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SDH, MDH AND LDH ENZYMATIC ACTIVITY IN FISH *LABEO ROHITA* EXPOSED TO BIFETHRIN 10% EC

[1]V.CHAITHANYA KUMARI, [2]ANITHA SMRUTHI CH AND [3]V.VENKATARATHNAMMA

Research scholar[1,2] Department of Zoology and Aquaculture

Acharya Nagarjuna University, Guntur-522510. A.P., India

ABSTRACT

Now a days synthetic pyrethroids are widely available in the field of agricultural sector to achieve the highest yield. Among the pyrethroids bifethrin has good persistence and insecticidal activity. Bifethrin enters through different routes into aquatic organisms and then accumulate in various vital organs of fresh water fish *Labeo rohita* which ultimately reaches the humans through food chain and shows its adverse effects. SDH, MDH and LDH are the enzymes secreted in the organisms for the oxidation of glucose molecules and for the liberation of usable chemical energy in the form of ATP. In the present study test fish *Labeo rohita* was exposed to sub lethal concentrations of bifethrin for different exposure periods i.e for 24hrs, 5days and 10 days to evaluate the enzymatic activity of Succinate Dehydrogenase(SDH), Malate Dehydrogenase(MDH) enzymes which participate in aerobic respiration and Lactate Dehydrogenase (LDH) enzyme which participate in the anaerobic respiration in various organs of fish such as brain, gill, liver, kidney and muscle. In this case study the levels of the enzyme LDH was elevated due to the requirement of quick energy resource (ATP) for the organism in all exposure periods whereas the enzymatic activity of SDH and MDH were depleted in all the tissues of the experimental fish *Labeo rohita* because of the reason that it takes time to oxidize glucose molecule in aerobic respiration. Under the exposure of Bifethrin in Sub-lethal concentrations on the10thday maximum percentage of elevation of LDH was observed in liver(31.57%) and minimum percentage of elevation of LDH was observed in muscle (18.36%) . On 10th day , the maximum percentage of depletion of SDH was seen in kidney (-30.77%), minimum depletion of SDH was observed in brain (-16.90%), MDH level was maximum in liver (-22.78%) and minimum in kidney (-17.02%) was observed when all the exposures were compared with controls.

KEY WORDS: pyrethroid, bifethrin, *Labeo rohita,* mitochondria, LDH, SDH, MDH and Enzymatic activity.

INTRODUCTION:

The use of chemicals in the sector of agriculture has got increased in today's world to achieve the highest yield. Pesticides which are used in pest control are responsible for physiological and biochemical changes in the fresh water organisms and it influences the activities of several enzymes and metabolites. Fresh water fishes which gets exposed to pesticides for even a short span of time might get affected physiologically and enzymes are very much affected by pesticidal toxicity because they are sensitive in nature. All the enzymes are metabolic fuels in the biological systems. The information on the sub lethal effects of xenobiotic compounds on certain antioxidant enzyme activities such as LDH (Lactate Dehydrogenase), SDH (succinateDehydrogenase), MDH (Malate Dehydrogenase) are very important to measure the damage of tissue and organ damage due to pesticidal toxicity. Lactate dehyrogenase is an enzyme that transfers a hydride from one molecule to another and it is seen more in blood and heart tissues, which are released during tissue damage. It's presence is the common marker of injuries and diseases. LDH and MDH isoenzymes are major stress related enzymes found in fishes (Neelanjana et al., 2017).Enzyme bioassays how ever remains as a useful technique in looking for sublethal effects of toxic pollution. It may also provide an early warning signal in stressed organism (Folmar, 1993). Changes in enzyme activity because of cell death, increases or decreases the enzyme production. Changes in enzymes profiles are important toxicity indices. Maintenance of Internal homeostasis through biochemical processes in the Kreb's cycle may be reflected by varying in the levels of the enzymes due to cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs (Heath, 1991). Hence this study is aimed to find out the biochemical changes in *Labeo rohita* exposed to sublethal concentrations of Bifethrin 10% EC in different exposure periods.

MATERIALS AND METHODS

The freshwater fish *Labeo rohita* were collected from Kuchipudi, Guntur District. They were brought in plastic containers and acclimatized to the laboratory conditions for 15days in large plastic tanks with unchlorinated ground water at a room temperature of $28+2^0$ C prior to experimentation. LC_{50} for 96hrs was found out by using probit method(Finney, 1971). For biochemical studies fishes were exposed in sublethal concentration for the periods of 24hrs, 5days and 10 days. After completion of exposure periods tissues like gill, brain, liver, muscle and kidney were separated and preserved in ice cold solution. The enzymatic activity of LDH was estimated by the method of (Srikanthan and Krishnamurthy, 1955). Enzymes such as SDH and MDH were estimated by the method of (Nachlas, 1960) with slight modifications.

RESULT AND DISCUSSION

The study on the biochemical changes in fish has become a prominent tool for monitoring environmental exposureto contaminants. The calculated values for *Labeo rohita* exposed to sublethal concentrations i.e., (1/10th of the lethal concentration (2.2µg/l) of Bifethrin 10% EC, the activities of the enzymes LDH, SDH and MDH along with standard deviation and percent change over control is given in Table1, Table 2, Table 3 and it is represented graphically in Fig1, Fig2, Fig 3. In the present study it has been noticed that the LDH level was elevated whereas SDH and MDH levels were depleted in the tissues of liver, muscle, kidney, gill and brain in test fish *Labeo rohita* which were been exposed to Bifethrin 10% EC, in sublethal concentrations for 24hrs, 5days and 10 days and are compared with controls. The levels of LDH, SDH and MDH has got decreased in all the tissues compared to control.

Liver is the vital organ of carbohydrate metabolism. Carbohydrate metabolism is mainly concerns with the fulfilment of energy demands in animals by undergoing aerobic and anaerobic segment processes. Higher glycogen content was observed in liver. This is mainly due to the involvement of liver in glycogen synthesis and utilization. Glycogen is the major storage form of energy in liver and muscle. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis within the muscle itself (Lehninger principles 2008). Though brain tissue is metabolically active, lower levels of glycogen content is observed because it lacks the potentiality to store glycogen and it depends on blood glucose for all its metabolic activities. As per the above explanation it is observed that, Carbohydrates are the primary and immediate sources of energy in stress condition. These carbohydrate reserves get depleted to meet the energy demand. Hence the depletion of glycogen levels due to direct utilization for the energy generation and the demand is caused by pesticidal stress (Tilak K.S, et al., 2009).Total depletion of glycogen would result in the disruption of the enzymes which are associated with carbohydrate metabolism (kamalaveni, k. et al., 2001). So the inhibition of the enzymes LDH, SDH and MDH is observed and it indicates that pesticides significantly inhibits aerobic, as well as anaerobic metabolism in exposed organism. It is observed that the level of deprovement in MDH is seen in the tissues in the order of Liver>Brain> Gill> Kidney > muscle . From all these studies, it is evident that Bifethrin has an effect on the oxidative metabolism in test fish *Labeo rohita* even at sublethal concentrations.

Lactate Dehydrogenase activity(LDH):

Lactate Dehydrogenase (LDH) is a hydrogen transferring enzyme which catalysed the oxidation of L-Lactate to pyruvate in the mediation of NAD^+ which acts as hydrogen acceptor. The enzyme LDH plays an important role in Carbohydrate metabolism for the production of body energy. Its is terminal enzyme of anaerobic glycolysis and found in almost all the tissues. Tissues release LDH into the blood stream if there is any damage or disease. So the level of increased LDH in the blood provides us direct or indirect source to

understand the cellular damage. Normal activities of LDH patterns were altered in the test fish due to chemical stress that leakage of LDH is the marker of membrane permeability or cell death. Disturbances in their catalytic process due to xenobiotic compounds can cause cellular homeostasis affecting different enzymatic systems, which leads to show effects at higher levels of biological organization such as tissues, organs or individuals (Orrego et al., 2011). It has crucial importance in the muscular physiology, particularly in the conditions of chemical stress, when high levels of energy may be required in a short period of time(Baghi M et al.,1995).LDH is released from tissues of liver, lungs, kidney, muscle and heart after the cellular damage(Coppola J.A et al.,2002).Disruption of respiratory epithelium might have caused tissue hypoxia resulting in a decrease in oxidative metabolism which may be responsible for increase in LDH activity in toxicant stress(Gill TS et al).Any change in protein and Carbohydrate metabolism may cause change in the LDH activity (Abston PA et al.,1976). The activity of LDH was highly elevated due to profenofos and carbosulfan exposure in the tissues of fish *Labeo rohita* indicating that the anaerobic respiration arrived and aerobic respiration inhibited so as to meet the increased metabolic stress and to overcome the toxic stress(Bantu et al., 2017). A similar elevation of LDH activity was observed in the tissues of fish *Labeo rohita* on exposure periods of sublethal concentrations of Deltamethrin (Mohan et al., 2017). LDH activity was increased after exposure to *Colisafasciatus* in lethal concentration of cypermethrin (Shailendra et al., 2010).

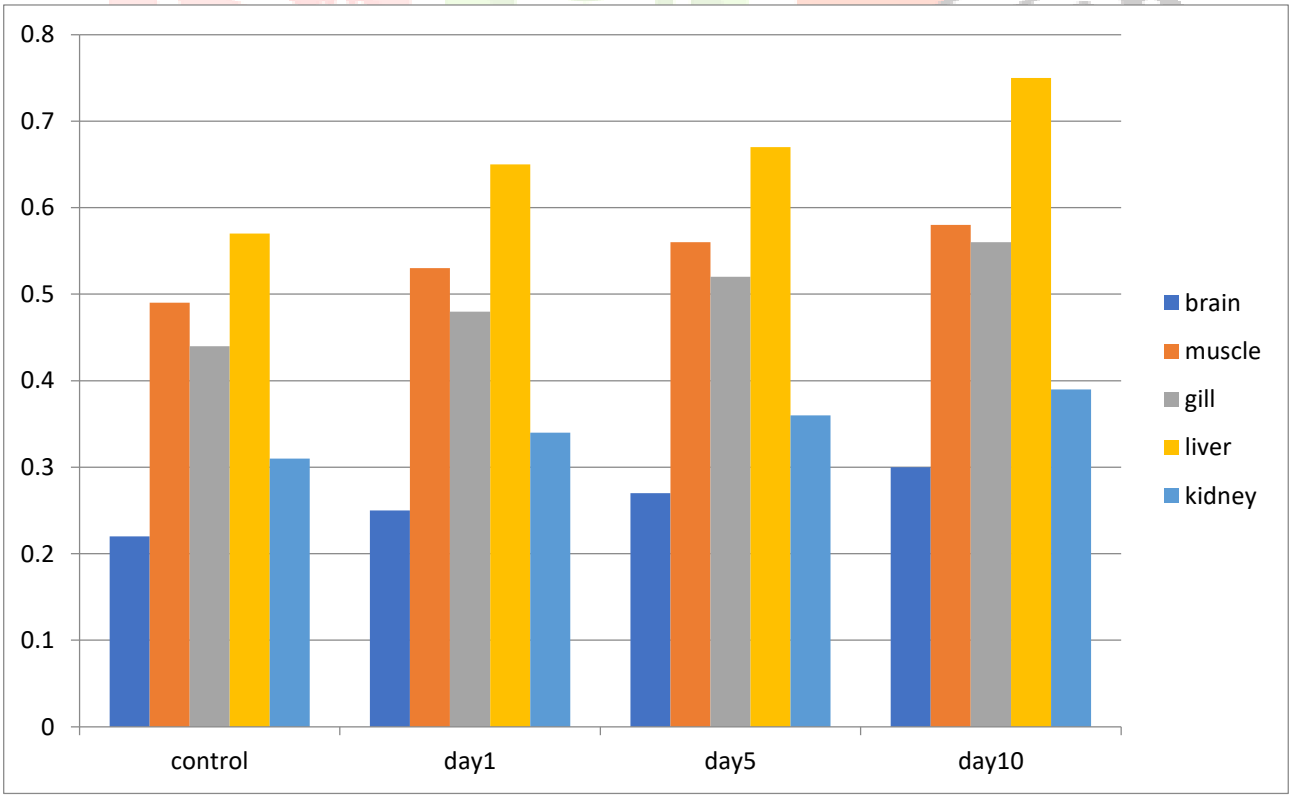
In the Present study tissues of control fish, *Labeo rohita* LDH was observed in the order: Liver > Muscle > Gill > kidney > Brain for the periods of 24hrs, 5days and 10days. The activity levels of Lactate Dehyrogenase in *Labeo* rohita exposed to Bifethrin was expressed as micro moles µ of formazan/mg protein/hr. In the present study the LDH levels were elevated in the tissues of the test fish when exposed to Bifethrin in Sub-lethal concentrations were compared with controls in (Table 1 and Fig 1).

Table 1. Change in the Specific activity levels of LDH (µ molesof formazan/mg protein/h) and % change over the control in differenttissues of *Labeo rohit*on exposure to sub lethal concentrations of Bifethrin (10% EC)

Tissue	Control	Exposure period (days)					
		1 day		5 days		10 days	
		Sub lethal	% change	Sub lethal	% change	Sub lethal	% change
Brain	0.22	0.25 ±0.02	13.63	0.27 ±0.01	22.72	0.30 ±0.01	36.36
Muscle	0.49	0.58 ±0.01	8.16	0.56 ±0.01	14.28	0.58 ±0.03	18.36
Gill	0.44	0.48 ±0.01	9.09	0.52 ±0.01	18.18	0.56 ±0.01	27.27
Liver	0.57	0.65 ±0.02	14.03	0.67 ±0.03	17.54	0.75 ±0.02	31.57
Kidney	0.31	0.34 ±0.01	6.45	0.36 ±0.01	16.12	0.39 ±0.01	25.80

The Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. The Values are significant P< 0.05.

Fig.1. Change in LDH activity (µ moles of formazan/mg prote in/hr) in different tissues of *Labeo rohita* on exposure to sub lethal concentration of Bifethrin (10% EC).



In the present study the significant alterations were observed in LDH activity, it specifies that the damage of the organs producing particular enzymes either liver or kidney, because LDH is an important glycolytic enzyme and is present in all the body tissues. Increasing with ammonia concentration, there was progressive increase in LDH activity in gill, liver, kidney and brain of the exposed fingerlings *Cirrhinusmrigala,* and this might be due to induced stress and increase in the rate of glycolysis and the pyruvate is not routed to Kreb's cycle, rather catalyses to lactate; there by shifting the respiratory metabolism from aerobiosis to anaerobiosis (Das et al., 2004). it was observed that the LDH activity in the freshwater fish *Ctenopharyngodon idella* under the exposure to sublethal concentration of λ-cyhalothrin was elevated which indicates that the increased anaerobic respiration so as to meet the energy demands where aerobic oxidation is lowered. It suggests that aerobic catabolism of glycogen and glucose has shifted towards the formation of lactate, which might show adverse impact on the organism (Rajeshwari G et al., 2020). The increased LDH activity in brain and liver tissues of *Labeo rohita* treated with cypermethrin for 96hrs (Das and Mukherjee., 2003). All the above findings supports the present study that LDH is associated with cellular metabolic action, particularly in conditions of chemical exposure and stress when high levels of energy required in a short period of time. So, the levels of LDH also increases in the tissues of the experimental organism which acts as the mechanism to over come the toxic stress.

succinate Dehydrogenase (SDH) activity

Succinate Dehydrogenase catalyses the oxidation of Succinate into fumarate in the Kreb's cycle and can be found in the inner mitochondrial membrane. These enzymes which are present inside mitochondria converts the energy from food into a form the cell can use. With in the mitochondria, the SDH enzyme links two important cellular pathways in energy conversion: citric acid cycle and oxidative phosphorylation. Respiratory chain is inhibited in the absence of this enzyme and cause for the cessation of oxidative process. In controls, SDH activity was more in liver > muscle > kidney > gill > brain. When compared to their respective controls the calculated values of Succinate dehydrogenase activity and the percent change over control along with standard deviation are given in Table 2 and Figure 2. The calculated values of SDH and standard deviation along with percent change over the controls is tissue specific like brain, liver, muscle, gill and kidney of fish *Labeo rohita* exposed to sublethal concentrations of Bifethrin 10% EC for 10 days is in the order of kidney > Liver > gill > muscle > brain. It has been observed that the level of SDH in exposed fish has got decreased gradually when compared with controls from day 1-day 10. The SDH value is maximum in liver (-15.95 % change) and minimum in brain (-7.04 % change) For 24hrs period of exposure where as it is maximum in kidney (-21 % change) and minimum in brain (-12.68 % change) for the period of 5days exposure. The SDH value is maximum in kidney (-30.77 % change) and minimum is in brain (-16.90 % change) For 10 days period of exposure. Higher activity of SDH in liver and muscle suggests higher distribution of mitochondria in these tissues, since SDH is mitochondrial localized enzyme (MichMichaelM.Cox). Similar decrement in the SDH activity was also observed by the various researchers in

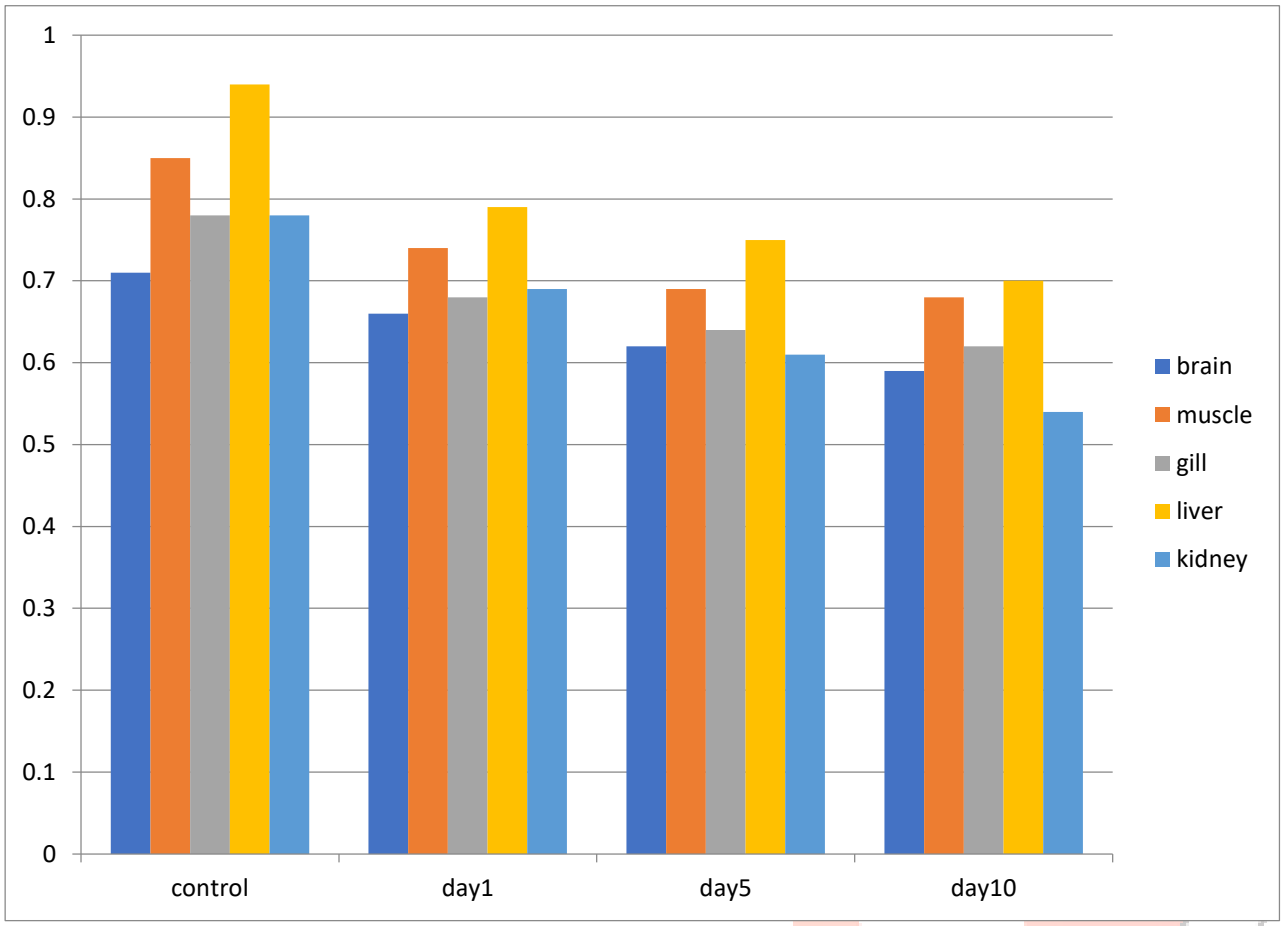
different species of the fish exposed to different pesticides. Due to pesticidal stress SDH oxidative enzyme was significantly affected and It indicates the degree of impairment caused to the respiratory metabolism in the tissues of the test fish *Labeo rohita*. As SDH is also an enzyme of significance in oxidative metabolism of cell, any alteration in its activity is possible to upset the co-ordination of TCA cycle and other metabolic pathways (Lehninger, 1993). Radhakrisnanh et al., (1992) offer a strong support for the present observations. The general decrease in SDH activity during pesticides stress was associated with the inhibition of mitochondrial respiratory mechanism or dearrangement in ultra structure, architectural integrity and permeability of mitochondria (Tripathi, G et al., 2004). This hinders the transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity and shifting the aerobic metabolism to anaerobiosis (Shailendra kumar singh et al., 2010.)

Table 2. Change in the Specific activity levels of SDH (µ moles of formazan/mg protein/hr) and % change over the control in different tissues of *Labeo rohita* on exposure to sub lethal concentrations of Bifethrin (10% EC).

Tissue	Control	Exposure period (days)					
		1 day		5 days		10 days	
		Sub lethal	% Change	Sub lethal	% Change	Sub lethal	% change
Brain	0.71 ±0.01	0.66 ±0.01	-7.04	0.62 ±0.01	-12.68	0.57	-16.90
Muscle	0.85 ±0.03	0.74 ±0.05	-10.58	0.69 ±0.03	-18.82	0.68	-20.00
Gill	0.78 ±0.03	0.68 ±0.02	-12.82	0.64 ±0.04	-17.94	0.62	-20.51
Liver	0.94 ±0.01	0.79 ±0.01	-15.95	0.75 ±0.02	-20.21	0.70	-25.53
Kidney	0.78 ±0.02*//	0.69 ±0.06	-11.53	0.61 ±0.01	-21.79	0.54	-30.77

The Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. The Values are significant P< 0.05.

Fig.2. Change in SDH activity (µ moles of formazan/mg protein/hr) in different tissues of *Labeo rohita* on exposure to sub lethal concentration of Bifethrin 10%EC.



So, It is observed that the level of SDH decreases in tissues of organisms which are under chemical stress. There is a rapid depletion in SDH activity in all tissues of fish *Labeo rohita* treated with sublethal and lethal concentrations of profenofos and carbosulfan due to pesticidal stress was associated with the inhibition of mitochondrial respiratory mechanism of dearrangement on ultra-structure, architectural integrity and permeability of mitochondria (Bantu et al., 2017).

MalateDehydrogenase (MDH) activity:-

Malate dehydrogenase is an NAD dependent enzyme And it helps in the conversion of malate to oxaloacetate and also catalyses the reversible oxidation of fumarate to malate. It exists in two isozymic forms: (a) mitochondrial (b) cytosolic. This enzyme not only converts malate to oxaloacetate but also plays a prominent role in fixing CO_2 and in gluconeogenesis (Lehninger, 2008).

Malate +NAD^+---------- Oxaloacetate +$NADH^+$+ H^+

Any alterations in mitochondrial structure inhibits the activity of MDH.Most of the TCA cycle enzymes are of mitochondrial origin and if any structural change in these enzymes induced by the pesticide might influence their activity levels. Pesticides are known for their effect on mitochondrial structure which in turn alters the enzyme activities associated with it (Venkata

Rathnamma et al., 2008). Tissue of control fish, of *Labeo rohita* MDH activity was in the order of: Liver > Muscle > Gill > Brain > kidney. The MDH value is maximum in gill (-8.77 % change) and minimum in brain (-2.22 % change) For 24hrs period of exposure where as it is maximum in liver (-12.65 % change) and minimum in brain (-6.66 % change) for the period of 5days exposure. The SDH value is maximum in liver (-22.78 % change) and minimum is in brain (-17.02 % change) For 10days period of exposure. In the Present study, the MDH activity was depleted in all the tissues of test fish exposed to Bifethrin for 1day, 5days and 10 daysin sublethal concentrations and were compared with controls and the percentage change is noted down in the Table 3 and Fig. 3.

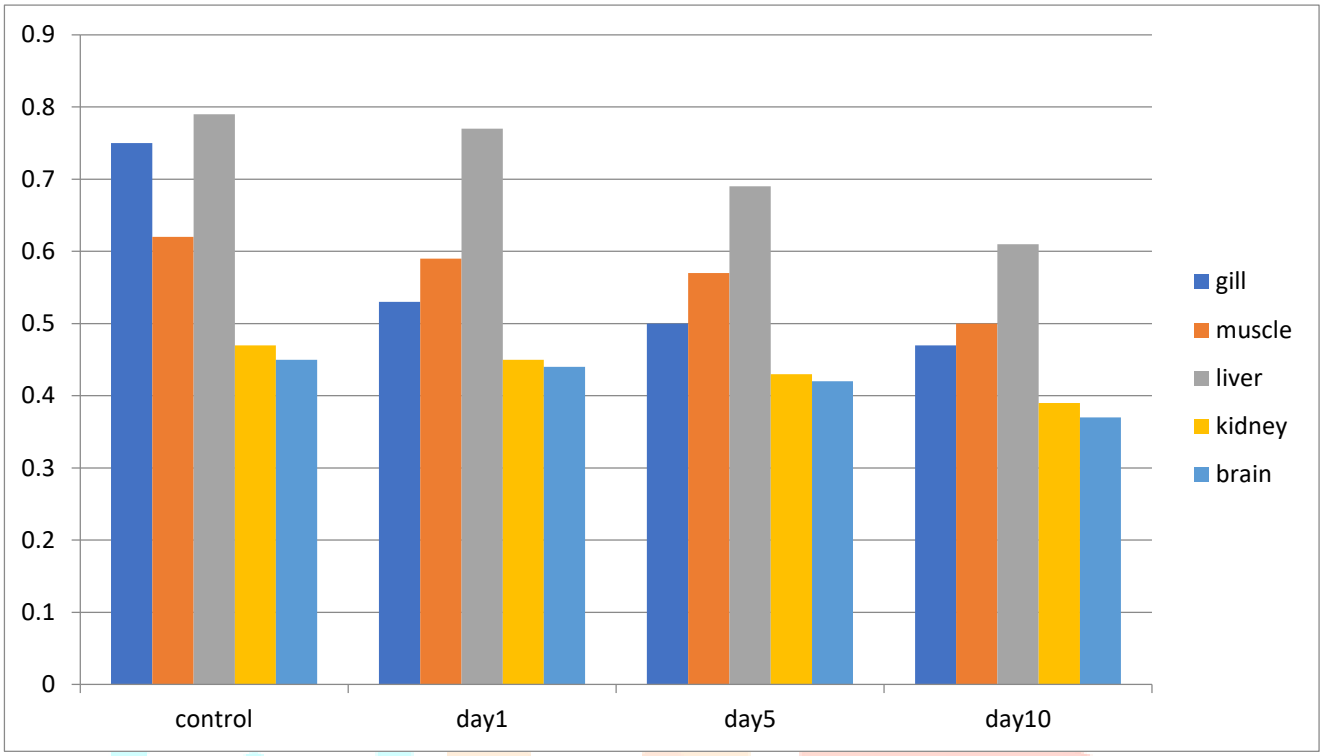
The decrease in the levels of malate dehydrogenase activity is similar to the decreased activity as encountered for SDH. The gradual decrease in the malate dehydrogenase activity is in line with the decrease of succinate dehydrogenase activity which indicates us the suppressed oxidative metabolism, which in turn lowers fumarate-malate conversions. Similarly the decrement in MDH activity was observed in the tissues of fish *Labeo rohita* on exposure periods of sublethal concentrations of Deltamethrin, which suggests that the lower level of functioning of Krebs cycle due to inadequate supply of substrate or decreased oxygen uptake at the tissue level during toxicant toxicity stress (Mohan et al., 2017).

Table 3. Change in the Specific activity levels of MDH (µ moles of formazan/mg protein/hr) and % change over the control in different tissues of *Labeo rohit*on exposure to sub lethal concentrations of Bifethrin (10% EC).

Tissue	Control	Exposure period (days)					
		1 day		5 days		10 days	
		Sub lethal	% Change	Sub lethal	% change	Sub lethal	% change
Gill	0.57 ±0.02	0.53 ±0.02	-8.77	0.50 ±0.02	-12.2	0.47 ±0.01	-17.54
Muscle	0.62 ±0.04	0.59 ±0.02	-4.83	0.57 ±0.01	-8.06	0.50 ±0.01	-19.35
Liver	0.79 ±0.01	0.77 ±0.01	-2.53	0.69 ±0.01	-12.65	0.61 ±0.02	-22.78
Kidney	0.47 ±0.01	0.45 ±0.03	-4.25	0.43 ±0.01	-11.11	0.39 ±0.01	-17.02
Brain	0.45 ±0.02	0.44 ±0.01	-2.22	0.42 ±0.02	-6.66	0.37 ±0.01	-17.78

The Results are the mean values of five observations and the Standard Deviation is indicated as ± and figures in % changeover control and sub lethal respectively. The Values are significant P< 0.05.

Fig.3. Change in MDH activity (µ moles of formazan/mg protein/hr) in different tissues of *Labeo rohita* on exposure to sub lethal concentration of Bifethrin 10%EC.



In the present finding the decreased MDH activity might suggest the lower level of functioning of TCA cycle due toinadequate supply ofsubstrate or decreased oxygen uptake to the tissue in the test fish *Labeo rohita* during the exposure of Bifethrin toxicity stress.

CONCLUSION

In the present finding it has been revealed that the synthetic pyrethroid Bifethrin 10% EC exposed in sub lethal concentrations for the period of 24hrs, 5days and 10days caused toxicity which has resulted in metabolic impairment in the experimental organism *Labeo rohit.* It has been observed that the activity of the enzyme LDH has got increased due to the requirement of immediate oxygen when exposed to the toxicant . Similarly the levels of the respiratory enzymes such as SDH and MDH has got decreased gradually due to the cessation of the aerobic respiration and shifting towards the anaerobic respiration due to the pesticidal stress. This might be the mechanism by which the fish overcomes toxic stress. Hence the present study indicates that the enzymatic activities can be potential tool for the Study of alterations in the carbohydrate metabolism in the fish *Labeo rohita* when exposed to the test chemical Bifethrin 10% EC in sublethal concentrations.

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