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Evaluation of Lactate Dehydrogenase on exposure to Endosulfan and Fenvalerate by using Native-PAGE

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Abstract:

Pollution of the aquatic environment is a serious and growing problem. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment having led to various deleterious effects on the aquatic organisms including fish. In the present study, freshwater fish Labeo rohita was exposed to two pesticides i.e., Endosulfan(35% EC) an organchlorine and Fenvalerate(20% EC) a synthetic pyrethroid. The LC_{50} values determined for endosulfan and fenvalerate at 24 h were 0.6876, 0.4749 μgL^{-1} respectively. The $1/10^{th}$ of 24 h LC_{50} of both the pesticides was selected as sublethal concentrations. LDH is a key enzyme in carbohydrate metabolism and occurs virtually in all tissues. It is indicative of variation in tissue functioning as a consequence of presence, increase or decrease in the concentration of the toxicant. The fish were exposed to sublethal concentrations for 8 days and expression of lactate dehydrogenase isoenzymes in different tissues were determined by using native gel electrophoresis.

Key words: Endosulfan, Fenvalerate, Lactate dehydrogenase and *Labeo rohita*

Introduction

Pesticides play an important role in modern agriculture by providing dependable, persistent and relatively complete

control against harmful pests with less expense and effort but also they have, no doubt, increased crop yields by killing different types of pests, which are known to cause substantial or total crop damage. On the other hand, these chemicals are considered as potent pollutants of the water environment with undesirable effects on non-target organisms such as fish and water animals (Atamanalp and Yanık, 2001).

Endosulfan(6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-odioxa-thiepin-3-oxide) is a chlorinated cyclodiene insecticide which acts as a contact poison in a wide variety of insects and mites (Naqvi and Vaishnavi, 1993). Endosulfan is particularly toxic to fish as indicated by acetyl cholinesterase inhibition in bluegill sun fish (Dutta and Arends, 2003).

Synthetic pyrethriods are another type of pesticides; these are also toxic to aquatic organisms. Fenvalerate is the most widely used compound of the synthetic pyrethroid pesticides and is registered for use in agriculture to protect a wide variety of crops including cotton, soybeans, corn, vegetables, apples, peaches, pears and nuts from insect pests (Casida and Quistad, 1998). In India, the pesticide is used primarily to control pests of cotton and vegetables (Madan et al. 2000). The pesticide enters aquatic ecosystems through various routes and poses a risk to many non-target aquatic organisms, particularly those inhabiting water bodies adjacent to agricultural fields. Although synthetic pyrethroids have been claimed as safe and environmentally friendly because of their selective toxicity to insects, low persistence and low toxicity to mammals and birds, they are highly toxic to a number of other non-target organisms including fish, lobster, shrimp, mayfly nymphs and many species of zooplankton (Oudou et al. 2004)

Lactate dehydrogenase (EC 3.1.1.27) is one of the chief enzyme of carbohydrate metabolism which catalyses the oxidation of lactate and reduction of pyruvate during anaerobic glycolysis. It is a tetrameric molecule consists of two separate

loci which code for A and B subunits of this enzyme. The A and B subunits indiscriminately associate and form five tetrameric isozymes (A4, A3B1, A2B2, A1B3 and B4) (Fujio and Kaneko, 1980). Isozymes are multiple forms of a single enzyme, which often have different isoelectric points and therefore can be separated by electrophoresis. Electrophoretic studies were done extensively on the different tissues of various animals from which it reveals that the enzyme exhibit in multi molecular forms and functions (Markert and Moller, 1959). LDH electrophoretic patterns could help in investigating and to locating the pesticide stress. Stress reflects on respiratory metabolism as LDH is a key enzyme in carbohydrate metabolism and occurs virtually in all tissues. It is indicative of variation in tissue functioning as a consequence of presence, increase or decrease in the concentration of the toxicant (Jyothirmayee et al. 2005)

The aim of this work was to evaluate the expression of lactate dehydrogenase isoenzymes in different tissues of fresh water fish *Labeo rohita* after exposure to sublethal concentrations of endosulfan and fenvalerate for eight days by using native gel electrophoresis.

Material and Methods

The freshwater fish *Labeo rohita* (Hamilton) is an edible and commercially valuable fish. Live fish of size 6-7 ±1cm and 6-8 g weight were brought from a local fish farm and acclimatized at 28 ± 2 ^{0}C in the laboratory for one week. The stock solutions for Endosulfan 35% Emulsifiable Concentrate (EC) and Fenvalerate 20% Emulsifiable Concentrate (EC) were prepared in 95% acetone to yield a concentration of 100mg/100ml which were further diluted with distilled water to get a working solution. The water used for acclimatization and conducting experiments was clear unchlorinated ground water. In each test ten fish were introduced in toxicant glass chambers with a

capacity of ten liters. The data on the mortality rate of fish was recorded. The dead fish were removed immediately. The toxic tests were conducted to choose the mortality range from ten percent to ninety percent for 24 hrs in static tests. The concentration that produced fifty percent mortality in test species noted. LC_{50} values were calculated by Finney's Probit analysis (1971).

Lactate dehydrogenase by Native PAGE

Native polyacrylamide gel electrophoresis was performed to analyze LDH. Electrophoresis was carried out at 4°C for 3 h, applying a current of 100 mV. The procedure for LDH staining was based on method of Worthington with modifications (Mishra and Shukla, 1999). The staining solution for LDH comprised 0.1M Tris HCl buffer (pH-8.4), 1 mg ml^{-1} adenine dinucleotide (NAD^+), 0.5 mg ml^{-1} Nitro blue tetrazolium (NBT), 0.1 mg ml^{-1} Phenozine metho sulphate (PMS) and 0.05M lithium lactate.

Results and Discussion:

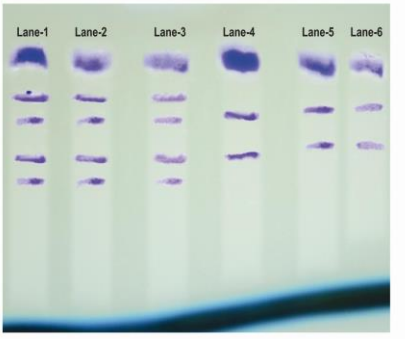
Lactate dehydrogenase enzyme in fish has always been the subject of much attention (Coquelle et al. 2007). It is to be noted that, not much information was available with the changes in the expression of LDH on toxicity with endosulfan and fenvalerate. The aim of this work was to evaluate the expression of lactate dehydrogenase isoenzymes in different tissues of fresh water fish *Labeo rohita* after exposure to sublethal concentrations of endosulfan and fenvalerate for eight days by using native gel electrophoresis.

The LDH profile in the gill and kidney tissues of control and exposed tissues Fig- I, lane 1- 6. The gill showed three bands in control, endosulfan exposed and fenvalerate exposed (lane 1-3), amongst all lanes, lane 1 was the most intense. All

bands in pesticide exposed tissues showed decrease in intensity when compared to control. The kidney showed two bands (lane 4-6) in control, endosulfan exposed and fenvalerate exposed, amongst all lanes, lane 4 was the most intense. All bands in exposed tissues showed decrease in intensity when compared to control. The difference of the distribution of LDH isoenzymes in tissues is known to reflect differences in their metabolic activity.

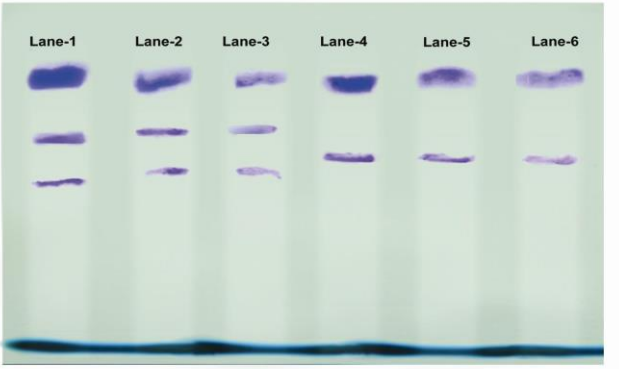
The LDH profile in the muscle and liver tissues of control and exposed tissues was showed in Fig- II, lane 1-6. The muscle showed five bands in control, endosulfan exposed and fenvalerate exposed (lane 1-3), amongst all lanes, lane 1 was found to be most intense. All bands in exposed tissues showed decrease in intensity when compared to control. The liver showed three bands (lane 4-6) in control, endosulfan exposed and fenvalerate exposed, amongst all lanes, lane 4 was the most intense. All bands in exposed tissues showed decrease in intensity when compared to control.

Fig-1: Changes in the activity of Lactate Dehydrogenase in Liver and Muscle Tissues of *Labeo rohita* on Exposure to Sublethal concetrations of Endosulfan and Fenvalerate for 15 Days by Native-PAGE



Lane-1 Control Muscle
Lane-2 Endosulfan Muscle
Lane-3 Fenvalerate Muscle
Lane-4 Control Liver
Lane-5 Endosulfan Liver
Lane-6 Fenvalerate Liver

Fig-2 : Changes in the activity of Lactate Dehydrogenase in Gill and Kidney tissues of *Labeo rohita* on Exposure to Sublethal concetrations of Endosulfan and Fenvalerate for 15 Days by Native-PAGE



Lane-1 Control Gill **Lane-4 Control Kidney**
Lane-2 Endosulfan Gill **Lane-5 Endosulfan Kidney**
Lane-3 Fenvalerate Gill **Lane-6 Fenvalerate Kidney**

Jyothirmayee *et al.* (2006) had done polyacrylamide gel electrophoresis for endosulfan induced changes in LDH pattern in freshwater fish *Anabas testundineus* and *Clarias batrachus.* The protein subunits showed decreasintrend in intensity of all the fractions throughout the exposure period demonstrating an inhibitory effect of endosulfan on kidney and muscle LDH. The differing inhibition of the LDH isoenzyme activity in muscle and in the bovine heart tissues of rabbits were studied by Young et al. (1999) after exposure to pentachlorophenol. An elevated level of LDH was observed in brain and liver tissues of lymphoma bearing mice by using Native-PAGE (Brij Bharti and Rajinikant mishra, 2008).

Alterations in the LDH isoenzyme of tissues induced by toxics reflect a metabolic cellular dysfunction of these tissues (Ribeiro et al. 1999; Arai et al. 2003; Tripathi and Verma, 2004). Analysis of each tissue revealed characteristic changes in

LDH isoenzyme patterns indicating organ-specific tissue damage. Ramesh et al.(1991) observed characteristic changes in LDH isoenzyme patterns *invivo* exposure to carbofuran.

Conclusion:

In the present study, all the pesticide samples showed a steady decreasing trend in intensity compared to control, demonstrating decreased activity of LDH on exposure to endosulfan and fenvalerate. The decrease in intensity was more in fenvalerate exposed tissues than endosulfan due to more pesticide stress.

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