THE POSSIBLE IMPACTS OF MALATHION ON BIOCHEMICAL PARAMETERS OF A FRESHWATER FISH, *Crrhinus mrigala*

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Abstract: Both the aquatic and terrestrial ecosystems are interconnected. Deterioration of one can affect the productivity of the other. To investigate the possible effect of malathion widely used as pesticide in agricultural fields of Pakistan on the aquatic organisms a laboratory study was carried out in small concrete ponds by using normal tap water. For this purpose a successful Indian major carp specie, ***Crrhinus mrigala*** was exposed to different concentrations of malathion for different durations. The physico-chemical analyses of ponds water was carried out by following APHA methods. The time duration used was 24, 48, 72 and 96 hours acute toxicity tests. The malathion concentrations used were 5 µl/L (one day), 4 µl/L (2 days), 3 µl/L (3 days), 2 µl/L (4 days). At the end of experimental periods fish blood was collected and analysed. On analysis of fish blood **decreased** levels of glucose, cholesterol, triglyceride, high density lipid, low density lipid, total protein, lactate dehydrogenase while **increased** concentrations of alkaline phosphatase, alanine amino transferase were recorded.

The **results** suggest the exposure of the fish to sub lethal concentrations have altered the metabolism of the target animal.

The study gives scope to further biochemical studies for the assessment of effects of pesticide on other organisms, as pesticides are becoming alarming part of our ecosystem, because of increasing human food demands.

Key words: lipid, fish, toxicity. Malathion, pesticides, cholesterol, glucose, total protein.

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INTRODUCTION

Food and Agriculture Organization (FAO) has defined the term pesticide as: any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit.Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAO, 2002).

Pesticides production started in 1952 in India by establishing a plant for production of BHC near Calcutta. After china, the second largest manufacturer of pesticide was India and globally it ranks twelfth. Used of pesticide in India was different from that of the world. In India 76% of pesticide used as insecticide as against globally (Murthy *et al.*, 2013). In 1958 the production of pesticide in India was 5,000 metric tons and in 1998 it was 102,240 metric tons. In 1996-97 the demand for pesticide was considered to be about Rs. 22 billion (USD 0.5 billion), which was about 2% of the total world market (Aktar *et al*., 2009).

Aquatic pollution is a serious problem caused by pesticides.Direct and indirect contamination of water by pesticide lead to kill fish and productivity of fish was reduced, edible fish tissues were contaminated with chemicals which affect the health of humans when they consume these fish (Cerejeira, 2003). Pesticides in residue amounts have been found in food, water and increase the possible threats to human health by exposing to these chemicals.Surface waters were contaminated with pesticides and constitute a major problem at regional national and global levels (Spalding, 2003). These compounds were discovered by German scientist in late 1930's. It was a by-product of nerve gas research. In this group insecticides with general purposes were included having a wide range of toxicity to fish (malathion, diazinon, parathion, phosmet and chlorpyrifos), as well as compounds that work as fumigants and as systematic (dimethoate, ronnel and disulfiton).Organophosphate causes nerve poison, they

block the active site of enzyme acetylcholinesterase (ACHE) which break the neurotransmitter substance, Ach(acetylcholine) from the nerve synapse. Increase of acetylcholine dangerous and responsible for hyperactivity, tremors, and convulsions and eventually death.Larger use of OP compounds spread these chemicals over a great range or extent in the environment. They were lesser retainable than organochlorines and do not gather in the fatty tissues.Organophosphorus compounds replaced the organ chlorine compounds which were more persistent due to their faster biodegradation and lesser persistency in the environment (Anees, 1975).To control domestic and agricultural insect pests organ phosphorus insecticides were used all over the world.Organophosphorus insecticides were also used in medicine and industry due to lesser persistency and biodegradability (Fahmy, 2012).Malathion is one of the earliest organophosphate insecticide which was non systematic, wide spectrum developed in 1950.Organophosphate insecticides were used are over the world because they are less toxic to mammals and high selectively as compared to other insecticides.In earlier researches it was clearly observed that malathion has adverse effect on organisms including fish(Kushwaha *et al.*, 2000).

MATERIALS AND METHODS

The fresh water fish ***Cirrhinus mrigala*** size 12-14 cm and weight 13-15 g were brought from Sherabad hatchery District Peshawar, Pakistan. The fish were fed daily with commercial fish pellets except Sunday a week and acclimatized to the laboratory conditions at 28 °C for 15 days. Tube well water was used for conducting experiments and fish acclitimization. The fish ponds were made by concrete and were of 700 litre capacity. Malathion 45% was purchased from the local market in Peshawar Pakistan. All the experiments were performed to assess the acute toxicity (Short term toxicity).

Acute Toxicity

For acute toxicity 35 fish were taken and were divided into five groups out of which 3 fishes were collected from each tank for blood collection.

Group 1: This was control group and was kept without any treatment.

Group 2: This group was treated with Malathion i.e. 5 µl/L, for one day (24 hours).

Group 3: This group was treated with Malathion concentration of 4 µl/L, for two days (48 hours).

Group 4: This group was treated with Malathion at a rate of 3 µl/L, for three days (72 hours).

Group 5: This group was treated with Malathion at a concentration of 2 µl/L, for four days (96 hours).

After exposure to Malathion, blood was collected and analysed and was compared with control group. Anesthesia (MS222) was used to produce unconsciousness in fish. Blood samples were collected by using hypodermic syringes from caudal vein of fish. Blood was collected in EDTA tubes. The blood in EDTA tubes was rotated gently to get mixed with EDTA and prevent blood clotting. EDTA tubes were shifted to icebox and taken to the laboratory for analysing. Samples were centrifuged in centrifugation machine. After centrifugation clear serum was obtained in a gel tube at the top while blood cells were settled down at the bottom of the tube.

General Procedure for Measurement of parameters

- In these tests two reagents were used. First reagent was taken in a test tube about 50 µl and 2nd reagent was taken in same test tube of about 50 µl with the help of micropipette.
- To this test tube having reagents, 10µl serum was added with the help of micropipette.
- It was mixed by rotating the test tube and then test tube was put in thermostat so that temperature became equal to room temperature.
- After that the sample was run through biochemical analyzer machine.
- Reading was obtained on display
- This whole process was completed in about 10 minutes.

  Percentage of mean value of experimental groups was drawn out by subtracting mean value of experimental group from mean value of control group. The result of subtraction are now divided by control mean and then multiplied with 100.

RESULTS

In the present investigation ***Cirrhinus*** *mrigala* has shown different responses for different periods to the toxicant. The results suggest that the toxicity increased by increasing the time. Low quantity can hamper the biochemical activity with longer time, so the less is the concentration required for causing sublethal toxicity. Glucose, Cholesterol, Triglyceride, High density lipid, Low density lipid, Total protein, Lactate dehydrogenase decreased concentrations were observed as compared to control group (Fig I and Table 1) while increased concentration of Alkaline phosphatase, Alaninine amino transferase were recorded after exposure of fish to Malathion pesticide (Fig. II and Table 2). Tube well water was used

for rearing fish. Results of Physico-chemical parameters of fish ponds water taken after intervals are mentioned in Table. 3. Standard methods adopted by APHA (1985) were followed for the estimation of ponds water physico-chemical parameters.

Table 1. Showing biochemical parameters of control group of fishes.

S.no	Biochemical Parameters	Fish 1	Fish 2	Fish 3	Mean±SE
1	Glucose (mmol/L)	65.5	63.3	61.8	63.5±1.07
2	Cholesterol (mmol/L)	180.4	181.7	182.5	181.5±0.61
3	Triglyceride (mmol/L)	145.0	144.5	146.1	145.2±0.47
4	High Density Lipid (mmol/L)	135.5	134.2	136.5	135.4±0.66
5	Low Density Lipid (mmol/L)	45.0	44.4	43.5	44.3±0.43
6	Total Protein (g/L)	6.3	6.9	6.1	6.4±0.24
7	Lactate Dehydrogenase (IU/ml)	197.8	194.5	190.2	194.16±2.20
8	Alanine Amino Transferase (mmol/L)	46.5	46.00	46.9	46.5±0.26
9	Alkaline Phosphatase (IU/ml)	110.5	110.9	110.1	110.5±0.23

Table 2. Showing biochemical parameters of intoxicated fishes

	Groups	**Days**	**Fish 1**	**Fish 2**	**Fish 3**	**Mean±SE**	**Percent decrease(%age)**
Glucose (mmol/L)	2	1	53.9	56.1	54.5	54.8±0.65	13.7
	3	2	36.6	34.9	31.5	34.2±1.48	46.1
	4	3	23.0	27.1	21.9	24.2±1.53	61.9
	5	4	15.3	14.8	15.0	15.0±0.14	76.3

	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent decrease(%age)
Cholesterol (mmol/L)	2	1	160.4	162.3	161.8	161.5±0.5	11.0
	3	2	120.5	121.2	122.7	121.4±0.65	33.1
	4	3	80.5	79.6	81.4	80.5±0.51	55.7
	5	4	60.5	61.5	62.7	61.6±0.63	66.1

	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent decrease(%age)
Triglyceride (mmol/L)	2	1	120.2	119.5	121.5	120.4±0.58	17.1
	3	2	87.1	86.5	85.9	86.5±0.34	40.4
	4	3	60.3	61.5	62.4	61.7±0.71	57.4
	5	4	51.2	50.4	52.5	51.4±0.61	64.6

	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent decrease(%age)
High Density Lipids (mmol/L)	2	1	102.2	101.5	103.4	102.4±0.55	22.7
	3	2	90.5	91.4	92.2	91.4±0.49	32.5
	4	3	70.5	71.2	72.2	71.3±0.49	47.3
	5	4	69.2	69.5	69.4	69.4±0.08	48.8

	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent decrease(%age)
Low Density Lipid (mmol/L)	2	1	30.5	31.4	32.2	31.4±0.49	29.2
	3	2	35.5	36.6	34.5	35.5±0.60	19.7
	4	3	20.5	21.4	22.2	21.4±0.49	51.7
	5	4	15.4	16.1	14.9	16.2±0.9	63.5

	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent decrease(%age)
Total Protein (g/L)	2	1	5.5	5.9	5.6	5.7±0.12	11.9
	3	2	4.9	4.6	4.4	4.6±0.14	27.9
	4	3	4.6	4.9	4.3	4.6±0.17	28.5
	5	4	3.9	3.4	3.6	3.6±0.14	43.5

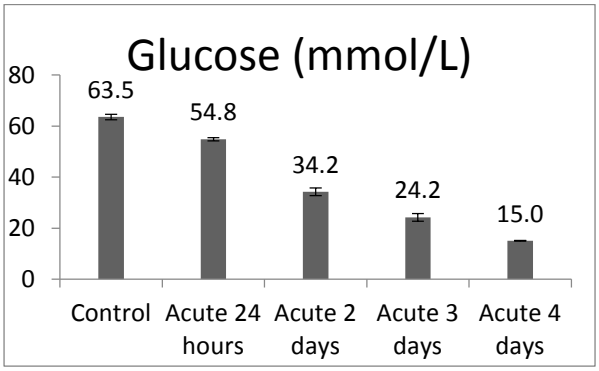
	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent decrease(%age)
Lactate dehydro genase (IU/ml)	2	1	186.6	184.5	180.2	183.8±1.88	5.4
	3	2	175.5	173.4	171.1	173.3±1.27	10.7
	4	3	165.6	169.6	164.1	166.8±1.58	14.1
	5	4	154.5	155.6	157.8	155.9±0.97	19.7

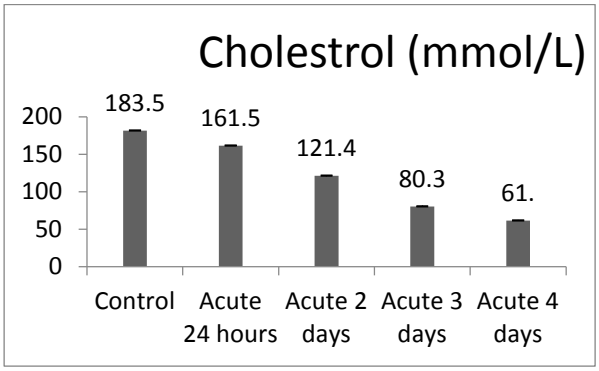
	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent increase (%age)
Alanine amino transferase (mmol/L)	2	1	49.3	49.9	49.6	49.6±0.17	6.75
	3	2	53.5	53.9	53.0	53.5±0.26	15.1
	4	3	63.5	63.9	63.1	63.5±0.23	36.7
	5	4	65.5	65.1	65.9	65.5±0.23	40.9

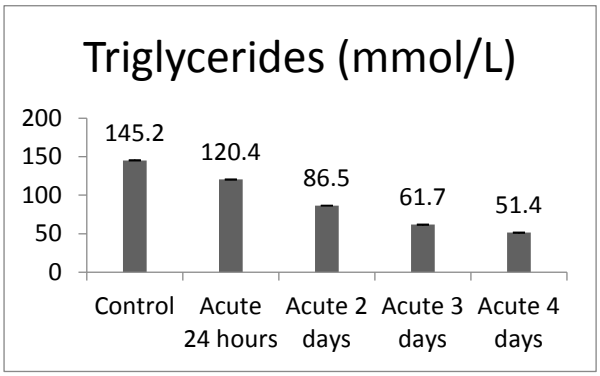
	Groups	Days	Fish 1	Fish 2	Fish 3	Mean±SE	Percent increase (%age)
Alkaline phos phatase (IU/ml)	2	1	144.5	146.4	145.5	145.5±0.54	31.6
	3	2	160.5	161.4	162.3	161.4±0.51	46.1
	4	3	170.5	171.9	173.4	171.9±0.83	55.6
	5	4	190.5	191.4	192.3	191.4±0.51	73.2

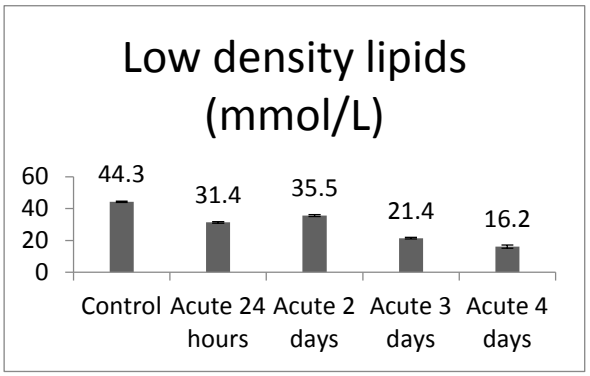
Tab 3. Physico-chemical parameters of fish ponds during the study period.

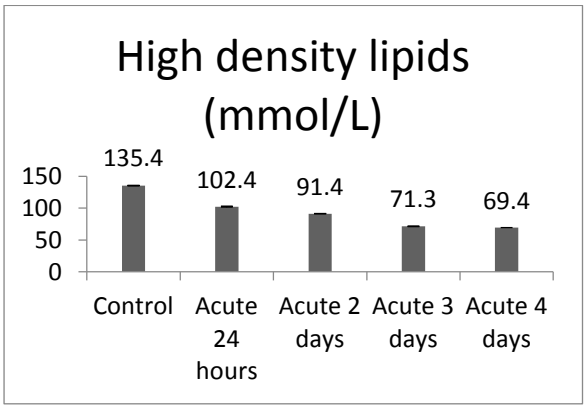
Parameters	Sample 1	Sample 2	Sample 3	Mean±S.E
PH	7.7	7.6	7.7	7.7±0.03
Water Temp ©	18.2	18	18	18.1±0.06
Total Hardness (mg/L)	100	90	95	95±2.88
Calcium Hardness (mg/L)	60	65	60	61.7±1.66
Magnesium Hardness (mg/L)	40	25	35	33.3±4.40
Water Conductivity (µS/cm)	428	435	432	431.7±2.02
Dissolved Oxygen (DO) (ppm)	7.2	7.3	7.5	7.3±0.08
Total Solids (TS) (mg/L)	318	324	323	321.7±1.85
Total Dissolved Solids (TDS) (mg/L)	218	222	225	221.7±2.02
Total suspended solids (TSS) (mg/L)	100	102	98	100±1.15
Total Alkalinity (mg/L)	161	161	168	163.3±2.33
Chloride (mg/L)	19	20	22	20.3±0.88
Sulphate (mg/L)	14	15	18	15.7±1.20

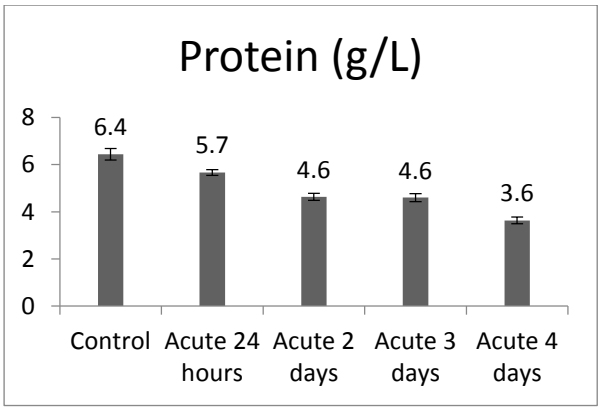

Glucose (mmol/L)
80
60
40
20
0
63.5
54.8
34.2
24.2
15.0
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days


Cholestrol (mmol/L)
200
150
100
50
0
183.5
161.5
121.4
80.3
61.
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days


Triglycerides (mmol/L)
200
150
100
50
0
145.2
120.4
86.5
61.7
51.4
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days


Low density lipids (mmol/L)
60
40
20
0
44.3
31.4
35.5
21.4
16.2
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days


High density lipids (mmol/L)
150
100
50
0
135.4
102.4
91.4
71.3
69.4
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days


Protein (g/L)
8
6
4
2
0
6.4
5.7
4.6
4.6
3.6
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days

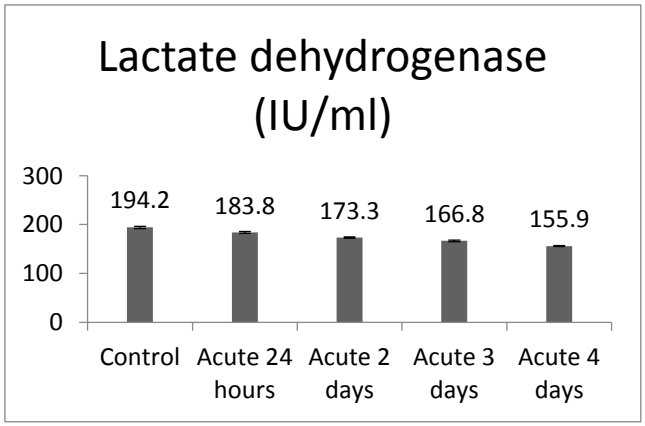

Lactate dehydrogenase (IU/ml)
300
200
100
0
194.2
183.8
173.3
166.8
155.9
Control
Acute 24 hours
Acute 2 days
Acute 3 days
Acute 4 days

Fig. I. Showing decreased concentration after feeding Malathion to *Cirrhinus mrrigala* for mentioned days.

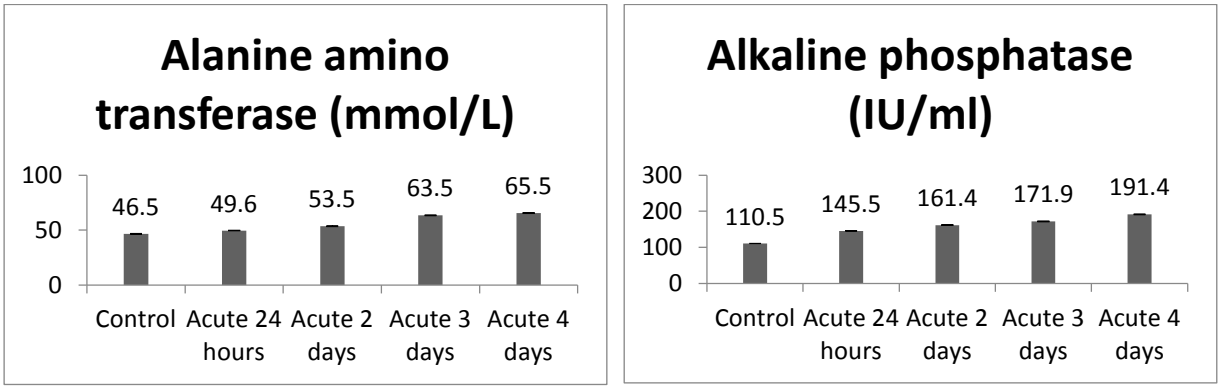


Fig. II. Showing increase in parameters concentration after feeding Malathion to *Cirrhinus mrrigala* for mentioned days.

DISCUSSION

Water pollution has one of the critical environmental problems of the centuries. Physiological and biochemical aspects of fish in aquatic environment have been affected by pesticides (Webb and Brett, 1973; Palanichamy *et al*., 1986, 1987; Seshagiri Rao *et al*., 1987; Vasanthi and Ramasamy, 1987; Vasanthi *et al*., 1990). Changes in composition of fish body when exposed to pesticide has been reported in many studies (Sivaprasanna Rao and Ramana Rao, 1979; Rath and Misra, 1980; Palanichamy *et al*., 1986, 1987; Vasanthi and Ramaswamy, 1987; Seshagiri Rao *et al*., 1987; Babu *et al*., 1988; Malla Reddy and Bashamohideen, 1988; Begum and Vijayaraghavan, 1995).

Pesticide toxic effect cause extremely irritating condition which make

the worse or effective the metabolic and physiological activities of fish. Glucose is one of the important source of energy, its level reflect the effect of environmental stress on fish body (Kavitha *et al*., 2010), its concentration after treatment in *cirrhinus mrigala* for 1 day was 54.8±0.65 mmol/L (decrease of 13.7%), for 2 days was 34.2±1.48 mmol/L (decrease of 46.1), for 3 day was 24.2±1.53 mmol/L (decrease of 61.9 %) and for 4 day was 15.0±0.14 mmol/L (decrease of 76.3%). So it is evident that glucose value of acute toxicity group on first day showed less decrease as compare to control group but significant decrease was found for 2, 3 , and 4 days dose, Glucose concentration decreased with increase in number of days in acute toxicity case, this decrease was due to pesticide concentration, oxygen deficiency and increased energy was utilized during metabolic processes. Sastry and Siddique (1982) noticed hypoglycaemia when exposed fish to sublethal concentration of Malathion. In Indian cat fish same results has been observed when exposed to malathion dose of Lc_{50} methyl Parathion by Srivastava and Singh (1999). Low glucose level indicates the lack of potential to store glycogen and was totally dependent on glucose present in blood to perform various metabolic activities (Lehninger, 2004). On the other hand (Pickering, 1981), reported increase glucose level after short and long term exposure to stress condition (Tintos *et al*, 2006).

Cholesterol involve in building of cell membrane as well as plasma protein layer (Yang and Chen, 2003). Cholesterol level after treatment in C*irrhinus mrigala* for 1 day was 161.5±0.5 mmol/L (decrease of 11.03%), for 2 day was 121.4±0.65 mmol/L (decrease of 33.1%), for 3 days was 80.5±0.51 mmol/L (decrease of 55.7%) and for 4 days was 61.6±0.63 mmol/L (decrease of 66.1%) in acute toxicity case, it is clear that on 1st day acute toxicity group showed non-significant decrease as compared to control group, but on 2 ,3 and 4 day significant decrease were observed in cholesterol level in serum of fish. Reduced level of cholesterol may be due to low concentration of cholesterol biosynthesis in the liver or due to absorption level of dietary cholesterol reduced (Kanagaraj *et al.,* 1993). It has been reported that cholesterol reduction may also occur due to pollutants when fish used fatty deposits from the body instead of glucose to deal with stress condition (Remia *et al.,* 2008). Biochemical changes observed in the liver of *Cyprinus carpio* by Virk and Sharma (1999) and they observed depletion of cholesterol content in the liver. Decrease level of cholesterol in liver and muscles of freshwater fish (*Punctius ticto*) has also

been reported by Ganeshwade (2012), while increase in blood cholesterol level of male rats were reported when treated with fungicide Carbendazim (Selmanoglu *et al*., 2001).

Main function of triglyceride was to provide cellular energy and signals the nutritional status. In the diagnostic treatment of such lipid metabolism involved diseases, triglyceride was much important. Triglyceride level after treatment of *Cirrhinus mrigala* for 1 day was 120.4±0.58 mmol/L (decrease of 17.1%), for 2 day was 86.5±0.34 mmol/L (decrease of 40.4%), for 3 days was 61.7±0.71 mmol/L (decrease of 57.4%), and for 4 days was 51.4±0.61mmol/L (decrease of 64.6%) in acute toxicity group. So on comparison acute toxicity group with control group it was clear that on 1st day acute toxicity group showed 17.1% decrease as compared to control group, but on 2, 3 and 4 days decrease percent was 40.4%, 57.4%, 64.6% in serum of fish. Decline of triglyceride level increased with increase in number of days of exposure. Serum triglyceride decrease was supported by report of Kori-Siakpere *et al*., (2007) where paraquat resulted in plasma triglyceride decrease in *Clarias gariepinus* after the exposure period of 96h.

Lipid provides energy to metabolic processes and plays an important role in energy metabolism. They are very important for cellular and sub-cellular membrane as well. It is also used as storage of energy, being stored and transported in the form of glycerol ester. HDL concentration after exposure to malathion for one day in *cirrhinus mrigala was* 102.4±0.55 mmol/L (decrease of 22.7%), for 2 day was 91.4±0.49 mmol/L (decrease of 32.5%), for 3 days was 71.3±0.49 mmol/L (decrease of 47.3%) and for 4 days was 69.4±0.08 mmol/L (decrease of 48.8%) for acute toxicity group. So on comparison acute toxicity group with control group it was cleared that on 1st day acute toxicity group shows non-significant decrease as compared to control group, but on 2,3 and 4 day significant decrease were observed in lipid level in serum of fish. Decline in lipid content of fish body showed negative effect of stressors (Svobodova *et al*., 2006).

LDL concentration after exposure to Malathion in *cirrhinus mrigala* for one day was 31.4±0.49 mmol/L (decrease of 29.2%), for 2 day was 35.5±0.60 mmol/L (decrease of 19.7%), for 3 days was 21.4±0.49 mmol/L (decrease of 51.7%) and for 4 days was 16.2±0.9 mmol/L (decrease of 63.5%), for acute toxicity group. So on comparison acute toxicity group with control group it was cleared that on 1 and 2 days of acute toxicity

group shows non-significant decrease as compared to control group,but on 3 and 4 day significant decrease were observed in lipid level in serum of fish. Similar findings were given by Patil and Patole (2012) the effect of the Malathion and Cypermethrin on the lipid components of freshwater fish *Lepidocephalicchthys guntea.* They observed that, the lipid level content decreased sufficiently in all treated groups. Lipid level decreases in a sufficient amount because the liver is the principle spot of detoxifiation in fishes (Dixith, 2005). Patil and Kulkarni (1995) found decrease of lipid content in fresh water fish *Channa punctatus* when exposed to pesticide summach. So decrease of total lipids was probably due to the abundant lypolysis and subsequently used for glucose synthesis. The above results evidently indicate that Malathion has a toxic effect over fresh water fish *Channa punctatus*

Protein were considered to be the main factor for maintaining mechanism of stressed organism, Protein concentration after treatment of *Cirrhinus mrigala* for 1 day was 5.7±0.12 g/L (decrease of 11.9%) for 2 day was 4.6±0.14 g/L (decrease of 27.9%), for 3 days was 4.6±0.17 g/L (decrease of 28.5%) and for 4 days was 3.6±0.14 g/L (decrease of 43.5%) So on comparison acute toxicty group with control group it was cleared that on 1st day acute toxicity group shows lesser decrease as compared to control group,with increasing days decrease of protein level was increased in acute toxicity. Our results show agreement with results of (Premadas and Anderson, 1963; Sivaprasanna Rao and Ramana Rao, 1979; Bakthavalsalam, 1980; Ram and Sathyanasan, 1984; Ganguly *et al.,* 1997; Ramani, 2001). Reduction in protein level was due to difference in energy level when fish was under stress condition reported by Manoharan and Subbiah (1982).Decrease in protein level shows that carbohydrate metabolism was disturbed as a response protein breakdown occur to combat such situation and inhibit the Protein synthesis and ATP synthesis.Glucose,protein and amino acid level gradually decrease with increasing days, and reduction of protein level may also be due to utilization of protein in the form of amino acid in the body to deal with stress condition (Borah, 1996). On the contrary (Sahib *et al.,* 1984) reported increased level of protein when exposed fresh water fish *Tilapia mossambica* to Malathion.

Lactic dehydrogenase concentration after treatment in *Cirrhinus mrigala* in acute toxicity group for 1 day was 183.8±1.88 IU/mL (decrease of 5.4%), for 2 days was 173.3±1.27 IU/mL (decrease of 10.7%), for 3 days was 166.8±1.58 IU/mL(decrease of 14.1%) and

for 4 days was 155.9±0.97 IU/mL(decrease of 19.7%), it was cleared that acute toxicity group shows non-significant decrease as compared to control group. This reduction shows inhibition of LDH activity so that Malathion inhibits the aerobic and anaerobic metabolic process in exposed animals. During anaerobic respiration lactic dehydrogenase catalyses the interconversion of lactic acid and pyruvic acid. Drop of LDH activity as compare to control group shows decrease glycolytic process and lower metabolic process as a result of Malathion. Similar results were made by Asztalos *et al.,* (1990) on observing reduced activity of LDH in *Cyprinus carpio*.LDH level indicate anaerobic respiration which enable animal to deal with oxygen deficiency (Masopust, 2000). This decreased activity was because of the higher rate of glycolysis, which was the only pathway for energy-producing for the animal when under stressed condition. LDH was changed by oxygen stress and was valued glycolytic enzyme in the biochemical systems. Alanine amino transferase concentration after treatment of *Cirrhinus mrigala* in acute toxicity group for 1 day was 49.6±0.17 mmol/L (increase of 6.75%), for 2 days was 53.5±0.26 mmol/L (increase of 15.1%), for 3 days was 63.5±0.23 mmol/L (increase of 36.7%) and for 4 days was 65.5±0.23 mmol/L (increase of 40.9 %). So it is clear that lesser change in parameters due to one day dose occurred as compared to control group and on 2 and 3 days duration the increase was more as compared to control group. An elevated level of ALT activity in fish exposed to Malathion and Porkiabiglosa pods extracts respectively has been reported by Abalaka *et al*., (2011) and Zaki *et al.,* (2009). Their opinion was that this increased activity in the exposed fish was indicative of hepatic damages leading to their circulation leakage (Mousa *et al.,* 2008) and/or synthesis of enzyme in the liver increased. An increase in ALAT and AAT activity levels in fish following fenvalerate intoxication was reported (Radaiah, 1988; Ayyanna, 1988). Alkaline phosphatase play a very important role in carbohydrate metabolic process and transferring prosophyl mechanism (Singh, 1977). Its concentration after treatment of *Cirrhinus mrigala* in acute toxicity group for 1 day was 145.5±0.54 IU/mL (increase of 31.6%), for 2 days was 161.4±0.51 IU/mL (increase of 46.1%), for 3 days was 171.9±0.83 IU/mL (increase of 55.6%), and for 4 days was 191.4±0.51 IU/mL (increase of 73.2%). So on comparing acute toxicty group with control group it was clear that lesser change was observed as compared to control in acute group 1 day dose, but with increasing dose in acute toxicity group the alkaline phosphate

concentration was also increased and at 4 day dose of malathion raised the alkaline phosphatise level up to 73.2%. Similarly increased activity of alkaline phosphatase had observed in fishes after exposure to gallium due to toxic stress of pesticide on fish liver (Yang, 2003). Elevated activity of alkaline phosphatase activity of Freshwater fish *Ctenopharyngodon idella* was reported when exposed to sublethal dose of fenvalerate (Shakoori *et al*., 1996). Increased ALP activity has been reported by (Goel *et al.,* 1984) in *Clarias batrachus* .when exposed to Alachlor due to tissue damage. So elevated ALP activity may be due to necrosis of liver cells. On the contrary there was no significant change in the carp (*Cyprinus carpio*) alkaline phosphatase activity when exposed to diazinon on comparison with control fishes (Luskova, 2002). Rashatwar and Ilyas, (1983) reported declined activity of alkaline phosphatase in treated fishes.

Conclusion

Results indicate that after exposure to pesticides, the activity of glucose, cholesterol, triglycerides, protein, LDL, HDL, and lactate dehydrogenase in the blood of *Cirrhinus mrigala* decreased, while the activity of alanine amino transferse and alkaline phosphatase decreased, which reflects that the insecticide stress have altered the activities of enzymes and other biochemical parameters. The results suggest that the exposure of the fish to sub lethal concentrations have altered the fish metabolism, and thus giving scope to further studies on other living organisms to assess the toxic effects.

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