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Study the Impact of Endosulfan Pesticide on behavioral responses in the Fresh Water Fish *Labeo Rohita*

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ABSTRACT

Static renewal test was conducted to determine the toxicity of technical grade (91.06% purity) insecticide endosulfan on the fresh water fish Labeo Rohita. Fishes were exposed to various concentrations of insecticide endosulfan for 96 hours and the percent mortality was recorded. Behavioral responses and morphological deformities were studies in the experimental periods. Fish in toxic media exhibited irregular erratic and darting swimming movements. The behavioral and morphological changes may be due to the formation of amino acids by degradation of proteins with concentration of pesticide.

Keywords: Endosulfan, *Labeo Rohita*, Behavioural changes, Amino acids and Insecticides.

INTRODUCTION

Endosulfan is the patent organo chlorine insecticide and acaricide that is being phased out globally. Endosulfan becomes a highly controversial agrichemical[1] due to acute toxicity. Potential for bioaccumulation, and role as an endocrine disruptor, because of its threats to human health and the environment, a global ban on the manufacture and use of endosulfan was negotiated under the Stockholm convention in April 2011. More than 80 countries including the European Union, Australia, Newzealand several west African nations, the United States, Brazil and Canada had already banned it or announced phase outs by the item the Stockholm convention ban was agreed upon[2]. It is still used extensively in India, China and few other countries. It is produced by Makhteshim Agan and several manufacturers in India and China.

Endosulfan is a derivative of hexa chlorocyclo penta diene and is Known as aldrine, chlordane and heptachlor and it is obtained by Diels – Alder reaction[3] Endosulfan is one of the most toxic pesticides in the market today. It is used in agriculture sector to control insect pests including white fly, aphids, leafhoppers, Colorado potato beetles and cabbage worms.

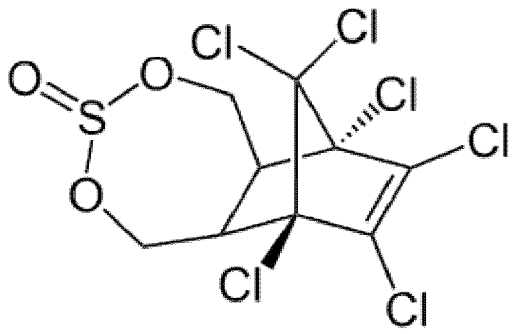

Figure 1. Structure of Endosulfan

A major part of the world's commercial food is being supplied from fish source and it is essential to secure the health of fishes. In India, as much as 70% of the chemical formulations employed in agricultural practices are believed to affect non-target organisms and to find their way to fresh water bodies ultimately polluting them.

MATERIALS AND METHODS

Labeo Rohita finger lings weighing 5±0.5 grams and average length of 7 cm were collected from the Government fish seed form at Ponnur, Guntur Dist, Andhra Pradesh, India and acclimatized to laboratory conditions for 7 days in large plastic tubs previously washed with potassium permanganate to free walls from any microbial growth physic chemical characters of water was followed APHA Method[4]. Acclimatized conditions are temperature 27± 1^0c, pH: 6.8±0.05 at 27^0c and dissolved Oxygen (D.O) 6.9 to 7.4 mg L^{-1}. In accumatisation time, we have supplied food regularly by commercially available fish feed. For this investigation technical grade Endosulfan (91.06%) was used and brought from Agro Industries Limited Guntur, A.P. India. Pesticide column 250 mm length and 4.8 mm diameter samples filtered through PALL life sciences filter paper 0.45 mic. membrane 13 mm diameter size.

Preparation of sample solution: We have taken the effected organs form pesticide induced fish into boiling test tube and added the pure Hexane (AR) then boiled. After heated fish absorbed pesticide settled at inside of the test tube walls, then removed the fish organ from the test tube bottom and again added hexane for dissolving the pesticide in the test tube walls. This sample solution is used for the behavioral study of pesticide induced fish by using HPLC analysis.

Analysis of Endosulfan by HPLC: Concentrations of Endosulfan in the test medium was confirmed by High performance liquid chromatography (HPLC) and this method described by Mr. John son[5]. The HPLC analysis was opertunated by using a UV detector with a mobile phase. Mobile phase consisting of acetonitrile (20%), water (20%), and methanol (60%) then run though a C_{18} column with a flow rate of 1.0 ml/ minute and then analyzed the peaks of Endosulfan in sample solutions.

RESULTS AND DISCUSSION

Proteins are primary importance of the living world and only because of their peculiar biological specificity among various types and these are responsible for many metabolic changes in total fish organs. These proteins are main energy sources to play an important role in the maintenance of blood glucose[6] (Fig 2). It is the most fundamental biochemical constituent present abundantly in the body of fish and this result presented in Table 1.

Table 1. Concentration of endosulfan pesticide observed in fish organs.

Concentration of pesticide (ml)	Gill (mg/ml)	Head (mg/ml)	Digestive system (mg/ml)	Liver (mg/ml)	Muscle (mg/ml)	Total observed pesticide (mg/ml)
1.0 ml	0.0410	0.0460	0.0320	0.0300	0.0100	0.159
2.0 ml	0.0501	0.0470	0.0370	0.0310	0.0154	0.1805
3.0 ml	0.0517	0.0546	0.0398	0.0326	0.0281	0.2068
4.0 ml	0.0580	0.0601	0.0416	0.0291	0.0271	0.2159
5.0 ml	0.6210	0.0642	0.0450	0.0390	0.0306	0.7998
6.0 ml	0.0662	0.0690	0.0480	0.0410	0.0302	0.2544
7.0 ml	0.0710	0.0782	0.0485	0.0462	0.0317	0.2756

The protein changes are observed in various tissues after the pesticide induced along with control was graphically represented in Figure 2.

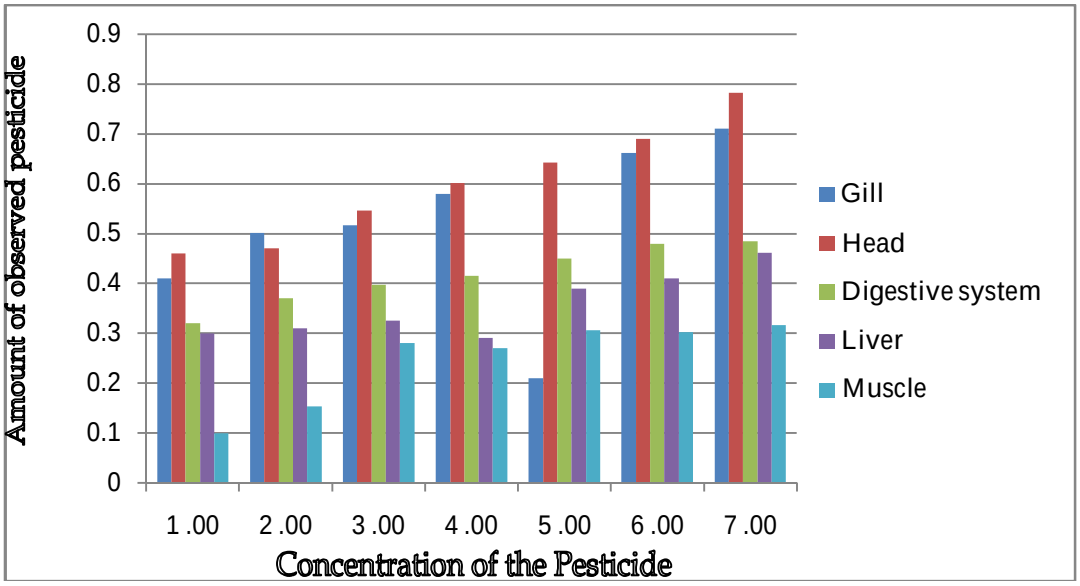

Figure 2. Pesticide absorbance by fish organs in different volumes.

The insignificant alteration of proteins at the end of 45 hours and it was observed in the tissues suggesting that the fish tend to resist the sudden stress for shorter duration[7]. Later with increases of time the decreases of protein content. The significant and maximum depletion was observed in the head and minimum in muscle. However

increases of consumer concentration of the pesticide decreases the protein in tissues of the fish *Labeo Rohita* the present analysis coincides with the reported data that the protein content in muscle and liver[8-12].

Formation of Amino Acids: In this research work, after the consumed the pesticides proteins are degraded and to form amino acids, then change the sequence of amino acids from original sequence in the isopropyl alcohol medium. In this paper we have report the measuring of amino acids quantity after degradation of proteins in fish body by applying the endosulfan pesticide in fish. These results are shown in Table 2.

Table 2. Endosulfan pesticide effect on fish body

Concentration of pesticide (mg/lit)	Leucine (mg/lit)	Tyrocine (mg/lit)	Tryptophan (mg/lit)	Valine (mg/lit)	Phenyl alanine (mg/lit)
1 .00	2.4	7.3	1.6	4.1	3.5
2 .00	3.7	8.1	1.7	5.6	4.1
3 .00	4.9	9.6	1.9	6.9	4.7
4 .00	6.2	14.4	2.3	8.5	6.1
5 .00	7.4	16	2.9	9.8	7.3
6 .00	8.8	17.8	3.2	11.5	9.4
7.00	11.5	20.2	3.8	15.3	12.2

Tryptophan amino acid is not effected in protein degradation at low concentrations. But Tyrocine, valine, Leucine are degrade at all concentration of endosulfan pesticide. These results are shown in figure 3.

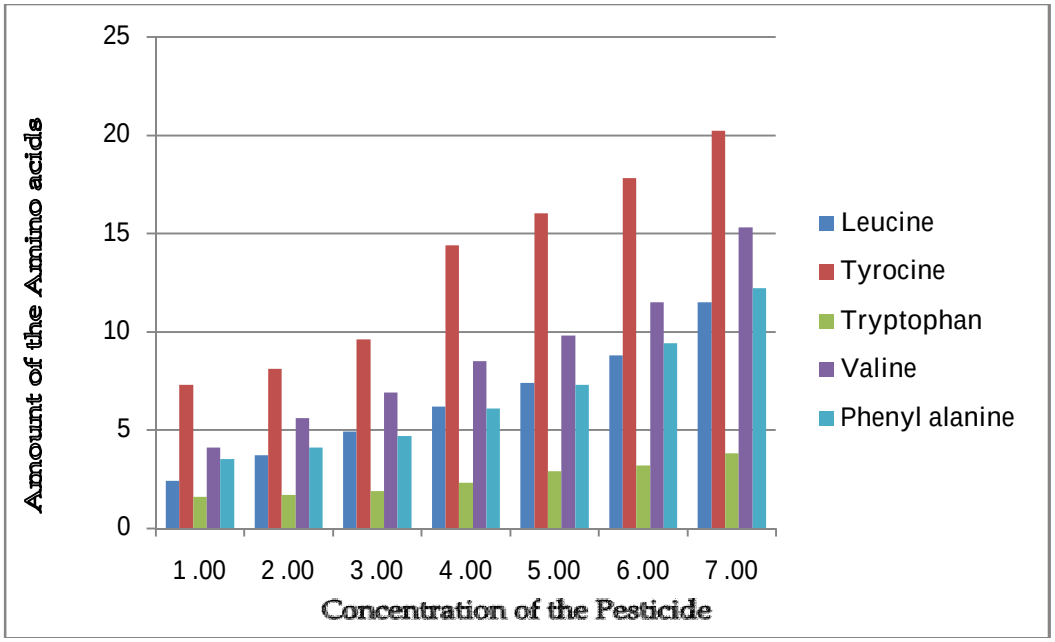

Figure 3. Change in Amino Acids by absorbing concentration of Pesticide

APPLICATIONS

Due to the formation of amino acids by degradation of proteins with concentration of pesticide the behavioral and morphological changes occur in the fresh water fish *labeo rohita.*

CONCLUSIONS

The present study proved that the Endosulfan is highly toxic and detrimental impact on the behavioral responses of *Labeo Rohita* at sub level concentration and any alterations caused by the pesticide may lead to variations of total proteins in fish body. Therefore the amount of Endosulfan pesticide in the aquatic systems should be monitored then control the usage pesticide because the decreasing nutrivalent value and mortality of fish.

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