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CYHALOTHRIN INDUCED BIO-CHEMICAL ALTERATIONS IN THE GRASS CARP *CTENOPHARYNGODON IDELLUS* (VALENCIENNES)

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ABSTRACT
The fresh water fish *Ctenopharyngodon idella* (Valenciennes) was exposed to Cyhalothrin a synthetic pyrethroid insecticide commercial grade 2.5% EC, which was used extensively in this region and the acute toxic effects were studied. The static and continuous flow-through LC_{50} values obtained for 24, 48, 72 and 96 h were 0.0589, 0.0574, 0.0556 and 0.0540 ppm respectively for commercial grade cyhalothrin whereas for continuous flow-through the acute LC_{50} values for commercial cyhalothrin were 0.0548, 0.0519, 0.0519 and 0.0493 respectively. Among the obtained above LC_{50} values, the 96 h LC_{50} values were taken as lethal and $1/10^{th}$ of 96 h LC_{50} Value was taken as sub-lethal concentration. The test fish *Ctenopharyngodon idella* was exposed to lethal and sub-lethal concentrations of commercial (EC) grade for 24 and 96 h and the bio-chemical changes such as glycogen, proteins and nucleic acids in the vital organs *viz*; Gill, brain, liver, muscle and kidney were estimated. The LC_{50} values indicate that the insecticide cyhalothrin commercial grade is highly toxic to the fish. The changes in bio-chemical constituents were also significant. The data obtained in all were discussed with the available literature.

KEYWORDS: Cyhalothrin, grass carp, Sub-lethal, Bio-chemical, and Continuous-flow.

INTRODUCTION
The tremendous growth in industrial production and the consequent improvement in the standard of living have provoked a worldwide discussion on environmental quality. To meet the needs of ever increasing population, it has become imperative for the man to reduce crop and stored grain losses by pests. One integral part of this is to resort to increased use of pesticides to reduce agricultural loss. All these anthropogenic chemicals eventually find their way into the aquatic environment, where they may prove to be toxic to many non-target organisms. The environmental persistance of organochlorines (new era pesticides) has led to the invention of less persistent pesticides like organophosphate, carbamate and synthetic pyrethreoids.

The problem of pesticidal impact on the ecosystem has assumed considerable proportions owing to the modernization of agricultural operations and the consequent widespread and indiscriminate permeation of the ecosystem with these pesticides. The effects of pesticides on aquatic fauna, particularly fishes, may be exhibited' in a variety of ways, since the majority of them are non-selective and produce detrimental and sometimes fatal side effects on non-target specie. As a consequence, the structure and functions of communities, eco-system and populations are altered. The reproductive potential, growth and other physiological activities, like behavior, hormonal secretions, thermo-regulation etc., will be changed. Among the pesticides of the three classes organochlorines, organophosphates and carbamates, organochlorines are more persistent Murty, (1986); O' Brien (1967); and Edwards (1973). But all the three are having more mammalian toxicity and hence, it has necessitated banning or restricting on their use and also attention was focused to synthesize new molecules (Veeraiah, 2001). As a consequence several new generation pesticides were introduced, for the last four decades.

It is apparent that human chemical additions have introduced or increased environmental stress for aquatic organisms and fishes in particular. Many of the effects of pesticides to fishes are subtle and insidious. Unlike. direct eradication of populations (eg fish kills), tfima more serious long—term. decline of stocks of fish are caused by indirect factors such as predation, disease and reproductive failure. Fish which are subjected to

unnatural stresses in any part of their life history may be rendered less capable of performing those functions necessary to fulfil their life cycle.

Lambda cyhalothrin is a synthetic pyrethroid (an insecticide) used for the eradication of several insects at home and agricultural fields aphids, beetles, butterfly larvae, cockroaches, mosquitoes, ticks and flies (Mergel, 2010; He *et al*., 2008). According to Moretto (1991), Lambda-cyhalothrin consists of a racemic mixture of two or more active of the four isomers of cyhalothrin. Changes in fish metabolic, physiological, pathological and behavioral response suggest stress condition. However, Lambda cyhalothrin is very toxic to fish (Mergel, 2010). Inyang *et al*. (2016a) reported that Lambda cyhalothrin elicit alteration in total protein and albumin in *Parpohiocephalus obscurus.* Other effects of ƛ-cyhalothrin in fisheries such as *Channa punctatus*, is neurotoxicant leading to alteration in acetyl cholinesterase (Kumar *et al*., 2009a), alterations in nucleic acids and protein contents (Kumar *et al*., 2008, 2009b), behavioral changes, skin color, intense hyperactivity, enhanced loss of balance, rapid swimming, amplified surfacing activity, rate of opercular and convulsions (Kumar *et al*., 2011). Also De Moraes *et al*. (2013) have reported behavioral changes in *Brycon amazonicus* exposed to ƛ-cyhalothrin. Muthukumarave *et al*. (2013) reported that Lambda cyhalothrin induced biochemical and histological changes in the liver of *Oreochromis mossambicus.* Okechukwu and Auta (2007) reported the effect lambda-cyhalothrin on biochemical parameters (serum glucose, protein, cholesterol, triglyceride, glutamic pyruvic acid transaminase, glutamic oxaloacetic acid transaminase and alkaline phosphatase) of *Clarias gariepinus.*

Hence, in the present study an attempt has been made to study the cyhalothrin induced bio-chemical changes in the vital organs such as brain, liver, gill, kidney and muscle of the fish *Ctenopharyngodon idellus*. The bio-chemical changes include glycogen, proteins, nucleic acids and the enzyme activities of lactate dehydrogenase, acid phosphotase and the amino transferases of aspartate and alkaline transferase activities.

MATERIALS AND METHODS

Test Species

The freshwater capture fish of *Ctenopharyngodon idellus* with a size range of 8-10 cm, irrespective of their sex; have been chosen as the test organism in the present study. The fish were acclimatized to the laboratory conditions in large plastic tanks with un-chlorinated ground water for two weeks at a room temperature of 28±2^{o}C. During the period of acclimatization fish were fed daily with fish meal on an average of 3% of their body weight. Feeding was stopped one day prior to the experimentation. All the precautions laid down by APHA (1998) were followed.

The stock solutions were made with acetone as solvent. Controls were maintained for each experiment and they were added with the quantity of acetone equal to the highest concentration used in the test (APHA, 1998).

In the present study 1/10th of 96 hrs LC_{50} value was selected as sub-lethal concentration to study the behavioral alterations and physiological alterations (As per the recommendations of committee on toxicity studies – Anon, 1975). Experiments were conducted to determine the toxicity of cyhalothrin in various concentrations with 2.5% EC formulation in static and continuous flow through systems. 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 10% to 90% for 24, 48, 72 and 96 h in static and continuous flow through systems.

Finney's probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LC_{50} values.

Biochemical methods

The glycogen content was estimated by the method of Kemp *et al*., (1954). Total protein content was estimated by the modified method of Lowry *et al*., (1951). The nucleic acids DNA & RNA were estimated by the method of Searchy and Mac Lnnis (1970). The Lactate Dehydrogenase activity (LDH) was estimated by the method of Srikanthan and Krishna Murthy (1955) with slight modifications. The activity of AAT and ALAT were determined by the method of Reitman and Frankel (1957).

RESULTS AND DISCUSSION

The results of the present work i,e, the 24, 48, 72 and 96 h LC_{50} values of cyhalothrin 2.5% EC formulation in static and continuous flow through systems for the fish, *Ctenopharyngodon idella* were:

Static: 0.0190 mg/l 0.0167 mg/l 0.0147 mg/l 0.0121 mg/l
C.F.M: 0.0132 mg/l 0.0110 mg/l 0.0092 mg/l 0.0076 mg/l
Static: 0.0228 mg/l 0.196 mg/l 0.188 mg/l 0.172 mg/l
C.F.M: 0.0164 mg/l 0.158 mg/l 0.0142 mg/l 0.0132 mg/l

The test fish Ctenopharyngodon idella is highly sensitive to cyhalothrin technical grade and 2.5% EC formulation. These findings are in agreement with Phipps and Holcombe (1985) for channel catfish *Ictalurus punctatus*, Ferrando *et al*., (1991) for *Angulla angulla* Holcombe *et al*., (1982) for *Pimephales promelas*, Phipps and Holcombe (1985) for *Pimephales promelas* and *Carrasius auratus* and Ferguson *et al*., (1966) for *Gambusia affinis*. The culture of the carps simulates the condition of static system (standing water) while in rest lotic system. In aquaculture practices to cure the fish diseases especially crustacean pests the fish farmers are directly spraying the synthetic pyrethroid pesticides. They may have effect on culture species. The

transportation into the nearby running water they may affect the lotic fish. Hence, any concentration above or below the lethal is not recommended for spraying. Lambda cyhalothrin is highly toxic to many fish and aquatic invertebrate species. Reported LC_{50}s in these species are as follows: bluegill sunfish, 0.21ug/L Kidd, and James,(1991).

Biochemical Changes

4.3.1. Glycogen

The results of the present study for glycogen in control and exposed fish were graphically represented in Fig1 and 2 respectively, for 24 and 96 h. In the tissues of control fish, *Ctenopharyngodon idella* glycogen content was in the order of:

Liver > Muscle > Gill > Brain > Kidney

Among the test tissues, higher glycogen was observed in liver. Highest glycogen content of liver is acceptable due to its involvement in glycogen synthesis and utilization. Glycogen is the major storage form of carbohydrate in animals which occurs mainly 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 (Harper, 1985). Though brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities (Lehningar, 1983).

Under exposure to lethal and sub-lethal concentrations of cyhalothrin 2.5% (EC) to the test fish *Ctenopharyngodon idella* for 24 and 96 h, the depletion in the levels of glycogen was observed and graphically represented in Figs. 1 and 2. The main storage form of polysaccharide in cells is glycogen. It is especially in liver of control fish the glycogen content was more and then follows Muscle, Gill, Brain and Kidney.

The leotropic gradation series in terms of per cent decrement at 24 h exposure was:

Sub-lethal -24h: Liver > Muscle > Gill >Brain > Kidney

Lethal-24h: Liver > Gill > Brain > Muscle > Kidney

The decrement at 96 h exposure was:

Sub-lethal -96h: Liver > Muscle >Gill > Brain > Kidney

Lethal-96h: Liver > Gill> Muscle > Brain > Kidney

In the present study it was observed that, the sub-lethal exposures are showing more effect on liver tissue glycogen followed by muscle, gill, brain and kidney, whereas, the lethal exposures are showing affect on liver glycogen, followed by gill muscle, kidney, and brain. The glycogen content after exposure of lethal concentration in fish *Ctenopharyngodon idella* at 24 and 96 h, the glycogen content was found to be the highest in liver and lowest in kidney and brain. In sub-lethal exposure, the highest glycogen content was observed in brain, liver and lowest content in Kidney. The depletion of glycogen may be due to utilization of stored glycogen in liver for energy production as a result of pesticide-induced hypoxia. A fall in total glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or Hexose mono-phosphate pathway (Cappon & Nicholas, 1975).

Pesticides are known to act on endocrine system (Edwards, 1973). Hence, it is assumed that decrease in glycogen content may be due to inhibition of hormones which contribute to glycogen synthesis. Decreased glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthetase, which mediates glycogen synthesis (Stamp and Lesker, 1967).

The earlier observations on the effect of pesticide on carbohydrate metabolism in various species indicate an attenuation of the energy reserve under pesticide stress (Radaiah (1988); Kabeer *et al*., (1979); Holden, 1974). It appears that exposure to cyhalothrin 2.5% triggers enhancement of energy requirements. Carbohydrates might be utilized more to meet the extra demand of energy during stress conditions. So the carbohydrate level was decreased. Since the carbohydrates are considered to be the first among the organic nutrients to be decreased under any physiological stress conditions imposed on the animal (Clarke, 1975) a drop in tissue carbohydrate content may also be either due to decreased synthesis as a consequence of toxic stress or breakdown (Dezwaan and Zendee, 1972). The significant decrease of glycogen was observed in the liver, kidney and muscle tissues of *Oreochromis mossambicus* after exposued to Dichlorvos.

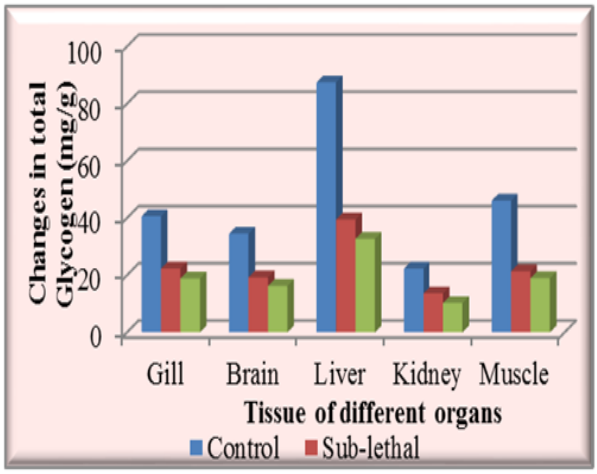


Fig. 1: a. 24 h Exposure.

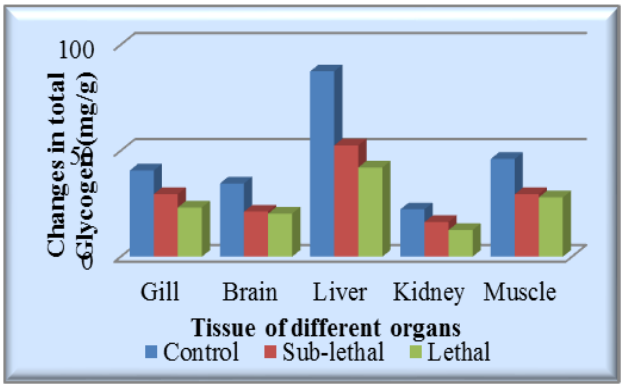


Fig. 1: b 96 h Exposure.

Fig. 1: Changes in the amount of Glycogen mg/g wet weight in tissues of *Ctenopharyngodon idella* exposed to Cyhalothrin 2.5% EC for 24 and 96 h.

Lakshmanan *et al.,* (2013), Veeraiah, and Durga Prasad (1998), reported that the total glycogen levels of brain, liver, muscle, gill and kidney of *Labeo rohita* were decreased on exposure to sub-lethal concentration of cypermethrin. Gijare *et al.*, (2011) reported notable alteration in liver and intestine glycogen of *Ophiocephalus punctatus* exposed to sub-lethal concentration of cypermethrin, Israel Stalin and Sam Manohar Das (2012) reported Glycogen mobilization is maximum in the liver tissue under exposure to Lebaycid (Fenthion). Quinolphos long-term exposure to sub-lethal concentrations decreased the glucose level in *Channa punctatus* (Sastry *et al.*, 1984). Cyhalothrin 2.5% sub-lethal exposure on freshwater mussel *Lemellidens marginalis* decreased the glycogen content (Moorthy *et al.*, 1985). Monocrotophos exposure to *Channa punctatus* reduced the glycogen levels (Miny Samuel and Sastry, 1989).

In muscle tissue of *Labeo rohita*, carbohydrate level in control was 46.60 mg/g and it was decreased to 44.80, 30.92 and 27.11 mg/g in 0.398 ppm of Dimethoate 30% EC exposures for 24,48 and 72 hours respectively (Binukumari.S and Vasanthi 2014). The liver glycogen content decreased progressively during exposure period, this may be due to toxic stress (Ganeshwade, 2011). Endosulfan 96 hrs exposure decreased the glycogen level in crab *Barytelphusa guerni* (Nagender Reddy *et al.*, 1991) and also in *Anabas scandens* (Yasmen *et al.*, 1991). Decrease of glycogen content in liver and muscle tissue of Atlantic salmon was observd under sub-lethal exposure of fenvalerate (Haya, 1989). Hexachlorocyclohexane on *Channa punctatus* (Ganathy *et al.*, 1994).

Sub-lethal concentrations of cypermethrin induced depletion of glycogen in *Tilapa mossambica* (Reddy and Yellamma, 1991); Anuradha (1993); Veeraiah and Durga Prasad (1998); Veeraiah, 2001) in *Cyprinus carpio* (Ravisankar *et al.*, 1992) and in *Channa punctatus* (Luther Das *et al.*, 1999). Sobha Rani *et al.*, (2000) observed a significant depletion in glucose and glycogen levels in various tissues of freshwater teleost *T. mossambica* under sub-lethal concentration of sodium arsenate had stated that these changes were tissue specific and time dependent. Bhamre *et al.*, (2001) observed decreased levels of glycogen under acute and chronic exposure of mercuric chloride in the tissues of whole body, foot digestive gland and mantle of fresh water mussel *Parreysia favidens.*

Herlinmayer *et al.*, (1970) and Smart (1978) stated that toxic stress results in the disruption of enzymes associated with carbohydrate metabolism. The decreased glycogen level is also attributed to the conversation of carbohydrates into aminoacids (Gaiton *et al.*, 1965). In the present study, it was observed that exposure to lethal and sub-lethal concentrations of cyhalothrin 2.5% EC, in the fish *Ctenopharyngdon idellus*, caused changes in the total glycogen level which may be attributed to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism.

Total Proteins

The calculated values for total proteins are graphically represented in Fig.3 and 4 respectively for 24, and 96 h. In the control fish *Ctenopharyngdon idellus*, the total protein content was in the order of,

Liver > Muscle > Brain > Gill > Kidney

The variation in distribution suggests gradual difference in metabolic calibers of various tissues. The liver is much in proteins, because of metabolic potential being oriented towards it and is the seat for the synthesis of various proteins, and also the regulating center of metabolism. The present trend in the tissues is justifiable in the wake of mechanical tissue of muscle intended for mobility and it does not participate in metabolism.

Under sub-lethal and lethal concentration of cyhalothrin 2.5% EC, the total protein was found to decrease in most of the tissues and the decrement in protein content at 24 h exposure, was in the order of:

Sub-lethal: Liver > Gill >Muscle > Brain > Kidney

Lethal: Liver > Gill > Muscle > Brain >Kidney

The leotropic gradation series in terms of per cent decrement at 96h exposure:

Sub-lethal -96h: Liver > Gill > Muscle > Brain > Kidney

Lethal-96h: Liver > Muscle > Gill > Brain > Kidney

Sastry and Siddiqui (1984) reported that the protein content was decreased in liver, muscle, kidney, intestine, brain and gill of *Channa punctatus* treated with quinalphos. The levels of protein decreased significantly in liver, kidney and muscle *Catla catla* treated with endosulfan (Gaiton *et al.*, 1965). Nagender Reddy *et al.*, (1991); Yeragi *et al.*, (2000) observed that the decreased levels of proteins in gills, testis, ovaries and muscle of marine crab *Uca marionis* exposed to acute and chronic levels of Malathion.

Aruna Khare *et al.*, (2000) observed that the sub-lethal concentrations of Malathion showed a significant increase in total protein content in kidney of exposed fish during the first week and thereafter a gradual decrease in protein content was observed in the later periods of exposure. The protein content was decreased when *Labeo rohita* exposed to cypermethrin (Aruna Khare *et al.*, (2000) in muscle, liver, kidney, brain and testis of *Rana hexadactyla* exposed to aldrin (Aruna Khare *et al.*, (2000) and Nirma a detergent (Pandi Bhaskaran *et al.*, 1991) also support the present study. The 96 hours exposure of Endosulfan 35% E.C. decreased the total and soluble protein content in crab (Pandi Bhaskaran *et al.*, 1991). The sub-lethal concentrations of fenvalerate deplete the protein content of *L. thermalis* (Pandi Bhaskaran *et al.*, 1991). The level of protein decreased significantly in liver, kidney and muscle when *Catla catla* was treated with Endosulfan (Rao, 1989). Sastry and Siddiqui (1984) reported that the protein content was decreased in liver, muscle, kidney, intenstine, brain and gill, of *Channa punctatus*, treated with quinalphos.

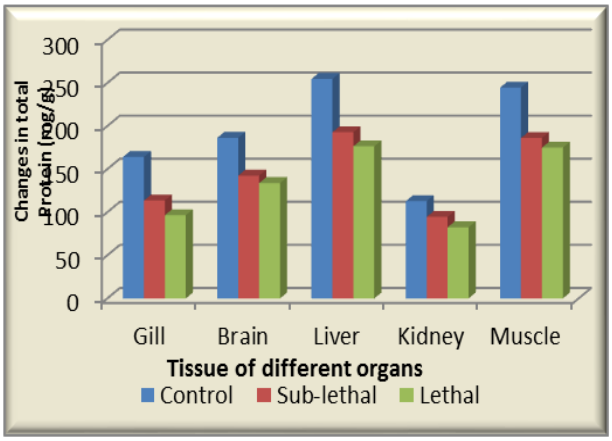


Fig. 2a. 24 h Exposure.

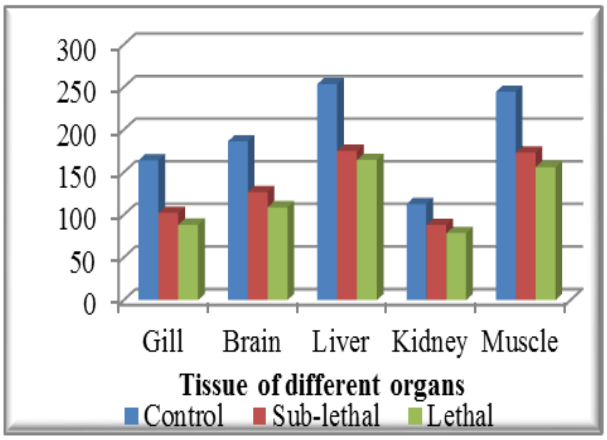


Fig. 2b 96 h Exposure.

Fig. 2a and 2b. Changes in the Protein content (mg/gram wet weight of the tissue) over the control in different tissues of *Ctenopharyngodon idella* on exposure to lethal and sub-lethal concentration of Cyhalothrin 2.5% EC, for 24 and 96 h.

Kidney tissue of *Ctenopharyngodon idella* evidenced a significant decrease in the protein content under sub-lethal and lethal concentrations of cyhalothrin 2.5%. The muscle tissue of *Ctenopharyngodon idella* showed significant decrease in protein under lethal and sub-lethal concentrations of cyhalothrin 2.5% lethal exposure. Changes in brain and kidney in the fish suggests that they are relatively less affected than hepatic tissue under cyhalothrin 2.5% toxicity.

The decreased trend of the protein content as observed in the present study in most of the fish tissue may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to directing free amino acid for the synthesis of necessary proteins or for the maintenance of osmotic and ionic regulation (Schmidt Nielson, 1975).

In the present investigation, the decrease was more apparent in sub-lethal concentrations than in lethal concentrations. It may be due to detoxification of enzymes or mechanism, which is apparently slow. Several other investigations also revealed a decreased trend in protein profiles with organophosphate compounds. All these investigations support the present study of decreasing trend of proteins in the fish *Ctenopharyngodon idella* exposed to lethal and sub-lethal concentrations of cyhalothrin 2.5% EC.

Nucleic Acids (DNA & RNA)

Nucleic acid and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating different activities of cells. In the present study, the DNA content was found to be increased in most of the tissue in response to cyhalothrin 2.5% EC sub-lethal exposure. Enhancement in the DNA level might be due to activation of some dormant regulating factors or increase in activity of the essential factors controlling DNA synthesis. Earlier reports by Natarajan (1981) revealed that the enlargement of nuclei in chloride-secreting cells in *C. striatus* exposed to Metasystox corroborates the absorption or degradation of either of these pesticides or their metabolites in different tissues of the fish.

DNA

The nucleic acid contents change over the control were presented in Fig.3.a and Fig.3.b. the DNA content in different tissues of control test fish, *Ctenopharyngodon idella* was in the order of:

Gill >Kidney >Brain > Liver > Muscle

Under exposure to sub-lethal concentrations of cyhalothrin 2.5% EC, for 24 h the amount of DNA content was found to decrease in most of the tissue of the fish *Ctenopharyngodon idella* and the decrement was in the order of:

Sub-lethal: Muscle > Liver > Gill > Brain > Kidney

Lethal: Liver > Muscle > Gill > Brain > Kidney

Under exposure to lethal and Sub-lethal concentrations of cyhalothrin 2.5% EC, for 96 h the depletion of DNA in various tissues of *Ctenopharyngodon idella* was in the order of:
Sub-lethal: Liver >Gill > Muscle > Kidney > Brain
Lethal: Liver > Muscle > Gill > Brain > Kidney

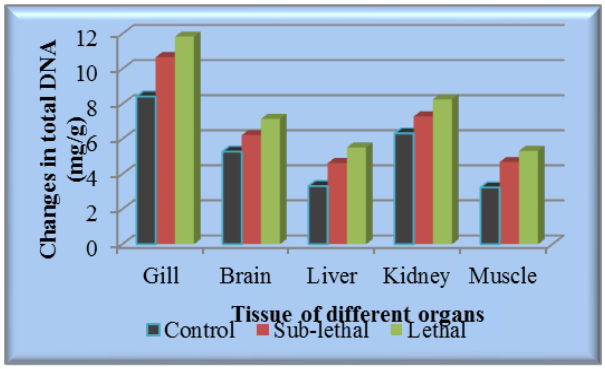


Fig. 3a 24 h Exposure.

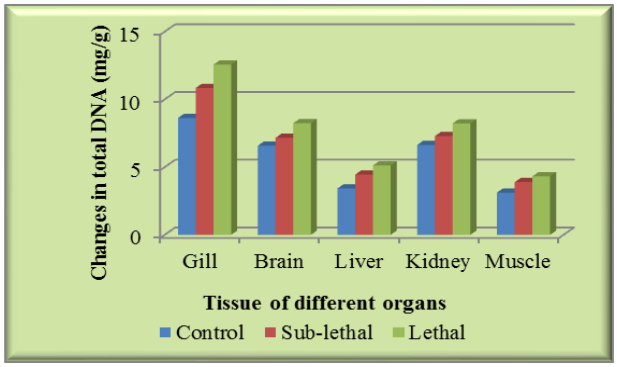


Fig. 3b 96 h Exposure.

Fig. 3a and 3b. Change in the amount of Deoxy ribonuclic acid (DNA) (mg/gram wet weight of the tissue) over the control in different tissue of *Ctenopharyngodon idella* on exposure to sub-lethal and lethal concentration of Cyhalothrin 2.5% EC for 24 and 96 h.

RNA

The calculated values of nucleic acid content RNA, and the change over the control fish were presented in Fig. 4.a and 4.b. The RNA content in different tissues of control test fish, *Ctenopharyngodon idella* was in the order of:
Liver >Gill > Kidney > Muscle > Brain

Under exposure to sub-lethal and lethal concentrations of cyhalothrin 2.5% 50% EC, for 24 h, the amount of RNA decreased in most of the tissue of the test fish, *Ctenopharyngodon idella* was:
Sub-lethal: Liver > Brain > Muscle > Gill > Kidney
Lethal: Liver > Gill > Muscle >Brain > Kidney

Under exposure to lethal and Sub-lethal concentrations of cyhalothrin 2.5% EC for 96 h, the depletion of RNA in various tissues of *Ctenopharyngodon idella* was in the order of:

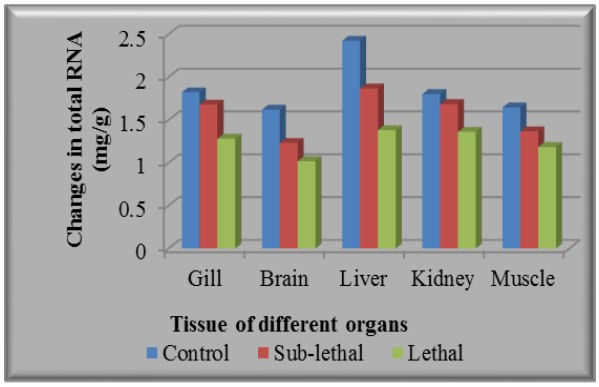


Fig. 4a 24 h Exposure.

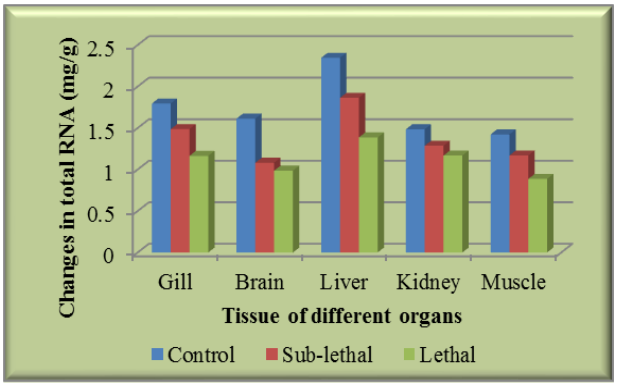


Fig. 4b 96 h Exposure.

Fig. 4a and 4b. Change in the amount of Ribonucleic acid (RNA) (mg/gram wet weight of the tissue) in different tissue of *Ctenopharyngodon idella* on exposure to sub-lethal and lethal concentration of Cyhalothrin 2.5% EC for 24 and 96 h.
Sub-lethal: Gill > Kidney >Brain > Liver > Muscle
Lethal: Liver> Brain> Gill > Kidney >Muscle

The level of RNA in liver is higher than brain, gill, muscle and kidney. Veeraiah *et al.*, (2013) observed that the RNA content in all the tissues of C*irrhinus mrigala* showed a very significant decrease over the control. The decreasing trend was more in sub-lethal concentration than in lethal concentrations of cypermethrin. Sunitha (2012) reported that, the freshwater fish *Channa striatus* exposed to sub lethal concentration of cypermethrin showed a significant decrease in the level of DNA and RNA. The RNA level reflects the intensity of protein synthesis (Brachet 1995) and metabolic caliber of the tissue (Bulow 1970).

The decrease of RNA supports the view of Holbrook (1980). The RNA levels reflects transcription status and intensity of protein synthesis (Brachet 1995), as well as the metabolic activity of the tissues (Bulow 1970). In contrast to DNA, RNA content declined significantly in both the tissues and protein (Lowry and Somero (1990) reported that Inhibitory effects of fenvalarate on DNA, RNA and protein content show extreme toxicity of pyrethroid compound on the main biochemical machinery of the fresh water catfish, *Clarias batrachus*.

The work of Durairaj and Selvarajan (1988) also supports the present study.

The decreases in total protein level suggest the high protein hydrolytic activity due to elevation of protease activity. Inhibition of DNA synthesis, thus, might affect both protein as well as amino acid levels by decreasing the level of RNA in protein synthesizing machinery. The results of the present study suggest that, the exposed cyhalothrin 2.5% EC compound is a potent inhibitor of DNA synthesis, which in turn results in the reduction of RNA level.

The effects of sub-lethal and lethal concentrations of cyhalothrin 2.5% EC on DNA and RNA contents show moderate toxicity on the main biochemical machinery of the freshwater fish *Ctenopharyngodon idella*.

Lactate Dehydrogenase activity (LDH)

The Lactate dehydrogenase activity (LDH) and the change over control was given in Fig.5a and Fig.5b the activity levels of lactate dehydrogenase in *Ctenopharyngodon idella* exposed to cyhalothrin 2.5% were expressed as micro moles of formazan/mg protein/h.

The LDH levels of muscle, brain, liver, gill and kidney of control fish were almost stable. The control values of LDH in different tissues of the fish *Ctenopharyngodon idella* was in the order of:

Liver > Muscle > Gill > Kidney > Brain

Under sub-lethal and lethal exposures to cyhalothrin 2.5% EC commercial grade for 24 h, the activity levels of LDH were found to increase in all the tissues of the fish *Ctenopharyngodon idella*. The per cent change in the activity levels of LDH, in the test fish was in the order of:

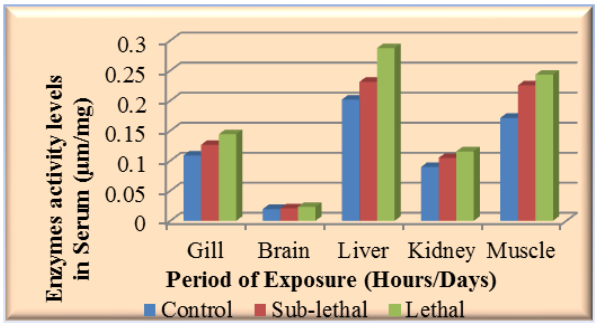
Sub-lethal: Muscle > Kidney > Gill > Liver > Brain

Lethal: Liver > Muscle > Gill > Kidney > Brain

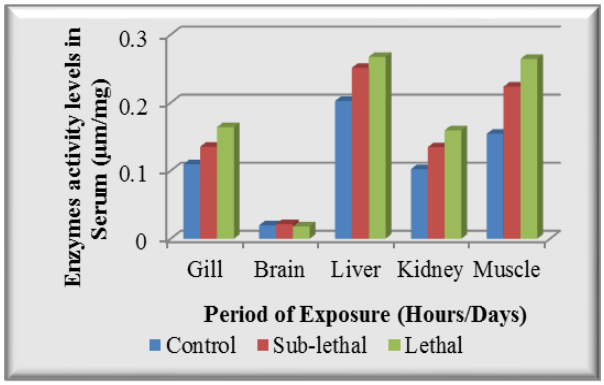
Under Sub-lethal and lethal exposure to cyhalothrin 2.5% commercial grade EC for 96 h further changes in the activity levels of LDH were noticed. The increased activity levels of LDH in the test fish were in the order of:

Sub-lethal: Kidney > Muscle > Liver > Gill > Brain

Lethal: Muscle > Kidney > Gill > Liver > Brain



5a 24 h Exposure.



5b 96 h Exposure.

Fig. 5a and 5b. Change in the specific activity levels of Lactate dehydrogenase (LDH) (µ moles of formazan/mg protein/h) and % change over the control in different tissues of *Ctenopharyngodon idella* on exposure to sub-lethal and lethal concentration of Cyhalothrin 2.5% EC for 24 and 96 h.

In the present study, it was observed that the activity of LDH was highly elevated following cyhalothrin 2.5% EC exposure indicating increased anaerobic respiration to meet the energy demands where, aerobic oxidation is lowered. Lactate dehydrogenase (LDH) coverts the lactate to pyruvate and has very important role in carbohydrate metabolism.

The LDH activity depends on its five isoenzymes and the activity changes under pathological conditions (Martin *et al.*, 1983). The increase of LDH activity during conditions favoring anaerobic respiration to meet the energy demands lowers the aerobic respiration (Martin *et al.*, 1983). The earlier reports on *Tilapia mossambica* (Anastasi and Bamistor (1980). Natarajan, (1984), reported increased LDH activity in gill, brain, muscle and liver tissues of *Labeo rohita* exposed to sub-lethal concentrations of metasystox. Ghosh, (1987) reported that sub-lethal toxicity of organophosphate (phosphamidon) on *Clarios batractus* showed an elevated LDH activity in gill, brain, liver and muscle tissues. Fluke (1972), explained that the raise of LDH activity increases the permeability of cells as well as necrosis. Sub-lethal copper toxicity on *Labeo rohita*, elevated the LDH activity levels (Venkataramana *et al.*, 1990).

Similar observations on LDH activity were made under pesticides stress by Satya Prasad (1983); Azhor Baig *et al.*, (1991); Mary Chandravathy and Reddy and yellamma (1991); Ganathy *et al.*, (1994); Asfia Parveen and Vasantha (1994); Nagabhushanam *et al.*, 1994; Veeraiah (2001).

In the present study also, it was observed that the activity of LDH in the fish *Ctenopharyngodon idella* under exposure to sub-lethal and lethal concentrations of cyhalothrin 2.5% EC commercial grade was elevated indicating that the anaerobic respirations arrived and

aerobic respiration inhibited so as to meet the increased metabolic stress and to overcome the toxic stress.

Amino Transferases

Asparate Amino-Transferase (SGOT/AAT) and Alanine Amino-Transferase (SGPT/ALAT) activity

The calculated values of amino-transferases and per cent change over control along with standard deviations were given in Fig.6a. Fig.6b. The changes in the levels of AAT and ALAT were studied in different tissue of brain, liver, muscle, gill and kidney in the test fish *Ctenopharyngodon idella* under sub-lethal and lethal concentrations of cyhalothrin 2.5% EC commercial grade after 24, and 96 h. The values were expressed mg of formazon formed /mg protein /h.

Aspartate Amino-Transferase (SGOT/AAT): The AAT activity in brain, liver, muscle, gill and kidney of the control fish *Ctenopharyngodon idella* was in the order of:
Gill > Liver > Brain > Kidney > Muscle

Under exposure to sub-lethal and lethal concentrations of cyhalothrin 2.5% EC commercial grade for 24 and 96 h the change in AAT activity of the fish *Ctenopharyngodon idella* was in the order of:
Gill > Liver > Brain > Kidney > Muscle

Under exposure to sub-lethal and lethal concentrations of methyl parathion 50% EC commercial grade for 24 and 96 h change in AAT activity in *Ctenopharyngodon idella* was in the order of:
24 h Exposure: Sub-lethal: Muscle > Brain > Liver > Gill > Kidney
Lethal: Muscle > Brain > Kidney > Liver > Gill
96 h Exposure: Sub-lethal: Muscle > Liver > Brain >Gill > Kidney
Lethal: Gill > Muscle > Kidney > Brain > Liver

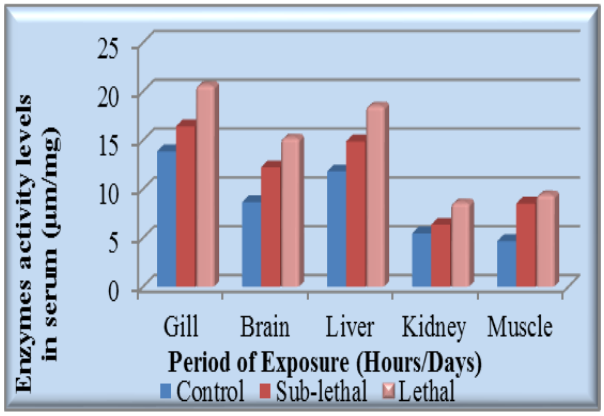
The AAT specific activity levels increased significantly 96 days exposure period.

The changes in exposed fish and change over control of ALAT activity are given in table Fig.7a and Fig.7b. The ALAT activity in different tissues of control fish was in the order of:
Liver > Brain > Muscle > Gill > Kidney

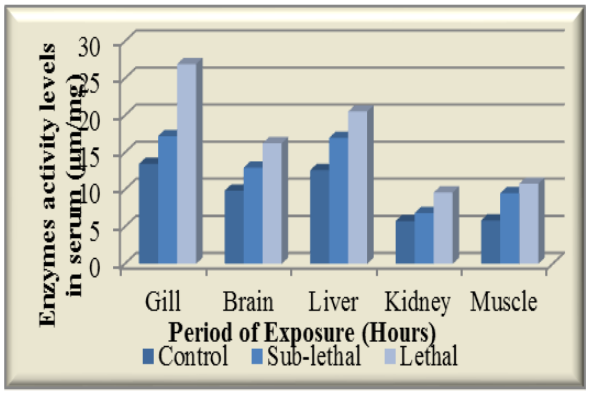
Under exposure to sub-lethal and lethal concentrations of cyhalothrin 2.5% EC for 24 and 96 h the change in ALAT activity was in the order of:
24 h Exposure: Sub-lethal: Kidney > Gill > Muscle > Liver > Brain
Lethal: Kidney > Gill > Muscle > Liver > Brain
96 h Exposure: Sub-lethal: Kidney > Muscle > Gill > Liver > Brain
Lethal: Kidney > Muscle > Gill > Liver > Brain

Since the pesticide stress was known to induce significant change in protein metabolism, it is likely that the aminotransferases were also considerably affected. Increased activities of AAT and ALAT in different tissues of fish suggest either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fatty acids during cyhalothrin 2.5% intoxication. The increase in activities of aminotransferases as observed in the present study were in agreement with earlier reports, demonstrating a consistent increase in the activities of these enzymes under conditions of enhanced gluconeogenesis. AAT and ALAT are located in both mitochondrial and cytosol fractions of the cell. A close relation appears to exist between the mitochondrial integrity and transaminase levels and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it. The alteration in the activities of AAT and ALAT as observed in the present study may also be due to the mitrocondrial distruption and damage as a result of cyhalothrin 2.5% induced stress.

The same trend was observed in cockroach, *Periplaneta americana* by Sesha Reddy and yellamma (1991). Kaviraj and Das (1994) reported an increased activity of aminotransferases due to lindane toxicity on *Anabas testudineus* during pesticide stress. The same trend was observed in cockroach, *Periplaneta americana* by Sesha Reddy and yellamma (1991). Increased levels of GOT and GPT in liver, muscle and blood under cadmium toxicity in *Channa punctatus* was observed by Sastry and Shukhla (1990). Kaviraj and Das (1994) reported an increased activity of aminotransferases due to lindane toxicity on *Anabas testudineus* during pesticide stress. GDH catalyses the reversible deamination of glutamate to α-ketoglutarate and ammonia. AAT catalyses reversible transamination of glutamate and oxaloacetate to α-ketoglutarate and aspartate, while ALAT catalyses the reversible.

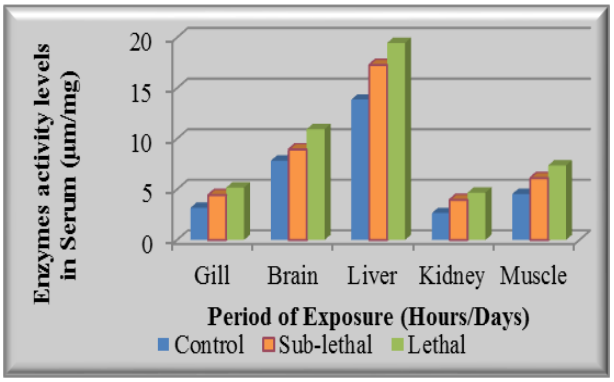


6a 24 h Exposure.

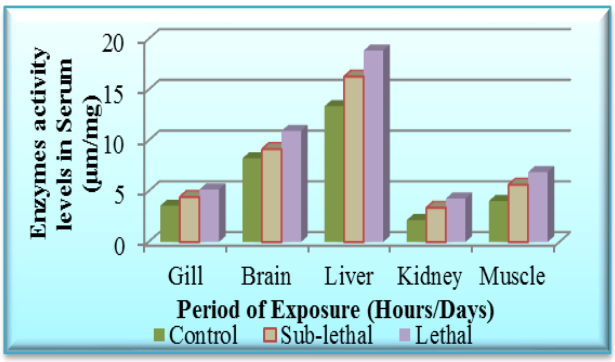


6b 96 h Exposure.

Fig. 6a and 6b. Change in the specific activity levels of Aspartate amino transferase (AAT) (µ moles of pyruvate formed/mg protein/h) in different tissues of *Ctenopharyngodon idella* on exposure to sub-lethal and lethal concentration of Cyhalothrin 2.5% EC for 24 and 96 h.



7a 24 h Exposure.



7b 96 h Exposure.

Fig. 7a and 7b. Change in the specific activity levels of Alanine amino transferase (ALAT) (µ moles of pyruvate formed/mg protein/h) in different tissues of *Ctenopharyngodon idella* on exposure to Cyhalothrin 2.5% EC for 24 and 96 h.

CONCLUSION

Insecticides are commonly used to eradicate insects in household and agricultural field. Pyrethroids based insecticides are mostly used. Lambda-cyhalothrin is major type of pyrethroids based insecticide. This study assessed the effect of lambda-cyhalothrin on biochemical constituents such as proteins glycogen, and Nucleic acids (DNA & RNA), and the enzyme activity levels of LDH, AAT, ALAT and acid phosphotases in some organs of *Ctenopharyngodon idella.* The findings showed that the bio-chemical contents and the enzyme activity levels were altered in the vital organs of the fish such as brain, Liver, Gill, Muscle and Kidney. Hence, care should be taken during use of lambda-cyhalothrin based insecticides in eradicating insects in the areas close to surface water sources.

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REFERENCES

1. Anastasi, A. and Bennister, J. N. (1980): "Effect of Pesticides on Mediterranean fish muscle enzyme". Acta. Adrief., 21(1): 119-124.
2. Anderson, J. M. and Peterson, M. R. (1969): "DDT sub-lethal effects on Brook trout nervous system". Science, 164-440.
3. Anon (1975): "Committee on methods of toxicity tests with fish macro invertebrates and amphibians". EPA, Oregon, 61.
4. APHA (1998): "American Public Health Association. In: Standard methods for the examination of water and waste water". APHA/AWWA/WPCF, Washington, DC. 2005.
5. Aruna Khare, Sudhasing and Keerthy Shrivastava, (2000): "Malathion induced biochemical changes in the kidney of freshwater fish *Clarias batrachus*". J. Ecotoxicol. Environ. Monit., 10(1): 11-14.
6. Asfia Parveen and Vasantha, N. (1994): "Evaluation of pesticide endosulfan impact on respiratory metabolism of fish *Clarias batrachus*". Ind. J. Comp. Ani. Physiol., 12(1): 83-89.
7. Azhar Baig, N. D. Vijayajoseph, K. and Jayantha Rao, K. (1991): "Heptachlor induced changes in the enzymes associated with carbohydrate metabolism in functionally different muscle of *Channa punctatus"*. J. Ecotoxicol. Environ. Monit., 12: 156-161.
8. Bhamre, P. R., Desai, A. E. and Deoray, B. M. (2001): "Effect of Mercuric chloride on glycogen content of the freshwater mussel *Parreysia faridens".* Poll. Res., 20(1): 13-15.
9. Binukumari, S. and Vasanthi, J. (2014): "Toxicity of the pesticide Dimethoate 30% EC on the Carbohydrate content of the Fresh water fish, Labeo rohita, Journal of Chemical, Biological and Physical Sciences, 4(1): 220-223.
10. Bodansky, A. (1932): "Phosphatase studies II. Determination of serum phosphatase. Factors influencing the accuracy of the determination". J. Biol. Chem., 101: 93.

11. Brachet (1995): "Studieds on Relativistic hydrodynamics of semiclassical quantum fluids Laboratoire de physique statistique". Ecole Normale superieure, Paris, France, 75005.
12. Bulow, F. J. (1970): "RNA-DNA ratios as indicators of recent growth rates of a fish". J. Fish. Res. Bd. Can., 27: 2343-2349.
13. Cappon, I. D. and Nicholas, D. M. (1975): "Factors involved in increased protein synthesis in liver Microsomes after administration of DDT". Pestic. Biochem. Physiol., 5: 109-118.
14. Clarke, D. H. (1975): "Exercise physiology". Prentice-Hall Inc., Englewood Cliffs, New Jersey.
15. De Moraes, F.D., Venturini, F.P., Cortella, L.R.X., Rossi, P.A. and Moraes, G. (2013). Acute toxicity of pyrethroid-based insecticides in the Neotropical freshwater fish *Brycon amazonicus". Ecotoxicol. Environ. Contam.*, 8(2): 59-64.
16. Dezwaan, A. and Zendee, D. I. (1972): The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* (L.) Comp. Biochem. Physiol., B43: 47-54.
17. Dezwaan, A. and Zendee, D.I. (1972). The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis* (L.) Comp. Biochem. Physiol., B43: 47-54.
18. Durai Raj, S. and Selvarajan, V. R. (1988): Changes in Nucleic Acid concentration in the brain of *Tilapia mossambica* exposed to the pesticide, phosphamidon. J. Com. Anim. Physiol., 6(2): 81-85.
19. Durai Raj, S. and Selvarajan, V.R. (1988). Changes in Nucleic Acid concentration in the brain of *Tilapia mossambica* exposed to the pesticide, phosphamidon. J. Com. Anim. Physiol, 6(2): 81-85.
20. Durga Prasad, M.K. and Veeraiah, K. (1998). Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish, *Labeo rohita* (Hamilton). Proc. Acad. Environ. Biol., 7(2): 143-148.
21. Edwards, C. A. (1973): Environmental Pollution by Pesticides. Plenum Press, London, New.
22. Edwards, C.A. (1973). Environmental Pollution by Pesticides 542 pp, Plenum Press. London and New York.
23. Ferguson, D.E., Ludke, J.L. and Murphy, G.C. (1966). Transactions of American Fisheries Society, 95: 315.
24. Ferrando, M.D. and Andreu-Moliner, E. (1991). Acute lethal toxicity of some pesticides to *Brachionus calyciflorus* and *Branchionus plicatilis*. Bull Environ. Contam. Toxicol., 47: 479-484.
25. Finney, D.J. (1971). "Probit Analysis" 3rd Ed., Cambridge Univ. Press, London/New York.
26. Fluke, D. J. (1972): Temperature dependence of the direct action of ionizing radiation on beef heart lactate dehydrogenase enzyme activity, substrate and coenzyme affinities. Radiat Res., 51(1): 56-71.
27. Gaiton, M.K., Dahl, D.R. and Elliott, K.A.C. (1965). Entry of glucose carbon into amino acids of rat brain and liver in vivo after injection of uniformly ^{14}C labelled glucose. Biochem. J., 94: 345-352.
28. Ganathy, V., Srinivasa Reddy, D., Reddy, S.L.N. and Shankaraiah, K. (1994). Effect of hexachlorocyclo- hexane on biochemical composition of the fish *Channa punctatus*. J. Ecotoxicol., Environ. Monit., 4(1): 015-020.
29. Ganeshwade, R. M. (2011): Biochemical Changes Induced by Dimethoate in the Liver of Freshwater Fish *Puntius ticto* (HAM), Biological Forum. An International Journal, 3(2): 65-68.
30. Ghosh, T.K. (1987). Toxic impact of three organophosphate pesticide on carbohydrate metabolism in a freshwater Indian catfish *Clarias batrachus*. Proc. Indian Natl. Sci. Acad. B53. No.2 pp.135-142.
31. Gijare shruti, S., Raja, I. A., Tantarpale, V. T. and Kulkarni, K. M. (2011): Modulation in glycogen of the freshwater fish *Ophiocephalus punctatus* exposed to cypermethrin an international quarterly journal of life sciences; The Bioscan, 2011; 6(1): 129-130.
32. Haya, K. (1989). Toxicity of pyrethroid insecticides to fish, Environmental Toxicology and Chemistry, 8: 381-391.
33. He, L-M., Troiano, J., Wang, A. and Goh, K. (2008). Environmental Chemistry, Ecotoxicity, and Fate of Lambda-Cyhalothrin. In: *Reviews of Environmental Contamination and Toxicology*. Whitacre, D.M. (ed.). Springer, 71–91.
34. Helinmayer, L.M.G., Francois, M., Hascheke, R.M. and Fisher, E.H. (1970). Control of phosphorylase activity in a muscle glycogen particle II Activities of calcium. J. Biol. Chem., 245: 6649-6656.
35. Holbrook (Jr.), D.J. (1980). Effects of toxicants on nucleic acid and protein metabolism In: Introduction to biochemical toxicology (Eds: E. Hodgson and F.E. Guthrie). Blackwell Scientific Publications, Oxford, 261-284.
36. Holcombe, G.W., Phipps, G.L. and Tanner, D.K. (1982). The acute toxicity of Kelthane, dursban, disulfoton, pydrin and permethrin to fathead minnows. Pimephales promelas and rainbow trout *Salmo gairdneri*, Environ Pollut., 29A: 167-178.
37. Holden, A.V. (1974). Effect of Pesticides on fish. In: Environmental pollution by Pesticides, (ed) C.A.Edwards, Plenum Press, New York, 213-253.
38. Israel Stalin, S., Sam Manohar, Das, S. (2012): Biochemical changes in certain tissues of *Cirrhina mrigala* (Hamilton) (Cyprinidae: Cyprniformes) exposed to fenthion. International Journal of Environmental Sciences, 2: 3.
39. Kabeer Ahammad, Sivaiah S. and Ramana Rao, K.V. (1979). Comp. Physiol. Ecol., 4(2): 81-83.
40. Kaviraj, A. and Das, B.K. (1994). Cadmium induced changes in fish and other aquatic organisms. J. Natcon., 6(2): 105-122.
41. Kemp A, Adrinene J M, Kits and Van Hejnenga (1954). A calorimetric method for the determination of glycogen in tissues. Bio. Chem. J., 56: 646-648.

42. Kemp A, Adrinene J M, Kits and Van Hejnenga (1954). A calorimetric method for the determination of glycogen in tissues. Bio. Chem. J., 56: 646-648.
43. Kidd, H. and James, D. (eds.) (1991). Agrochemicals Handbook. Third edition. Royal Society of Chemistry, Cambridge, England.
44. Kumar, A., Rai, D.K., Sharma, B. and Pandey, R.S. (2009a). ƛ-cyhalothrin and cypermethrin induced in vivo alterations in the activity of Acetyl cholinesterase in a freshwater fish, *Channa punctatus* (Bloch). *Pestic Bicochem Physiol.,* 93: 96–99.
45. Kumar, A., Sharma, B. and Pandey, R.S. (2009b). Cypermethrin and ƛ-cyhalothrin induced in vivo alterations in nucleic acids and protein contents in a freshwater catfish *Clarias batrachus* (Linnaeus; Family—Clariidae). *J. Environ. Sci. Health* B, 44: 564–570.
46. Kumar, A., Sharma, B. and Pandey, R.S. (2011). Assessment of acute toxicity of k-cyhalothrin to a freshwater catfish, *Clarias batrachus*. *Environ Chem Lett.*, 9: 43–46.
47. Kumar, A., Sharma, B. and Pandey, R.S. (2008). Cypermethrin and ƛ-cyhalothrin induced alterations in nucleic acids and protein contents in a freshwater fish Channa punctatus. *Fish Physiol. Biochem.*, 34: 331–338.
48. Lakshmanan, S., Rajendran, A., & Sivasubramaniyan, C. (2013): "Impact of Dichlorvos on tissue glycogen and protein content in fresh water fingerlings, *Oreochromis mossambicus* (Peters)". Int. J. Res. Environ. Sci and Technol, 3(1): 19-25.
49. Lehninger, A.L. 1978, 1983: Biochemistry kalyani publications Ludhiana, New Delhi.
50. Lowery, M. S. and Somero, G. N. (1990): Starvation effects on protein synthesis in red and white muscle of the barred sand bass, Paralabras nebulifer. Physiol. Zool., 63: 630-648.
51. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurment with Folin Phenol reagent. J. Biol. Chem., 193: 265-275.
52. Luther Das, V., Veeraiah, K. and Jeevaprada, P.N. (1999). Toxicity and effect of cypermethrin on biochemical constituents of the fish *Channa punctata*. J. Environ. Contam. Toxicol.
53. Martin, D.W., Mayers, P.A. and Rodwell, V.S. (1983). In; Harper's Review of Biochemistry Lange Medical Publications, Meruzen, Asia.
54. Mergel, M.(2010). Lambda-Cyhalothrin. http://www.toxipedia.org/display/ toxipedia/
55. Lambda-Cyhalothrin. Accessed December 4th, 2016.
56. Miny Samuel and Sastry, K.V. (1989). In vivo effect of monocrotophos on the carbohydrate metabolism of the freshwater snake head fish *Channa punctatus*. Pestic. Biochem. Physiol., 34: 1-8.
57. Mishra, J., & Srivastava, A. K. (1984): "Effects of chlordane on the blood and tissue chemistry of a teleost fish, *Heteropneustes fossilis"*. Cellular and molecular biology., 30(6): 519.
58. Moretto, A. (1991). Indoor spraying with the pyrethroid insecticide lambda-cyhalothrin: effects on spraymen and inhabitants of sprayed houses". *Bulletin of the World Health Organization*, 69(5): 591-594.
59. Murty *et al*., (1980). Improved ammonium molybdate method for thin layers chromatographic detection of organophosphate residues. J. Assoc. Of Analytical Chem., 63: 756-757.
60. Muthukumarave, K. Sathick, O.S. and Raveendran, S. (2013). Lambda cyhalothrin induced biochemical and histological changes in the liver of *Oreochromis mossambicus* (peters). *Int. J. Pure Appl. Zool.,* 1(1): 80-85.
61. Nagabhushanam, M., Suhasini, P.B., Rao, M.R. and Jayantha Rao, K. (1994). Effect of heptachlor on certain aspects of carbohydrate metabolism in Swiss albino mice. Bull. Environ. Contam. Toxicol, 52: 905-911.
62. Nagender Reddy, N., Venugopal, N.B.R.K. and Reddy, S.L.N. (1991). Effect of Endosulfan 35% EC on glycogen metabolism in the haemolymph and tissues of a freshwater field crab *Barytelphusa guerni*. Pestic. Biochem. Physiol., 40: 176-180.
63. Natarajan, G.M. (1981). Effect of lethal LC_{50} / 48 h Concentrations of metasystox on selected oxidative enzymes, tissue respiration and histology of gill of freshwater air breathing fish *Channa striatus*. Curr. Sci., 50(22): 985-991.
64. O'Brien, R.D. (1967). Insecticdes action and metabolism. Academic Press, London, New York.
65. Pandi Bhaskaran, (1991): Use of biochemical parameters in biomonitoring of pesticide pollution in some freshwater fishes. J. Ecotoxicol. Environ. Monit., 2: 101-104.
66. Phipps, G.L. and Holcombe, G.W. (1985). A method for aquatic multiple species toxicant testing: acute toxicity of 10 chemicals to 5 vertebrates and 2 invertebrtes. Environ. Pollut. Ser. A Ecol. Biol., 38: 141-157.
67. Ramamurthy, K. and Jayantha Rao, K. (1990). Toxic impact of heptachlor on carbohydrate metabolism in the tissues of a freshwater edible fish *Channa punctatus* (Bloch). Nateon. Publications, 3: 287-291.
68. Rao, D.M.R. (1989). Studies on the relative toxicity and metabolism of endosulfan to the Indian major carp *Catla catla* with special reference to some biochemical changes induced by the pesticide. Pestic. Biochem. Physiol., 33: 220-229.
69. Ravisankar, P., Sarala Waghray, S. and Indira Devi (1992). The effect of sublethal concentration of synthetic pyrethroid, cypermethrin to the common carp, *Cyprinus carpio*. J. Environ. Biol., 13(2): 89-94.
70. Reddy, A.T.V. and Yellamma, K. (1991). Perturbations in carbohydrate metabolism during cypermethrin toxicity in fish, *Tilapia mossambica* (Peters). J. Biochemistry, 23(4): 633-638.

71. Roberts, M. and Boyce, C.B.C. (1972). Methods in Microbiology (7-A ed). Norris, J.R. and Ribbows, 479 pp. D.W. Academic Press, New York.
72. Sastry, K. V. and Siddiqui (1984): "Some haematological, biochemical and enzymological parameters of a freshwater teleost fish, *Channa punctatus* 200 exposed to sublethal concentrations of quinolphos". Pestic. Biochem and Physiol., 22: 8-13.
73. Sastry, K. V. and Siddiqui (1984): Some haematological, biochemical and enzymological parameters of a freshwater teleost fish, *Channa punctatus* 200 exposed to sublethal concentrations of quinolphos. Pestic. Biochem and Physiol., 22: 8-13.
74. Sastry, K.V. and Adad Siddiqui (1984). Some haematological, biochemical and enzymological parameters of a freshwater teleost fish, *Channa punctatus*, exposed to sublethal concentrations of quinalphos. Pestic. Biochem. and Physiol., 22: 8-13.
75. Schmidt Nielson, B. (1975). Osmoregulation: Effect of salinity and heavy metal. Fed. Proc., 33: 2137-2146.
76. Searchy, D.G. and MacLenis, A.J. (1970a). Determination of DNA by the Burton Diphenylamine technique in experiments and techniques in parasitology Eds: Mac Lnnis A J and Voge M). W.H. Freeman and Co., San-Francisco, 190-191.
77. Smart, G.R. (1978). Investigation of toxic mechanisms of ammonia to fish - gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acute lethal concentrations. J. Fish. Biol., 12: 93-104.
78. Sobha Rani, A., Sudarshan, R., Reddy, T. N., Reddy, P. U. M. and Raju, T. N. (2000): "Effect of sodium arscnite on glucose and glycogen levels in fresh water teleost fish *Tilapia mosambica"*. Pollu. Res., 19(1): 129-131.
79. Srikanthan, T. N. and Krishna Murthy, C. R. (1955): "Tetrazolium test for dehydrogenases". J. Sci. Ind. Res, 14: 206.
80. Stamp WG, Lesker PA (1967): "Enzyme studies related to sex difference in mice with hereditary muscular dystrophy". Am. J. Physiol., 213: 587.
81. Sunitha, K. (2012): "Effects of endosulfan and fenvalerate on carbohydrate metabolism of the freshwater fish, *Labeo rohita* (hamilton)". Int. J. Pharm. Pharm. Sci., 4(1): 262-268.
82. U.S. EPA. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. U.S. Environmental Protection Agency, Washington, DC, EPA/600/6-87/008 (NTIS PB88179874), 1988.
83. Veeraiah, K. (2013). Heavy metal, cadmium chloride induced biochemical changes in the Indian major carp *cirrhinus mriqala* (Hamilton). International Journal of Bioassays, *2*(07): 1028-1033.
84. Veeraiah, K. and Durga Prasad, M. K. (1998): "Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish, *Labeo rohita* (Ham)". Proc. Acad. Environ. Bio., 7(2): 143-148.
85. Veeraiah, K. and Durga Prasad, M. K. (2001): "Studies on ventilatory patterns of fish under normal and stressed conditions using indigenously designed electronic recording instrument". Proceedings of the International Conference of ICIPACT-2001.
86. Veeraiah, K. and Durga Prasad, M.K. (1998). Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish, *Labeo rohita* (Ham). Proc. Acad. Environ. Bio., 7(2): 143-148.
87. Yeragi, S. G., Koli, V. A. and Yeragi, S. (2000): Effect of pesticide malathion on protein metabolism of the marine crab *Uca merionis*, J. Ecotoxicol. Environ. Monit, 10(1): 59-62.