** (1954): A modified ninhydrin reagent for the photometric determination of Aminoacids and related compounds. *J. Boil. Chem.,* **221:** 907.
28. **Munro, H.N** (1970). In: Mammalian Protein metabolism (ed: H.N. Munro). Academic Press, New York, London, **4:**291.
29. **Pickering, O.H., Henderson, C and A.E. Lemke** (1962): Toxicity of organophosphorous insecticides to different species of warm water fishes. *Trans. Am. Fish. Soc.,* **91(2):** 175-184.
30. **Sanchez-Fortun S and Barahona MV**. 2005. Comparative study on the environmental risk induced by several pyrethroids in estuarine and freshwater invertebrate organisms. **Chemosphere., 59**: 553-559.
31. **Sivasankar R, Subahan M, Khalinder Basha K, Chennaiah K and Vijayalakshmi N**. (2014). Modulations in the carbohydrate metabolism by nicotine and red grape extract in the liver tissue of male albino rat with reference to aging. Biolife. 2(1); 313-323.
32. **Stuart, S. N. *et al***., 2005. Science, Amphibian Conservation Action Plan IUCN/SSC proceedings Fugler, C. M., *Fish. Inf. Bull*., **1(4)**, 1–51.
33. **Subbareddy S.V.** (2007). Survey of Amphibians and impact of Synthetic pyrethroid Fenvalerate on *RANA TIGRINA* (Indian Bull frog).). Ph.D. Thesis ,Sri Venkateswara University.Tirupathi.
34. **Vijaya Joseph, K and K. Jayantha Rao** (1987): Conservation of frogs an immediate step to a balanced ecosystem. Proc. Natl. Cont. Futurology.
35. **WHO** (Ed): (1991) Occupational exposure in insecticide application, and some pesticides. International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 53, 309- 328.

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