

# The Toxicity Effect of Monocrotophos 36% E.C on The Biochemical Changes Labeo Rohita (Hamilton, 1882)

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**Abstract**— Pesticides are stable compounds and they enter the aquatic ecosystem through the agriculture run off. The evaluation of nature and degree of harmful effects produced by toxic substance in the aquatic organisms are evaluated by toxic tests. The 96 hour LC50 values have generally been found to be satisfactory for the measurement of acute toxicity. The differences in 96 hours LC50 of the same toxicant in different fishes may be attributed to individual traits including those of behavior and additional structure such as accessory respiratory organs. The individual characters such as size and weight, sex and biological behavior are important determination for variation in LC50 values. Therefore the present study is an attempt to study the toxicity of the pesticide with respect to biochemical of carbohydrate, protein, lipid of fish *Labeo rohita* (Ham). The Monocrotophos affects not only fishes but also organisms in the food chain through the process of consumption of one by the other. The pesticide, which enters the body tissues of the fish, affects the physiological activities.

**Key words:** Monocrotophos, Hamilton, *Labeo Rohita*

## I. INTRODUCTION

Adversely human activities are directly or indirectly affect the environment. Developed and developing countries which are progressing rapidly in the field of agriculture, technology and industries are continuously releasing various kinds of harmful substances into the biosphere and thereby causing a severe threat to the environment (APHA 1995, Abbasi et al 1998).

The major sources of water pollution are domestic, agricultural and industrial wastes which are discharged into natural water bodies (De, 1996). Domestic sewages are run off from agriculture fields loaded with pesticides and fertilizers, pollute the water bodies. Commonly used pesticides can be harmful living organisms, pets, and their environment.

Pesticides are widely used in modern agriculture to aid in the production of high quality food. However, some pesticides have the potential to cause serious health and environment damage. (Tamizhazhagan.V 2015). Repeated exposure to sub-lethal doses of some pesticides can cause physiological and behavioral changes in fish that reduce populations, such as abandonment of nests and broods, decreased immunity to disease and increased failure to avoid predators (Veeraiyah et al., 2012). The increasing use of pesticides causes chemical pollution results potential health hazards to live stock, especially to fish, birds, frogs, and mammals (Nagaraju et al., 2013).

The most common cause of water pollution in developing countries is domestic and industrial waste that is directly released into streams or ponds without treatment. These wastes mostly contain various types of pollutants such as heavy metals, radioactive elements, pesticides, herbicides

and corrosive substances like acids and bases (Mhadhbi et al., 2012).

Aquatic water bodies are frequently polluted with a multiple of potentially hazardous substance (Gattaglin and Fairchild,2002). Now a days farmers are using variety of pesticide, insecticide, herbicide using agricultural field. The pesticide mainly two types organochlorine and organophosphate in recent year Monocrotophos is organophosphate using their field of controlling the insect pest.

Fishes play an important role in human nutrition. Fish proteins are well balanced with essential amino acids and are comparable to other proteins of animals origin (Tont,1977).further fishes contains lipids especially omega fatty acids from the human nutritious point of view.

Toxicity data for a variety of pesticides such as organophosphate, organochlorine, carbamide and pyrethroid pesticides have been reported for number of fish species by various author's (Anees,1975; Arunachalam and Palanichamy 1982 ; Arunachalam et al.,1980, Baskaran et al.,1989, Sing et al.,1981 ; Malla Reddy and Basha Mohideen 1989; Gurusamy and Ramadoss,2000 ; Sapna Shrinivasta, 2002 ; Nishar et al.,2004; Visvanathan et al.,2009).

The natural physiological functioning of an organisms gets distributed on exposure to toxicants, stress, it induces its effect first at cellular or even at molecular level, but ultimately cause physiological, pathological and biochemical alteration ( Venkata Rathnamma et al ., 2013).Although toxicant impairs the metabolic and physiological studies alone do not satisfy the completed understanding of pathological conditions of tissue under toxic stress. Hence, it is useful to have an insight into histological analysis (Muthukumaravel et al.,2013).

The biochemical changes occurring in the body of the organisms give first indication of stress. Several investigators have reported a number of changes in biochemical parameters of aquatic organisms due to pesticidal exposure (Mule and Lomte, 1992; Muley et al., 1996; Kumari and Kumar, 1996; Tilak and Rao, 2003; Maruthanayagam and Sharmila, 2004; Remia et al., 2008; Patil and David, 2007 and Vijakumar et al., 2009).

Carbohydrates form one of the major sources of energy precursor under any stress condition. Total carbohydrate content decreased during the exposure to Monocrotophos in the air breathing fish *Anabas scandens* maximum decrease in the brain tissues observed on 21st day (Yasmeen, et al., 1991). Decreased carbohydrate level has been noted in the liver and muscle of *Heteropneustes fossilis* exposed to Butachlor (Sangeetha Sharma and Agarwal, 2004). Chlorpyrifos, an organophosphate compound decreased hepatic glycogen levels due to inactivation of enzymes involved in the carbohydrate metabolism in the fresh water fishes, such as *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* (Tilak et al.,2005).

Protein being the essential substance is needed for growth and development and also serves as energy source during the stress condition. The total protein level of muscle and liver decreased in freshwater teleost fish, *Channa punctatus* exposed to Nuvacron (Sastry and Dasgupta, 1991). Decreasing trends have been reported in gill, liver, muscle and brain tissues of *O. mossambicus* exposed to Quinalphos (Durairaj and Selvarajan, 1992).

Lipids are generally triglycerides that can serve as metabolic reserves. Phospholipids showed a rapid decrease since it is actively degraded due to the pesticidal stress (Herpert et al., 1977). Pant et al., (1987) reported decrease in liver lipid content of *Barbus chonchonius* exposed to Aldiocarb for 15 and 30 days.

In general, the end points used in toxicity studies are mortality, survival and growth with acute toxicity tests, the parameters are quite appropriate, but for long-term sublethal concentration's these relevant parameters are difficult to ascertain (Sulekha et al., 2002).

Organophosphate (OP) pesticides are finding increasing use in recent years since they are biodegradable therefore persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides are being practiced for the control of pests in agricultural fields and thereby large quantities find their way into water bodies (Jyothi and Narayan, 1999). Hence, In the present study is an estimation of biochemical components such as total carbohydrates, protein and total lipids, in the muscle liver kidney and Histology of Gill and Brain then hematology studies of the fresh water fish in *Labeo rohita* (Hamilton 1882). exposed in monocrotophos.

Now a days farmers are using monocrotophos in their field for controlling the insect pest. Residual of these pesticide alters in to the ecosystem and disturb the healthy environment and aquatic forms. Aquatic farm contains fish and other organism. But the fish is mostly affected by pesticide residuals

## II. MATERIALS AND METHODS

### A. Acclimatization:

Healthy freshwater fish, *Labeo rohita* of the weight ( $15 \pm 1g$ ) and length ( $8.0 \pm 0.5$  cm) were selected for the experiment and were collected from the local commercially culture farm near Kumbakonam. Fish were screened for any pathogenic infections. Glass contamination aquaria were washed with 1%  $KMnO_4$  to avoid fungal contamination and then dried in the sun light.. Healthy fishes were then transferred to glass aquaria ( $35 \times 20 \times 20$  cm) containing dechlorinated tap water. The characteristics feature of the tap water as follows :

Fish were acclimatized to laboratory conditions for 10 to 15 days prior to experimentation. The rate of mortality during acclimatization was less than 10%. They were regularly fed with commercial food. Chlorinated tap water was changed daily to remove feces and food remnants.

### B. Toxicity Test:

Toxicity tests were conducted in accordance with standard methods (APHA, 1992). Stock solution of monocrotophos 36% EC with a concentration of 0.1 ml per liter (equivalent to 1 ppm) was prepared in distilled water. Based on the progressive bisection of interval on a logarithmic scale, log

concentrations were fixed after conducting the range finding test. The fish were starved for 24 hours prior to their use in the experiments as recommended by storage to avoid any interference in the toxicity of pesticides by excretory products. After the addition of the toxicant into the test tank with 10 liters of water having twenty fish, mortality was recorded after 24, 48, 72 and 96 hours. Five replicates were maintained simultaneously. Percent mortality was calculated and the values were transferred into probit scale.

Probit analysis was carried out as suggested by Finney (1971). Regression lines of probit against logarithmic transformations of concentrations were made. Confidential limits (upper and lower) of the regression line with chi-square test were calculated by a computerized program SPSS Version 14 for Finney's (1971) probit analysis.

## III. BIOCHEMICAL ANALYSIS

### A. Total Carbohydrate Estimation:

The total carbohydrate content was estimated by the technique of Roe (1955). A 10% homogenate of tissue was prepared using 5% TCA and this was centrifuged at 3000 rpm for 10 minutes. Samples were cooled in the dark at room temperature for 30 minutes. The supernatant was collect and the optical density was measured in a spectrophotometer (Hitachi 2205) at a wavelength of 620 nm a blank reading. Blank was prepared by mixing 1 ml of distilled water with 4 ml of Biuret reagent. The total carbohydrate content in mg/g of tissue.

### B. Total Protein Estimation:

Protein was estimated by the method of Lowry et al., (1951). 1% tissue homogenate were prepared in 10% TCA and centrifuged at 3000 rpm for 15 minutes. The gel set was dissolved in 1 ml of 1N NaOH to the above 5 ml of alkaline copper reagent was added and after 10 minutes, 0.5 ml of folin phenol reagent was measured after was added and rapidly The moisture content was estimated by subtracting the dry weight (dried in a hot air oven) of the muscle tissue from the known wet of the muscle tissue.

### C. Lipid Estimation:

The total lipids were extracted by the method of Floch et al., (1957) to find out total lipid, known volume of experiment samples were homogenized with 1 ml of methanol and 2 ml of chloroform to which again 2ml of chloroform : methanol (2:1 v/v) was added and mixed thoroughly. To this, 0.2 ml-0.09 % sodium chloride solution was added. The above mixture was poured into separately funnel, mixed and allowed to stand for few hours.

The lower phase was separated and 0.5 ml of extract was measured and poured into a clean test tube. It was allowed to dry in vacuum desiccators over silica gel, dissolved in 0.5 ml concentrated sulphuric acid and mixed well. The tube was plugged with non- absorbent cotton wool and placed in a boiling water bath for 10 minutes and the tubes were cooled at room temperature. 0.3 ml of this acid digest was taken for experimental analysis. 0.5 mg of cholesterol For stand and, 0.5 ml of distilled water for blank separately. To each tube, 5ml of vanillin reagent was added. Mixed well and allowed to stand for half an hour and the developed color were measured at 250 nm.

IV. RESULTS

A. Carbohydrate :

During the course of experiments observation were made to estimate how the animal reacts to the toxicity effect of the pesticide Monocrotophos. From the table (1) it is evident that the normal carbohydrate in muscle 7.54 mg/g after the exposure period of 24 hrs. The carbohydrate was reduced to 6.83 mg/g and 5.91 mg/g 4.77 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the carbohydrate was reduced to 24 hrs 6.27 mg/g and 96 hrs 3.81 mg/g respectively for hours of exposure period. The pesticide Monocrotophos the carbohydrate content decreased order. Like liver carbohydrate From the table (2) it is evident that the normal carbohydrate in liver 18.55 mg/g after the exposure period of 24 hrs. The carbohydrate was reduced to 18.48 mg/g and 18.45 mg/g 18.39 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the carbohydrate was reduced to 24 hrs 14.51 mg/g and 96 hrs 13.71 mg/g respectively for hours of exposure period.

From the table (3) it is evident that the normal carbohydrate in kidney 1.55 mg/g after the exposure period of 24 hrs. The carbohydrate was reduced to 1.18 mg/g and 1.05 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the carbohydrate was reduced to 24 hrs 1.05 mg/g and 96 hrs 0.53 mg/g respectively for hours of exposure period.

B. Protein:

From the table (1) it is evident that the normal protein in muscle 24.27 mg/g after the exposure period of 24 hrs. The carbohydrate was reduced to 21.98 mg/g and 20.12 mg/g 18.82 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the protein was reduced to 24 hrs 22.75 mg/g and 96 hrs 17.40 mg/g respectively for hours of exposure period. The pesticide Monocrotophos the protein content decreased order. From the table (2) it is evident that the normal protein in liver 24.24 mg/g after the exposure period of 24 hrs. The protein was reduced to 24.0 mg/g and 23.77 mg/g 23.55 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the protein was reduced to 24 hrs 19.95 mg/g and 96 hrs 19.41 mg/g respectively for hours of exposure period. The pesticide Monocrotophos the protein content decreased order.

From the table (3) it is evident that the normal protein in kidney 10.71 mg/g after the exposure period of 24 hrs. The protein was reduced to 10.59 mg/g and 10.41 mg/g 10.33 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the protein was reduced to 24 hrs 8.25 mg/g and 96 hrs 7.83 mg/g respectively for hours of exposure period. The pesticide Monocrotophos the protein content decreased order.

C. Lipid:

From the table (1) it is evident that the normal lipid in muscle 1.95 mg/g after the exposure period of 24 hrs. The lipid was reduced to 1.85 mg/g and 1.83 mg/g 1.65 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the protein was reduced to 24 hrs 1.89 mg/g and 96 hrs 1.53 mg/g respectively for

hours of exposure period. The pesticide Monocrotophos the lipid content decreased order. From the table (2) it is evident that the normal lipid in liver 8.1 mg/g after the exposure period of 24 hrs. The lipid was reduced to 7.51 mg/g and 6.94 mg/g 6.54 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the lipid was reduced to 24 hrs 6.15 mg/g and 96 hrs 5.55 mg/g respectively for hours of exposure period. The pesticide Monocrotophos the lipid content decreased order.

From the table (3) it is evident that the normal lipid in kidney 3.09 mg/g after the exposure period of 24 hrs. The lipid was reduced to 2.85 mg/g and 2.73 mg/g 2.61 mg/g respectively for 48, 72, 96 hrs of exposure period. The fish was introduced at sublethal concentration 0.40ppm the lipid was reduced to 24 hrs 2.43 mg/g and 96 hrs 2.01 mg/g respectively for hours of exposure period. The pesticide Monocrotophos the lipid content decreased order.

Table 1: Total Carbohydrate ,protein ,lipid contents in the muscles of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

Muscles	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Carbohydrate				
Control	7.54 ± 0.19	6.83 ± 0.19	5.91 ± 0.04	4.77 ± 0.09
Treatment	6.27 ± 0.61	6.05 ± 0.02	4.86 ± 0.03	3.81 ± 0.02
% Changes	16.8	11.0	17.7	20.12

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Muscles	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Protein				
Control	24.27 ± 0.05	21.98 ± 0.02	20.12 ± 0.04	18.82 ± 0.03
Treatment	22.75 ± 0.03	19.89 ± 0.12	19.25 ± 0.06	18.40 ± 0.08
% Changes	6.2	9.5	4.32	2.23

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Muscles	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Lipid				
Control	1.95 ± 0.01	1.85 ± 0.01	1.83 ± 0.01	1.65 ± 0.01
Treatment	1.89 ± 0.02	1.88 ± 0.01	1.79 ± 0.01	1.53 ± 0.01
% Changes	3.07	-1.62	2.18	7.27

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Table 2: Total Carbohydrate ,protein ,lipid contents in the Liver of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

Liver	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Carbohydrate				
Control	18.55 ± 0.02	18.48 ± 0.02	18.45 ± 0.01	18.39 ± 0.02
Treatment	14.51 ± 0.02	14.13 ± 0.01	13.99 ± 0.02	13.71 ± 0.01
% Changes	21.7	23.5	24.17	25.4

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Liver	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Protein				
Control	24.24 ± 0.02	24.00 ± 0.02	23.77 ± 0.01	23.55 ± 0.01
Treatment	19.95 ± 0.01	19.89 ± 0.01	19.59 ± 0.01	19.41 ± 0.01
% Changes	17.6	17.12	17.58	17.57

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Liver	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Lipid				
Control	8.11 ± 0.19	7.51 ± 0.19	6.54 ± 0.13	5.95 ± 0.05
Treatment	6.15 ± 0.01	5.91 ± 0.05	5.79 ± 0.01	5.55 ± 0.01
% Changes	31.86	21.30	11.46	6.72

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Table 3: Total Carbohydrate, protein ,lipid contents in the Kidney of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

Kidney	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Carbohydrate				
Control	1.55 ± 0.03	1.18 ± 0.019	1.05 ± 0.01	0.87 ± 0.03
Treatment	1.05 ± 0.01	0.89 ± 0.01	0.66 ± 0.13	0.53 ± 0.02
% Changes	32.25	24.57	37.14	39.08

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Kidney	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Protein				
Control	10.71 ± 0.01	10.59 ± 0.01	10.41 ± 0.01	10.33 ± 0.01
Treatment	8.25 ± 0.02	8.13 ± 0.01	8.01 ± 0.01	7.83 ± 0.01
% Changes	22.96	23.22	23.05	24.20

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

Kidney	Hours of Exposure			
	24 Hours	48 Hours	72 Hours	96 Hours
Lipid				
Control	3.09 ± 0.01	2.85 ± 0.01	2.73 ± 0.01	2.61 ± 0.09
Treatment	2.43 ± 0.01	2.31 ± 0.01	2.13 ± 0.01	2.01 ± 0.01
% Changes	21.35	18.94	21.97	22.98

Value are Mean ± SD of six observation – or + indicate percentage decrease or increase over control.

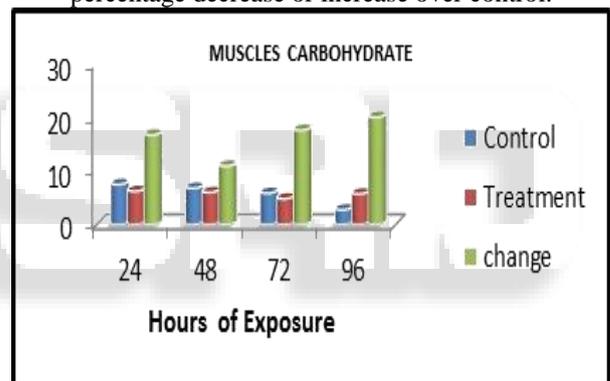


Fig. 1: Total Carbohydrate contents in the muscles of L. rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

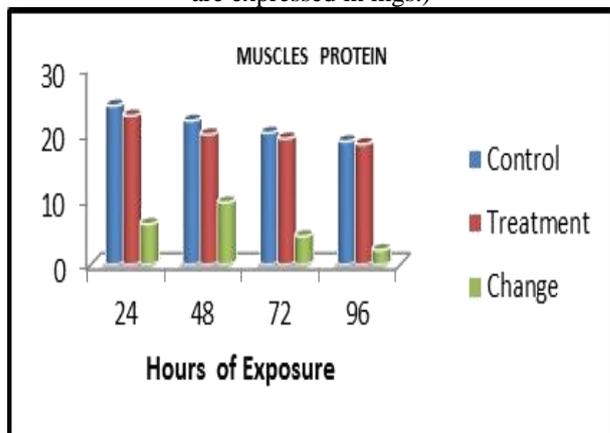


Fig. 2: Total Protein contents in the muscles of L. rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

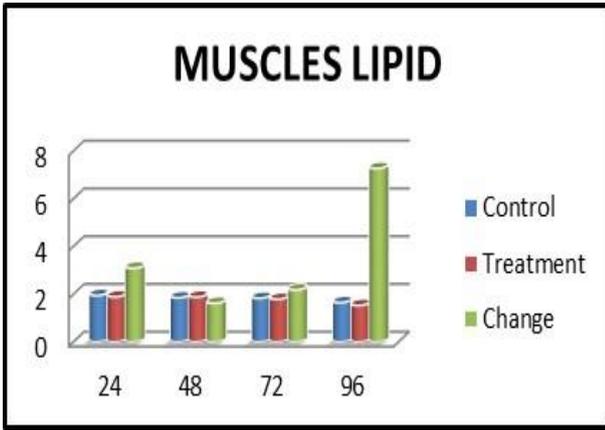


Fig. 3: Total Lipid contents in the muscles of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

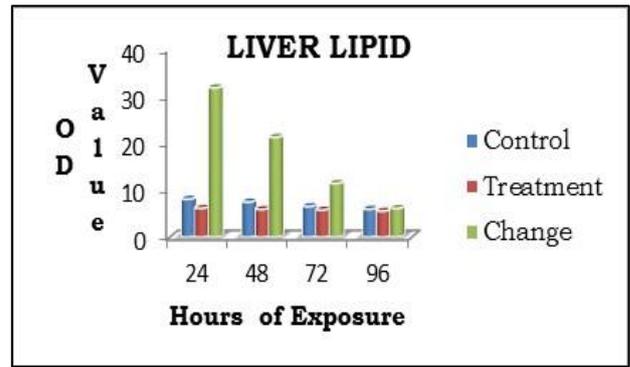


Fig. 6: Total Lipid contents in the Liver of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

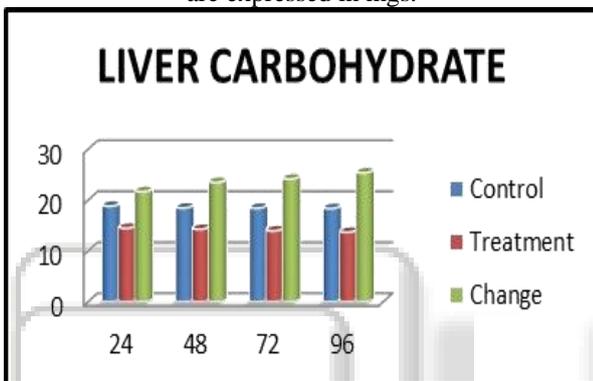


Fig. 4: Total Carbohydrate contents in the Liver of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

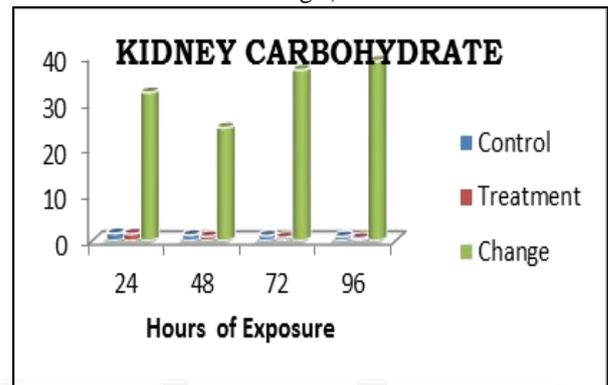


Fig. 7: Total Carbohydrate contents in the Kidney of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

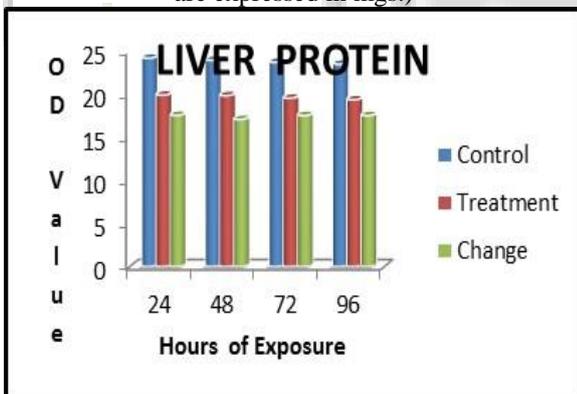


Fig. 5: Total Protein contents in the Liver of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

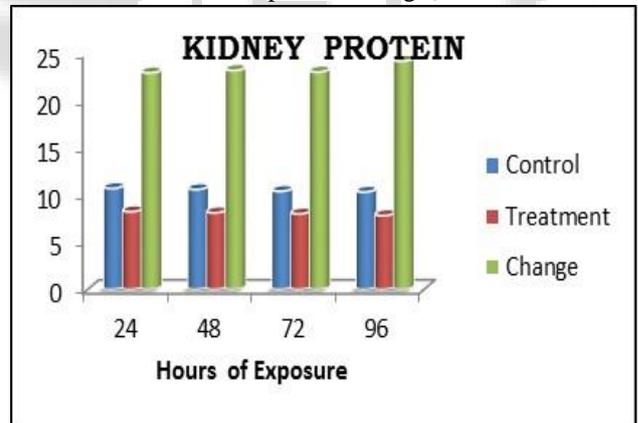


Fig. 8: Total Protein contents in the Kidney of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

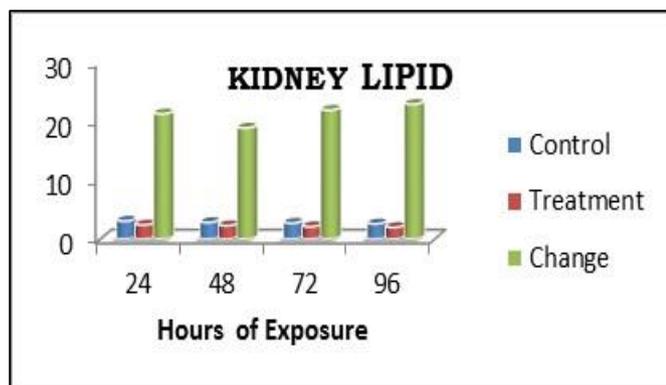


Fig 9 : Total Lipid contents in the Kidney of L.rohita control and 0.40ppm sublethal concentration of monocrotophos 36%.EC. at various time intervals (values are expressed in mgs.)

## V. DISCUSSION

The movements in animal were effected with the help of muscle. The following are the principal constituents of muscle : water 75%, protein 20%, minerals and organic compounds 5%. Proteins are perhaps the most essential and typical of all the constitution's of living cells. Proteins constitute the fabric material of protoplasm. Apart from formation of protoplasm, protein is an important constituent of the various cellular membranes in conjugation with lipids. Most of the biological active compounds are proteins including enzymes. The use of protein fuels is limited because they cannot be stored like lipids and carbohydrates.

Proteins are complex substance with high molecular weight forming not only the structural framework, but also gears and levers of the operating machinery in the living body. Proteins are useful for the polypeptide chains of amino acid molecules. The proteins are useful for the transport and storage. Specific proteins transport many small molecule and ions. A protein complex that guides the formation of neural networks in higher organisms. The primary function of protein food is to supply the amino acids needed for the growth, repair and general maintenance of the structural and catalytic machineries of living.

Carbohydrate and protein are the chief nutrients of the animals. They have a variety of functions. The carbohydrate supplies energy in the form of ATP molecules, which are formed during TCA cycle. The proteins in different tissues differ in composition and properties.

In the present study the protein content in the muscle and liver kidney of labeo rohita is decreased with the low concentration of pesticide Monocrotophos. Even with the same concentration longer exposure resulted in decreased amount of protein content (Table : 1,2 & 3) which indicates that the tissue protein undergoes proteolysis. The result in the production of free amino acids, which are used in TCA cycle for energy production under, stresses (Muthukumaravel et al., 2013). There are similar reports of effects of toxicants on total protein in other fishes by (Veeraiah et al., 2013) (Nagaraju 2013) Mule et al.,(2007).

In the present study the result obtained clearly indicate that there was a decreased amount of protein and glycogen content to resist the effect of pesticides. That is to provide immediately energy to the fighting elements of the

body and protect all systems of the body from the harmful effect of the pesticide.

With regard to carbohydrate in the fish exposed to different hours of exposure of pesticide Monocrotophos there was no much change within 24 hours of treatment with low concentration 0.040 ppm fish kept prolonged exposure up to 96 hours the carbohydrate content was observed in decreased amount (table:1,2and 3) the carbohydrate content was found more and more in decreased.

Such reduction in stored carbohydrate content has been reported in Labeo rohita exposed to Monocrotophos (Venkata Rathnamma 2013), effect of Monocrotophos was reported by (Sulekha 2011). A fall in carbohydrate levels clearly indicates its rapid utilization to meet the enhanced energy demands in pesticides treated individuals through glycolysis or hexose monophosphate pathway (Nagaraju 2013).

The term lipid was used by the biochemist to describe that group of substances of animal origin, which is insoluble in water, but soluble in fat solvents. All cells contain lipid in the form of globules scattered in the cytoplasm. The concentration is much higher in cells forming adipose tissue. In the present study hours of exposure periods has decreased amount of lipid.

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