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Commercial Deltametrin: Its (Sublethal) Impact on Carbohydrate Metabolism of *Labeo rohita*

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ABSTRACT

The present study aimed to evaluate the effect of sublethal concentration of a synthetic pyrethroid pesticide, i.e., deltamethrin (DM) on enzymological responses in an Indian major carp, Labeo rohita. In this study, the LC50 values of DM for 96 h were found to be 0.09 ppb. Effect of sub-lethal dose of delamethrin was evaluated 1/10th of 96 h LC50 value, 0.009 ppb concentration of deltamethrin was evaluated on carbohydrate metabolism of L. rohita. The significant ($p<0.001$) decrease in glycogen levels were observed in liver as well as in muscles during the 24 h of incubation and gradual increase were observed when compared with control group. The increase of lactate dehydrogenase was progressive in all the tissues, i.e., gills, brain, liver, and kidney and reached highest at 96 h of exposure in gills. However, the significant decrease in succinate dehydrogenase and malate dehydrogenase was observed in collected tissues and decrease is less at 24 h and it was increased along the exposure period. These results suggest that the tested concentrations of DM could have significant adverse effects on the hormonal and enzymologicalparameters of fish L. rohita. The alterations of these parameters can be effectively used to monitor the impact of DM in aquatic ecosystem.

Key words: *Deltanethrin, LC 50, Sublethal effect, Carbohydrate metabolism, Labeo rohita.*

1. INTRODUCTION

The use of modern synthetic chemicals in agriculture has increased 40 folds to preserve the standing crops from the attack of pests and to boost up crop production, to meet the ever-increasing food demand of the rising human population [1,2]. Among these, synthetic chemicals pyrithroids are most important and have been classified into two distinct groups, Type I and Type II based on the behavior, neurophysiological, chemical, and biochemical profiles [3,4]. Type II synthetic pyrethroid has a wide acceptability for agricultural purposes [5], Ex. Deltamethrin (DM) (Figure 1). It was first described and marketed in 1974 and 1977, respectively [6,7]. The physicochemical properties of DM have been represented in Table 1. DM use was extended from agriculture and home formulations to outdoors on lawns, ornamental gardens, golf courses, and indoors as a spot, crack, and crevice treatment [8,9] and also used to control pests, insects, and vectors of endemic diseases and fighting household insects [10]. In addition, it has also been used as a substitute pesticide for the control of ectoparasites in cattle, sheep, and poultry forms [11].

Due to imprudent and indiscriminate use of these chemical pesticides, the natural water resources such as lakes, rivers, ponds, paddy fields, and other low lying areas are getting polluted worldwide [12,13] that ultimately leads to contamination of aquatic environment. The highest concentration of pesticide residues were found in aquatic ecosystem than terrestrial ecosystem [14]. Moreover, the extensive deposition of these chemicals has attracted the attention of ecologists to understand the impacts of these chemical pollutants on aquatic biotic communities [15]. Aquatic pollution has erupted as global problem in recent past. Koprucu and Aydin [16] and Lazartigues *et al.* [17] have been described the changes in the population of the fauna as a consequence of sublethal effects of aquatic pollutants on ecologically important species. The photostable synthetic pyrethroid insecticides are lipophilic in nature are relatively harmless to birds and mammals but are extremely toxic to many marine and freshwater forms including invertebrates, insects, and fishes [18]. However, in fish, the deficiency of enzymes for the hydrolysis of pyrethroids and the high rate of absorption of these insecticides through gills makes these insecticides highly susceptible to ecosystem

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Table 1: Physicochemical properties of deltamethrin.

Variable	Information
Appearance	Color less crystalline powder, white or slightly beige powder.
Chemical nature	Cyano (3-phenoxy-phenyl) methyl; 2-(2,2dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate (CA); alpha-cyano-m-phenoxybenzyl, (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl -cyclopropanl-carboxylate, (S)-alpha-cyano-3-phenoxybenzyl (1R)-*cis*-3-(2,2-dibromovinyl) -2,2-dimethylcyclopropane-carboxylate
CAS number	52918-63-5
Chemical formula	$C_{22}H_{19}Br_2NO_3$
Molecular weight	505.24
Water solubility	Less than 0.1 mg/L. Insoluble<1 ppm at room temperature. 0.002 mg/L at 20°C. Almost insoluble
Solubility in other solvents	In kerosene and isoalkanes, less than 0.5, isopropanol 0.6, ethanol 1.5, Xylene 25, methylene chloride 70 (all in g/100g at 20°C). In acetone 500 g/L, benzene 450 g/L, DMSO 450 g/L, cyclohexanone 750 g/L, dioxane 900 g/L, All at room temperature. Tolulene 250g/L.
Melting point	98-101°C
Vapor presser	$2X10^{-8}$ mbar at 25°C
Partition coefficient	4.6 (25°C)
Aquatic field test half-life (days)	<2
Terrestrial field test half-life (days)	14-231
Hydrolysis half-life (days)	<33

DMSO: Dimethyl sulfoxide, CAS: Chemical abstracts service

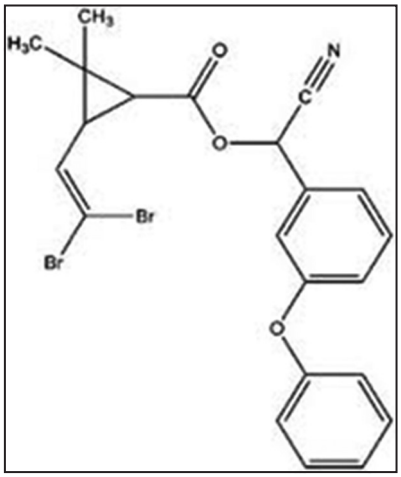

Figure 1: Chemical structure of deltamethrin.

toxicity [5,19]. Many investigators had extensively characterized, reviewed, and summarized on the levels of pesticide toxicity in different fishes [20-24].

DM and *Labeo rohita* was selected for the present study, because of uncontrolled usage of pesticide and very high consumption of fish in and around the Anantapuram (District) Andhra Pradesh. In this study, we evaluated the impact of sublethal concentrations of DM on carbohydrate metabolism of fresh water fish.

2. EXPERIMENTAL

2.1. Fish and Pesticide

The freshwater fish *L. rohita* is commonly called ROHU belongs to the family Cyprinidae. Live fish weighed about 10 ± 2 g were procured from local fisheries in and around Anantapur, Andhra Pradesh. The fish were stored in aquaria and water is aerated twice a day and before experiment the fishes were acclimatized to the laboratory conditions for a period of 10 days. Than the fish stock was maintained at natural photoperiod with ambient temperature. Tap water (free from chlorine) was used for the experiments and the physicochemical characteristics of the water were analyzed by standard methods.

Deltamethrin was selected for this study to analyze its impact on carbohydrate metabolism of freshwater fish *L. rohita*, because of wide range of applications to control pests, flies, and mosquitoes. Apart from this it also has high photo stability, degradability, non-persistent nature, and low mammalian toxicity. Commercial grade was used with 2.8% concentration.

2.2. Determination of LC50

Each experimental group consists of 10 number of fish and was exposed to different concentration of DM ranging from 0.073 to 0.097 ppb. In each test, fish introduced into toxicant glass chambers with a

capacity of ten liters and mortality rate of fish was recorded for up to 96 hrs exposure period. LC50 values were calculated by Finney's probit analysis [25]. The LC50/96 hrs was determined from the percent and probit mortality versus log concentration curves and was subsequently verified by Dragstedt and Behrens method as given by Carpentor [26]. After determination of LC50/96 h (0.09 ppb), the fish were exposed to sublethal concentration of DM ($1/10^{th}$ of LC50/96 h, i.e. 0.009 ppb).

2.3. Sample Collection

Blood sample collected from control and DM-treated groups by cardiac puncture by plastic disposable syringe fitted with 26 gauge needle moistured with heparin. The collected blood was expelled into separate heparinized plastic vials and kept immediately on ice. The whole blood was centrifuged for 15 min, at 10,000 rpm and plasma was withdrawn and transferred into clean vials for analysis. Further, after ion of blood, fish were washed with double-distilled water and blotted dry then fish were cut open, liver, and kidney was removed for the estimation of enzymatic activity.

2.4. Analytical Methods

In the present study, the levels of glycogen in liver and muscle and lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and Malate dehydrogenase were investigated in brain, liver, kidney, gills, and muscle of fish using the standard protocols at particular intervals of 24 h, 48 h, 72 h, and 96 h exposure to sublethal concentrations of DM. Each experiment was carried out in the organs of six individuals, and the mean of six values were taken into consideration.

2.4.1. Preparation of tissue homogenate

5% (w/v) and 10% homogenate of the various tissues were prepared in 0.25 M (21.4 of sucrose in 250 ml of distilled water) ice cold sucrose solution and centrifuged at 2500 rpm for 15 min and the supernatant was taken as the source of enzyme [27].

2.4.2. Estimation of glycogen

Glycogen concentration in various organs was estimated using anthrone reagent method as described by Caroll *et al*. [28]. To 0.2 ml of tissue digest, 1.8 ml of distilled water and 0.5 ml of 2% anthrone reagent was added and heated in boiling water bath exactly for 10 min. The mixture was cooled and O.D of the color developed was measured at 620 nm. A blank and glucose standards were also run similarly. The glycogen percent is expressed as mg/g wet weight of the organ.

2.4.3. LDH

LDH activity was estimated using the method of Srikanthan and Krishnamurthi [29] modified by Govindappa and Swami [30]. The reaction mixture consisting of 1 ml of 0.0001 M nicotinamide adenine dinucleotide (NAD), 0.1 ml of 0.004 M 2-(p-indophenol) 3-p-nitrophenyl-5-phenyltetrazolium chloride, and 0.5 ml of tissue homogenate. Then, mixture was incubated at 37°C for 30 min, and then, reaction was stopped by adding 6 ml of glacial acetic acid. The formozan formed was extracted into 6 ml of toluene overnight at 0°C and O.D was measured at 495 nm. A blank using 0.5 ml of distilled water and a control taking 0.5 ml of boiled enzyme were also run similarly. The enzyme activity was expressed as μ moles of formozan formed/mg protein/hour.

2.4.4. Succinate dehydraogenase

SDH activity was estimated using the method of Nachlas *et al.* [31]. The incubation mixture consisting of 0.2 ml of 0.4 M phosphate buffer (pH 7.7), 0.2 ml of 0.2 M sodium succinate, 1.0 ml of 0.004 M of 2-(p-indophenol) 3-p-nitrophenyl-5-phenyltetrazolium chloride (INT), 0.1 ml 0.005 M phenazine methosulphate, and 0.5 ml of 5% homogenate and incubated at 37°C for 30 min. Then, the reaction was stopped by adding 0.6 ml of glacial acetic acid. The formozan formed was extracted into 6 ml of toluene cooled overnight at 0°C and O.D of the color developed was measured at 495 nm. A blank 0.5 ml of distilled water and a control taking 0.5 ml of boiled enzyme were also run similarly. INT standards were prepared alongside for comparison. The enzyme activity was expressed as μ moles of formozan formed/mg protein/hour.

2.4.5. Malate dehydrogenase

Malate dehydrogenase (MDH) activity was estimated by the modified method of Nachlas *et al.* [31] 2 ml of incubated mixture contained 0.5 ml of sodium malate substrate (100 μ moles) 0.5 ml of INT (2 μ moles) 0.4 ml of phosphate buffer (pH 7.4) 0.1 ml of NHD (2 μ moles) and 0.5 ml of homogenate. After incubation for 30 min at 37°C, the reaction was arrested by the addition of glacial acetic acid. The formozen formed was extracted in 5 ml of toluene by leaving it overnight in a refrigerator. The O.D was recorded at 495 nm against a toluene blank. The enzyme activity was expressed as μ moles of formozan formed/mg protein/hour.

2.5. Statistical Analysis

The obtained data were analyzed statistically at $p<0.05$. The significance of sample means between control and DM-treated fish was tested using t-test. For sublethal studies, all values were expressed as means and analyzed by analysis of variance (ANOVA) to determine the significant differences ($p<0.05$) among the treatments and periods, and between the treatments and periods on each parameters.

3. RESULTS AND DICUSION

3.1. Determination of LC50

In the present study, the LC50/96 hrs was determined from the percent and probit mortality versus log

concentration curves [25] and was subsequently verified by Dragsted-Behrens method as given by Carpenter [26]. In this study, LC50 value for DM to freshwater fish *L. rohita* was determined as 0.09 ppb by exposing to four different exposure periods, i.e. ,24 h, 48 h, 72 h, and 96 h. The sublethal concentration of DM was determined as the 1/10 of LC50/96 h, i.e., 0.009 ppb. For further analysis used the sublethal concentration of DM to assess its efficacy on the carbohydrate metabolism. According to the 96 h, LC50 of DM to fish is ranging between 0.4 and 2.0 g/L [32]. Svobodova *et al.* [33] determined 96 h LC50 value for *Cyprinus carpio* as 0.058 µg/L. On the other hand, 24 h LC50 value of DM was 0.015 µg/L in *Clarias gariepinus* [34] and 0.016 ppm in *Poecilia reticulata* [35]. The differences in the LC50 values of DM to various other species will mainly depend on the species specificity [36] the capacity of species to tolerate pesticide stress [37], differences in size and weight [38], and also on nutritional state [39]. High absorption of DM through gills, liphophilicity nature and deficiency of hydrolyzing enzymes in fish are central to induce toxicity in the fish [40]. Moreover, these insecticides act mainly on the voltage-dependent Na+ channels of the nerve cell membrane and induce the toxicity [41].

3.2. Changes in Glycogen Levels

In the present study, it is observed that the glycogen content in the liver decreased at 24 h exposure of sublethal concentrations of DM when compared with controls by −30.566%, further exposure at 48 h, 72 h, and 96 h the liver glycogen content increased gradually by −18.14%, 12.39%, and +1.65%, respectively (Figure 2). These findings were found to be highly significant ($p<0.001$). Glycogen is the major storage form of carbohydrate in animals mainly in liver and muscle and is often called as animal starch. Alterations in liver and muscle glycogen under situations of stress have been reported and significant depletion in tissues is said to reflect the state of strenuous activity on the part of fish [42].

We also investigated the percentage change in muscle glycogen content in exposed fish was decreased at 24 h by −18.33% and same trend was observed at 48 h and 72 h exposure to decrease by −42.06% and 48.78%, respectively. Thereafter, the glycogen content was gradually increased by −20.88% at 96 h exposure (Figure 2). The decrease in the levels of liver and muscle glycogen on first-day exposure indicates the high energy demand associated with imposed DM stress. To overcome this animal tends to mobilize the blood glucose by stimulating the glycogenolysis.

Adrenal hormones such as glucocorticoids and catecholamines may be induced by pesticides, elevate the blood glucose level by conversion of stored glycogen into blood glucose [43,44]. The stepping

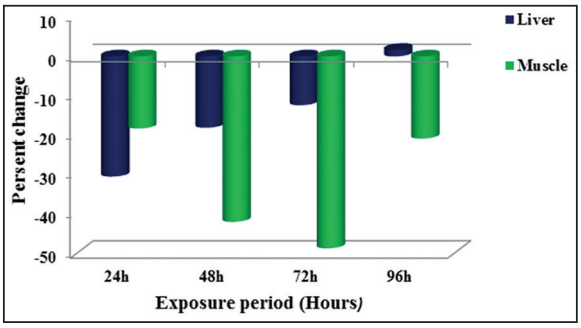

Figure 2: Percent changes over control in glycogen content in the various tissues at different exposure periods to sublethal concentration of deltamethrin.

up of glycogenolysis is evident from the fact that the decrease in glycogen content. Similar trend was observed in the same fish exposed to fenvalarate [45]. According to Rani *et al.* [46] decreased glycogen levels of liver, muscle, brain, gill, and kidney in *L. rohita was* observed on exposure to sublethal concentration of cypermethrin. Similarly, other investigators also reported that the tremendous decrease of glycogen levels in the tested fish models i.e., *L. rohita*, *Catla catla*, and *Cirrhinus mrigala* on the exposure of sub-lethal dose of chlorpyrifos [47]. Freshwater teleost *Tilapia mossambica* showed significant depletion in glucose and glycogen levels in various tissues on exposure to sublethal concentration of sodium arsenite and stated that these changes were tissue specific and time dependent [48]. Stalin and Das [49] reported initial decrease in liver glycogen content in various tissues and followed by its elevation in later exposure periods in the fish *Cirrhinus mrigala* on exposure to an organochloride fenthion. All these studies correlate initial elevation in blood glucose level followed by decrease in liver and muscle glycogen content.

3.3. LDH

From the data presented in Table 2 and Figure 3, it is found that the LDH activity was significantly increased in gill, muscle, liver, brain, and kidney during sublethal exposure of DM in various exposure periods, i.e., 24 h, 48 h, 72 h, and 96 h. The increase was progressive in the enzyme activity of all the tissues and reached highest at 96 h of exposure in gills. The maximum increase was observed in gills (35.16%) followed by liver, kidney, muscle, and brain (26.08%, 25.95%, 23.23%, and 12.09%, respectively). A cell harbors innumerable enzymes which are necessary for their vital functions. When cell's integrity is disrupted, enzymes are escaped into plasma/serum, where their activity be measured as a useful index of cell integrity [50]. LDH is catalyses the reversible conversion of lactate to pyruvate and vice versa. It is considered as an index for chemical evaluation of tissue damage in various diseases [51]. Changes in the enzyme activity may provide direct and indirect evidence of the cellular damage and can

Table 2: Lactate dehydrogenase activity in the selected tissue exposed to sublethal concentrations of deltamethrin during different exposure periods.

Name of the tissue	Exposure periods in hours (sublethal concentrations of DM)				
	Control	24	48	72	96
Brain					
Mean±SD	1.058±0.039	1.126±0.024	1.153±0.055	1.162±0.072	1.186±0.034
% change		6.427	8.980	9.830	12.098
t-test		<0.01	<0.01	<0.01	<0.001
Gill					
Mean±SD	0.182±0.021	0.207±0.015	0225±0.021	0.238±0.018	0.246±0.024
% change		13.736	23.626	30.770	35.165
t-test		<0.05	<0.01	<0.001	<0.001
Kidney					
Mean±SD	0.262±0028	0.298±0.022	0.312±0.023	0.326±0.026	0.330±0.022
% change		13.740	19.084	24.427	25.954
t-test		<0.05	<0.01	<0.01	<0.001
Liver					
Mean±SD	0.460±0.026	0.560±0.027	0.540±0.033	0551±0.031	0.580±0.038
% change		9.999	17.391	19.782	26.087
t-test		<0.001	<0.001	<0.001	<0.001
Muscle					
Mean±SD	0.099±0.010	0.115±0.014	0.118±0.012	0.119±0.010	0.122±0.014
% change		16.162	19.192	20.202	23.232
t-test		<0.05	<0.01	<0.01	<0.01

SD: Standard deviation, NS: Not significant, DM: Deltamethrin

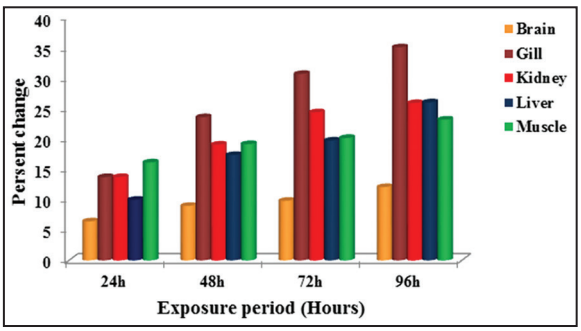

Figure 3: Percent change over control in lactate dehydrogenase activity in the exposed tissue at different exposure periods to sublethal concentrations of deltamethrin.

indicate the toxic mechanism. Thus, the significant changes in enzymes activity of LDH indicate damage to any or all organs producing this enzyme such as liver or kidney injuries [52,53].

An elevated level of LDH was also reported by different workers occupationally exposed to DDT (Dichloro Diphenyl Trichloro Ethane) [54]. According to UshaRani and Ramamurthi [55] LDH activity was increased in fresh water fish exposed to sublethal concentrations of cadmium. Significant decrease in LDH activity levels were also observed in the tissues of *Channa punctatus* exposed to *Euphorbia royeleana* latex [56]. Decrease in LDH activities was observed after exposure to endosulfan and fenvalerate on fresh water fish *Clarias batrachus*, which indicates decrease in aerobic and anaerobic capacity of fish [57].

3.4. SDH Activity

SDH is an important enzyme of citric acid cycle and catalyzes the reversible oxidation of succinate to fumarate. As shown in the Table 3 and Figure 4, SDH activity varied considerably during different hours of exposure span. In the present investigation, it can be visualized that there is a rapid depletion of SDH activity in all tissues of fish *L. rohita* exposed to sublethal concentrations of DM during 24 h, 48 h, 72 h, and 96 h when they compare with their respective controls. It indicated the depression in aerobic cellular metabolism in the fish. Being a key enzyme in the tricarboxylic acid (TCA) cycle, it is logical to assume that with the inhibition of SDH activity the metabolic path might have turned to anaerobic pathway to meet the increased energy demands during the pesticide stress. Among the four exposure periods of sublethal concentrations of DM SDH activity decreased in the tissue of fish *L. rohita*. However, the decrease is less

Table 3: SDH activity in the selected tissue exposed to sublethal concentrations of deltamethrin during different exposure periods.

Name of the tissue	Exposure periods in hours (sublethal concentrations of DM)				
	Control	24	48	72	96
Brain					
Mean±SD	0.751±0.050	0.675±0059	0.560±0.051	0.526±0.092	0.462±0.086
% change		−10.120	−25.433	−29.960	−38.482
t-test		<0.05	<0.001	<0.001	<0.001
Gill					
Mean±SD	0.146±0.014	0.130±0.010	0.124±0.011	0.114±0.013	0.109±0.015
% change		−10.958	−15.068	021.918	−25.342
t-test		<0.05	<0.01	<0.01	<0.001
Kidney					
Mean±SD	0.085±0.011	0.074±0.003	0.071±0.007	0.069±0.010	0.066±0.015
% change		−12.941	−16.471	−18.824	−22.535
t-test		<0.05	<0.05	<0.05	<0.05
Liver					
Mean±SD	0.351±0.030	0.293±0.020	0.241±0.026	0.197±0.080	0.189±0.069
% change		−16.524	−31.334	−18.824	−46.154
t-test		<0.01	<0.001	<0.05	<0.001
Muscle					
Mean±SD	0.095±0.009	0.082±0.005	0.079±0.007	0.072±0.008	0.068±0.006
% change		−13.684	−16.842	−24.211	−28.421
t-test		<0.01	<0.01	<0.001	<0.001

SD: Standard deviation, DM: Deltamethrin, SDH: Succinate dehydrogenase

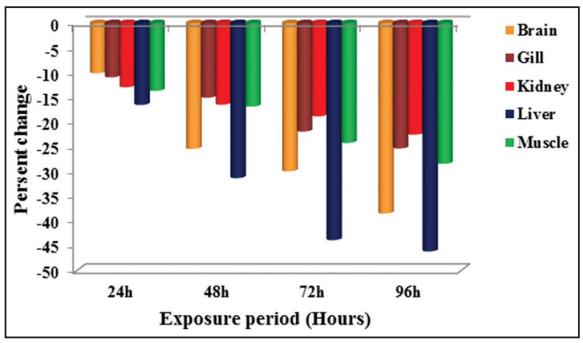

Figure 4: Percent change over control in succinate dehydrogenase activity in the exposed tissue at different exposure periods to sublethal concentrations of deltamethrin.

at 24 h exposure in the order of brain, gill, kidney, muscle, and liver and more at 96 h exposure periods in the order of liver, brain, muscle, gill, and kidney.

The general decrease in SDH activity during pesticides stress was associated with the inhibition of mitochondrial respiratory mechanism which prevents the transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity, and shifting the aerobic metabolism to anaerobiosis [58]. Similar results in the SDH activity was also observed by the various workers in different species of the fish exposed to different pesticides. Decrease in activities of LDH and SDH in fish *Colisa fasciatus* after exposure to Cypemethrin. Decreased activities of LDH and SDH were also observed in fish *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum* mill latex [59].

3.5. Malate de Hydrogenase Activity

Malate dehydrogenase is an NAD-dependent enzyme which converts malate to oxaloacetate and reversible oxidation of fumarate to malate and also plays a significant role in CO_2 fixation and in gluconeogenesis. In the present study, we observed that the significant decrease of MDH activity in treated fish at all the exposure periods (Table 4 and Figure 5) and this decrease was significant. MDH activity was decreased slowly and enhanced gradually over time of exposure and reached maximum at 96 h of exposure. Remarkable decrease was observed in gill (−36.06%) followed by brain (−28.45%), muscle (−27.58%), kidney (−25.45%), and liver (−24.31%).

The decreased MDH activity may also suggest the lower level of functioning of Krebs cycle due to inadequate supply of substrate or decreased oxygen

Table 4: Malate dehydrogenase activity in the selected tissue exposed to sublethal concentrations of deltamethrin during different exposure periods.

Name of the tissue	Exposure periods in hours (sublethal concentrations of DM)				
	Control	24	48	72	96
Brain					
Mean±SD	0.293±0.024	0.242±0.022	0.230±0.019	0.221±0.016	0.209±0.021
% change		−17.406	−21.502	−24.573	−28.669
t-test		<0.01	<0.001	<0.001	<0.001
Gill					
Mean±SD	0.122±0.012	0.098±0.014	0.092±0.010	0.081±0.012	0.078±0.009
% change		−19.672	−24.590	−33.607	−36.066
t-test		<0.01	<0.001	<0.001	<0.001
Kidney					
Mean±SD	0.165±0.013	0.145±0.011	0.136±0.009	0.130±0.010	0.123±0.007
% change		−12.121	−17.576	−21.212	−25.455
t-test		<0.01	0.01	<0.001	<0.001
Liver					
Mean±SD	0.292±0.023	0.262±0.018	0.245±0.019	0.232±0.016	0.221±0015
% change		−10.274	−16.096	−20.548	−24.315
t-test		<0.05	<0.01	<0.001	<0.001
Muscle					
Mean±SD	0.058±0.007	0.049±0.003	0.046±0005	0.044±0.006	0.042±0.004
% change		−15.517	−20.690	−24.138	−27.586
t-test		<0.05	<0.01	<0.01	<0.001

SD: Standard deviation, DM: Deltamethrin

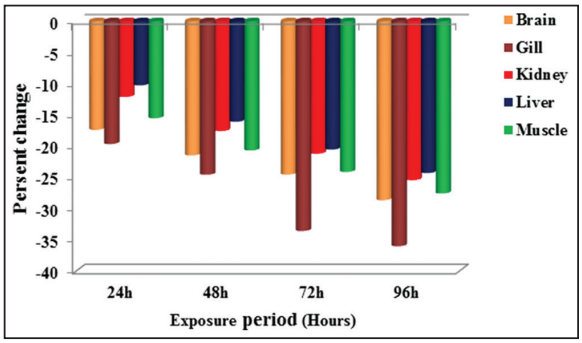

Figure 5: Percent change over control in malate dehydrogenase activity in the exposed tissue at different exposure periods to sublethal concentrations of deltamethrin.

uptake at the tissue level during DM toxicity stress. Several investigators correlated the decreased activities of TCA cycle enzymes to the changes in the integrity of mitochondria [60]. There are number of reports in the literature support of the results obtained in the present study. Tiwari *et al.* [59] was observed the decreased MDH activities in tissues of *Clarias batrachus* on exposure to endosulfan. Srinivasamoorthy [61] reported decreased MDH activity in tissue of muscle (*Lamellidens marginalis*) exposed to DM.

4. CONCLUSIONS

Exposure of *L. rohita* to sub lethal concentrations of DM has significantly altered the glycogen levels in liver, muscle, and enzymological (LDH, SDH, and MDH) responses. The present study reports that DM is a highly toxic pesticide to *L. rohita* and presence of DM even at very low concentrations in the aquatic environments may cause harmful effects on aquatic organisms. The parameters studied in this study could be used as potential biomarkers in assessing toxic effects of DM and also other pesticides. The findings of the present study can ascertain a safer level of this insecticide in the aquatic environment and protection of aquatic habitants.

5. REFERENCES

1. M. Chandola, M. Rathore, B. Kumar. (2011) Indigenous pest management practices prevalent along the hill farmers of Uttarakhand, ***Indian Journal of Traditional Knowledge***, **10(2):** 311-315.
2. S. K. Gupta, A. K. Pal, N. P. Sahu, N. Saharan, S. C. Mandal, M. S. Akhtar, C. Prakash, A. K. Prusty, (2012) Dietary microbial levan ameliorates stress and augments immunity in *Cyprinus carpio* fry (Linnaeus, 1758) exposed

to sub-lethal toxicity of fipronil, ***Aquaculture Research,*** **45(5):** 893-906.
3. J. R. Coats, (1990) Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides, ***Environmental Health Perspect,*** **87**: 255-262.
4. D. Verschoyle, W. N. Aldridge, (1980) Structure-activity relationships of some pyrethroids in rats, ***Archives of Toxicology*****, 45**: 325-329.
5. K. Haya, (1989) Toxicity of pyrethroid insecticides to fish, ***Environmental Toxicology and Chemistry*****, 8:** 381-391.
6. H. C. Pham, C. Navarro-Dalmasure, P. Clavel, G. Haverbeke, S. L. Cheav, (1984) Toxicological studies of deltamethrin, ***International Journal of Tissue Reactions,*** **6**: 127-133.
7. C. D. Tomlin, (2006) ***The Pesticide Manual: A World Compendium***, 14th ed. Farnham: British Crop Protection Council, p286-287.
8. W. J. Hayes, (1982) ***Pesticides Studied in Man,*** Baltimore, Maryland: Williams & Wilkins. p75-81.
9. M. Elliot, A. W. Farnham, N. F. Janes, P. H. Needham, D. A. Pulman, (1974) Synthetic insectide with a new order of activity, ***Nature,*** **248**: 710-711.
10. W. L. Braguini, S. M. Cadena, E. G. Carnieri, M. E. Rocha, M. B. de Oliveira, (2004) Effects of deltamethrin on functions of rat liver mitochondria and on native and synthetic model membranes, ***Toxicology Letters*****, 152:** 191-202.
11. S. K. Whyte, J. D. Westcott, D. Jimenez, C. W. Revie, K. L. Hammell, (2014) Assessment of sea lice (*Lepeophtheirus salmonis*) management in New Brunswick, Canada using deltamethrin (AlphaMax_) through clinical field treatment and laboratory bioassay responses, ***Aquaculture,*** **422-423:** 54-62.
12. S. K. Gupta, A. K. Pal, N. P. Sahu, A. K. Jha, M. S. Akhtar, S. C. Mandal, P. Das, A. K. Prusty, (2013) Supplementation of microbial levan in the diet of *Cyprinus carpio* fry (Linnaeus, 1758) exposed to sub lethal toxicity of fipronil: Effect on growth and metabolic responses, ***Fish Physiology Biochemistry*****, 39(6):** 1513-1524.
13. R. T. Ngidlo, (2013) Impacts of pesticides and fertilizers on soil, tail water and groundwater in three vegetable producing areas in the Cordillera Region, Northern Philippines, ***American Journal of Experimental Agriculture,*** **3(4):** 780-793.
14. W. W. Kilgore, (1975) Mingyuli environmental toxicology. In: ***Insecticide Biochemistry and Physiology***, New York: Olenum Press, p669.
15. R. A. Relyea, (2005) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities, ***Ecological Applications*****, 15(2):** 618-627.
16. K. Koprucu, R. Aydin, (2004) The toxic effects of pyrethroid deltamethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae, ***Pesticide Biochemistry and Physiology*****, 80:** 47-53.
17. A. Lazartigues, M. Thomas, C. Cren-Olive, J. Brun-Bellut, Y. L. Roux, D. Banas, C. Feidt, (2013) Pesticide pressure and fish farming in barrage pond in Northeastern France. Part II: Residues of 13 pesticides in water, sediments, edible fish and their relationships, ***Environmental Science and Pollution Research*****, 20(1):** 117-125.
18. T. Vani, M. Saharan, S. C. Mukherjee, R. Ranjan, R. Kumar, R. K. Brahmachari, (2011) Deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of Vitamin C, ***Pesticide Biochemistry and Physiology*****, 101**: 16-20.
19. I. Sayeed, S. Parvez, S. Pandey, B. Bin-Hafeez, R. Haque, S. Raisuddin, (2003) Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch, ***Ecotoxicology and Environmental Safety,*** **56**: 295-301.
20. N. Akerblom, (2004) ***Agricultural pesticide toxicity to aquatic organisms-a literature review***, California: Department of Environmental Assessment, Swedish University of Agricultural Sciences, Rapport.
21. M. E. de Lorenzo, G. I. Scott, P. E. Ross, (2001) Toxicity of pesticides to aquatic microorganisms: A review, ***Environmental Toxicology and Chemistry*****, 20**: 84-98.
22. M. G. Barron, K. B. Woodburn, (1995) Ecotoxicology of chlorpyrifos, ***Reviews of Environmental Contamination and Toxicology*****, 144**: 1-93.
23. R. Eisler, (1992) ***Fenvalerate Hazards to Fish, Wild Life and Invertebrates: A Synoptic Review. Contaminant Hazard Reviews, Report No. 24.*** Laurel, Maryland: US Fish and Wildlife Service, Patuxwnt Wildlife Research Centre, 20708.
24. D. J. Ecobichon, (1991) Toxic effects of pesticides. In: M.O. Amdur, J. Doull, C.D. Klaassen, (Eds.), ***Casarett and Doull's Toxicology: The Basic Science of Poisons,*** 4th ed. New York: McGraw-Hill Inc., p565-622.
25. D. J. Finney, (1971) ***Probit Analysis***, 3rd ed. London: Cambridge University Press.
26. P. L. Carpentor, (1975) ***Immunology and Serology,*** 3rd ed. Philadelphia, PA, London, Toronto: W.B. Saunders Company, p254.
27. G. H. Hogeboom, W. C. Schneider, M. J. Striebich, (1952) Cytochemical studies. V. On the isolation and biochemical properties of liver cell nuclei, ***Journal of Biological Chemistry,*** **196(1)**: 111-120.
28. N. V. Carrol, R. W. Longley, J. H. Row, (1956) Glycogeddetarmination in liver and musccle by

use of anthrone ragent, ***Journal of Biological Chemistry*****, 22**: 583-593.
29. T. N. Srikanthan, C. R. Krishnamurthi, (1955) Tetrazolium test for dehydrogenase, ***Journal of Scientific and Industrial Research*****, 14**: 206-207.
30. S. Govindappa, K. S. Swami, (1965) Electrophoritic characteristic of sub cellular components and their relation to enzyme activities in amphibian muscle bresh, ***Indian Journal of Experimantal Biology*****, 3(4)**: 209-212.
31. M. N. Nachlas, S. I. Marguiles, A. M. Seiligman, (1960) A colorimetric method for the estimation of succinic dehydrogenase activity, ***Journal of Biological Chemistry*****, 235**: 499-503.
32. D. J. Finney, (1971) ***Probit Analysis***, 3rd ed. London: Cambridge University Press.
33. WHO, World Health Organization, (1990) ***Environmental Health Criteria,*** Geneva: WHO, p97.
34. Z. Svobodova, V. Luskova, M. J. Drastichova, M. Svoboda, V. Labek, (2003) Effect of deltamethrin on haematological indices of common carp (*Cyprinus carpio L.*), ***Acta Veterinaria Brunensis,* 72:** 79-85.
35. M. Datta, A. Kaviraj, (2003) Acute toxicity of the synthetic pyrethroid deltamethrin to freshwater catfish *Clarias gariepinus*, ***Bulletin of Environmental Contamination and Toxicology*****, 70:** 296-299.
36. P. K. Mittal, T. Adak, V. P. Sharma, (1994) Comparative toxicity of certain mosquitocidal compounds to larvivorous fish, *Poecilia reticulata*, ***Indian Journal of Malariology*****, 31**: 43-47.
37. S. S. Jacob, N. B. Nair, N. K. B. Subramanian, (1982) Toxicity of certain pesticides found in the habitat to larvivorous fishes: *A. linealus* and *M. Cupanus*, ***Proceedings of the Indian Academy of Sciences Animal Sciences,* 91(3)**: 323-328.
38. K. J. Macek, W. A. McAllister, (1969) Inspecticide suscepibility of some common fish family representatives, ***Transactions of American Fisheries Society*****, 99**: 27-29.
39. A. K. Kumaraguru, F. W. H. Beamish, (1981) Lethal toxicity of permethrin to rainbow trout, *Salmo gairdneri* in relation to body weight and water temperature, ***Water Resources,* 15**: 503-505.
40. A. K. Pal, H. S. Kushwah, (1981) Apreliminary study on protective role of protein against endosulphon exposure. Symposium proceedings, ***Indian Journal of Biochemistry and Biophysics*****, 8(4)**: 4-10.
41. R. Viran, F. U. Erkoc, H. Polat, O. Kocak, (2003) Investigation of acute toxicity of deltamethrin on guppies (*Poecilia reticulata*), ***Ecotoxicology and Environmental Safety*****, 55**: 82-85.
42. M. E. Yonar, F. Sakin, (2011) Ameliorative effect of lycopene on antioxidant status in *Cyprinus carpio* during pyrethroid deltamethrin exposure, ***Pesticide Biochemistry and Physiology,* 99**: 226-231.
43. H. Tewari, T. S. Gill, J. Pant, (1987) Impact of chronic lead poisoning on the haematological and bochemical profile of a fish, *Barbus conchonius* (Ham), ***Bulletin of Environmental Contamination and Toxicology*****, 38:** 748-752.
44. R. C. Dalela, S. Ravi, V. Kumar, S. R. Verma, (1981) *In vivo* hematological alternations in a fresh water teleost Mystus following sub-acute exposure to pesticides and their combinations, ***Journal of Environmental Biology,* 2**: 79-86.
45. B. Asztolas, J. Nemasok, R. B. Gabrial, A. Szabo, J. Refarieo, (1990) The effect of pesticides on some biochemical parameters of carp *C. carpio*, ***Archives of Environmental Contamination and Toxicology*****, 19**: 257-280.
46. M. David, (1995) Effect of fenvalerate on behavioral***. Physiological and Biochemical Aspects of Freshwater Fish Labeo rohita***. Anantapur, India: Ph. D Thesis S.K. University.
47. A. S. Rani, R. Sudharshan, P. V. M. Reddy, T. N. Raju, (2000) Effect of Sodium arsenite on glucose and glycogen levels in freshwater teleost fish, *Tilapia mossambica*, ***Pollution Research*****, 19(1):** 129-131.
48. D. K. Rawat, V. S. Bais, N. C. Agrawal, (2002) A correlative study on liver glycogen and endosulfan toxicity in *Heteropneustes fossils,* ***Journal of Environmental Biology*****, 23(2):** 205-207.
49. R. K. Murray, D. K. Granner, V. W. Rodwell, (Eds.), (2006) ***Harper's Illustrated Biochemistry,*** 27th ed. Asia, Singapore: Ange Medical Publications.
50. S. I. Stalin, S. S. M. Das, (2012) Biochemical changes in certain tissues of *Cirrhinus mrigala* (Hamilton) exposed to fenthion, ***International Journal of Environmental Sciences*****, 2:** 3.
51. T. Arasta, V. B. Bais, P. Thakur, (1996) Effect of nuvan on some biochemical parameters of Indian catfish *Mystus vittatus*, ***Journal of Environmental Research,* 80:** 80-83.
52. A. L. Latner, (1975) ***Contarow and Tumer Clinical Biochemistry***, 7th ed. Philadelphia, PA: W.W. Saunders Co.
53. S. J. Young, A. Dowman, D. C. Cowell, (1999) The detection of pentachlorophenol by its inhibitory effectiveness on lactate dehydrogenase of rabbit muscle and bovine heart, ***Pesticide Biochemistry and Physiology*****, 64:** 1-8.
54. W. M. de Coen, C. R. Jansen, H. Segner, (2001) The use of biomarkers in *Daphnia magna* toxicity testing V. *In vivo* alternations in the carbohydrate metabolism of *Daphnia magna* exposed to sublethal concentrations of mercury and lindane, ***Ecotoxicology and Environmental Safety*****, 48:** 223-234.

55. S. Kossman, G. Wartalska, (1984) Activity of some enzymes in the urine of workers producing chlorinated hydrocarban pesticides, ***Medycyna Pracy***, **35**: 403-407.
56. A. U. Rani, P. Ramamurthy, (1987) Effect of sublethal concentration of cadmium on oxidative metabolism in the fresh water teleost, *Tilapia mossambica*, ***Indian Journal comparative Animal Physiology*****, 5(2)**: 71-74.
57. K. R. S. Rao, (1999) Pesticide Impact on Fish Metabolism, New Delhi, India: Discovery publishing House, p111-112.
58. A. Sweta, C. K. Pandey, G. Krishna, (2007) Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch), ***Pesticide Biochemistry and Physiology*****, 88(3)**: 268-272.
59. S. S. Kumar, S. S. Kumar, P. R. Yadav, (2010) Toxicological and biochemical alterations of cypermethrin (synthetic pyrethroids) against freshwater teleost fish *Colisa fasciatus* at different Season, ***World Journal of Zoology*****, 5(1)**: 25-32.
60. R. P. S. Tiwari, A. Singh, (2009) Changes in some biochemical parameters in the liver and muscle of *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum* mill *(*Lal kaner*)* latex, ***Natural Product Radiance*****, 8(1)**: 48-54.
61. R. Camber, M. A. Dianzani Mor, (1962) Mitochandrial biochemical damage in parathion, ***Folia Medica,*** **45:** 229.
62. K. Srinivasamoorthy, (1983) ***Modulatio of Carbohydrate and Associated Metabolism in the Selcted Tissue of Marine Prawn Penaeus indicus During Induced Methyl Parathion Stress***, Tirupathi, India: Ph.D Thesis. S. V. University.

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