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KEY WORDS: Imidacloprid, Acute Toxicity tests, biochemical changes hematological changes.

ACUTE TOXICITY OF IMIDACLOPRID TO FRESHWATER FISH *LABEO ROHITA* AND THE CONSEQUENTIAL BIOCHEMICAL CHANGES

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ABSTRACT

The fresh water fish *Labeo rohita* was exposed to the pesticide Imidacloprid, a nicotiniod Insecticide and determined the static and continuous flow through acute toxicity of the test toxicant for 24, 48, 72, and 96 hours. The per cent and probit values were taken and the LC_{50} values were calculated by Finney's probit analysis. The feeding of the test fish was stopped one day prior to the experimentation. The static LC_{50} values were 662.2 ppm, 654.6 ppm, 605.0 ppm, and 574.0 ppm respectively for 24, 48, 72 and 96 h. The continuous flow through values were 571.6 ppm, 538.7 ppm, 527 ppm and 509.7 ppm respectively for 24, 48, 72 and 96 h. The biochemical changes were estimated by following the standard protocols. Significant biochemical changes in different tissues were observed. The results obtained were discussed with the available literature.

INTRODUCTION

The increasing use of pesticides for the control of pests in agriculture including commercial and household production of vegetables cause potential health hazards to live stock, especially to fish, frogs, birds and mammals. Among the pesticides of the three classes' viz. organochlorines, organophosphates and carbamates, organochlorines are more persistent (Murthy, 1986; O' Brien, 1967; Edwards, 1973). Pesticides and related chemicals destroy the delicate balance between species that characterizes a functioning ecosystem. The qualitative and quantitative usage of chemicals is great concern ecologically. The discriminate use of chemicals is for the control of insect pests by elimination of target species whereas the indiscriminate usage has posed the problem on non-target organisms including man. Imidacloprid is relatively a new, systemic chloro-nicotinyl insecticide (Caroline Cox, 2001). It is used as a crop and structural insecticide, a seed treatement and a flea control treatment chemical. It is used for the control of sucking insects including rice hoppers, aphids, thrips, white flies, termites, soil insects and some beetles. It is most commonly used on rice, cereal, maize, potatoes, vegetables, sugar beet, fruit, cotton etc. Sarkar, (1999) reported that the hydrolysis half-life varies from 33 to 44 days at the same P^H and temperature. Imidacloprid was found to be stable in acidic and neutral water, but more readily hydrolyzed in alkaline water (Zheng et al., 1999). The formulation of the insecticide can affect the half-life. In wetable powder formulations, persistence increased by 3to 6 days compared to liquid formulation (Sarkar, 1999). Imidacloprid is designed to be effective by contact or ingestion (Tomlin, 2006). It is a systemic insecticide that trans locates rapidly through plant tissues following application (Tomlin, 2006; Fossen, 2006). Imidacloprid acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system (Buckingham et al., 1997).

Akhtar (1986) stated that the indiscriminate use of herbicides and other agrochemicals, careless handling, accidental spillage or discharges of treated effluents into natural water ways have harmful effects on the fish population and other forms of aquatic life and may contribute long-term effects in the environment. The usual measure of the environmental effect of any pollutant on an animal is mortality; however, other effects which are more delicate and indicate of physiological changes may ultimately detrimental to a population survival. The impairment of behavioral, physiological functioning could result in a gradual reduction in the adaptive capacity of species, leading to a decrease in their survival ability and population level. In order to have a thorough knowledge of the extent and type of damage caused by these insecticides, morphological and biochemical parameters should be established and pesticides not only induce morphological or pathological changes but also cause significant biochemical alterations in the living system (O"Brien, 1977; Edwards, 1973). According to Shakoori et al. (1976) in order to have a thorough knowledge of the extent and type of damage caused by these insecticides, morphological and biochemical parameters should be established. Pesticides cause significant bio-chemical alterations along with morphological or pathological changes in the living system (Edwards, 1973; 'O'Brien, 1977).

Hence, in the present study an attempt has been made to study the extent of damage caused by the test toxicant Imidacloprid to the test organism fresh water fish *Labeo rohita* (Hamilton) which is extensively cultured in this region and commonly named as rohu. Several aspects such as toxicity, Bio-chemical changes and hematological changes were studied.

MATERIALS AND METHODS:

The freshwater fish Labeo rohita measuring about 8-9 cm in length and 22.8±2 g in weight 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 282°C. During the period of acclimatization the fish were fed daily with fish meal rice bran or ground nut cake 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.

Preparations of Stock Solutions:

Imidacloprid, a soluble concentrate(SL) pesticide was dissolved in acetone without any agitation immediately prior to use. Doses of imidacloprid were prepared with 1gm of imida dissolved in 100ml of acetone as stock solution and working standard concentrations were prepared afresh at the time of experiment. (APHA, 1998).

Finney's probit analysis (Finney, 1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LC_{50} values. The respective probit values were taken from Table IX of Fisher and Yates. Blood glucose level was estimated by using the method of Folin and Wu (1962). The glycogen 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 amount of Cholesterol (lipid) was estimated by Folch et al., (1957) method.

RESULTS AND DISCUSSION:

The 24, 48, 72 and 96 h LC_{50} values of imidacloprid (17.8% SL)

(TATAMIDA) in static and continuous flow through methods for the fresh water fish Labeo rohita were;

Static: 662.2 ppm,654.6 ppm, 605.0 ppm, and 574.0 ppm.
C.F.M; 571.6 ppm, 538.7 ppm,527 ppm and 509.7 ppm.

Imidacloprid is moderately toxic to both aquatic and terrestrial organisms. In the present study, it was observed that the static LC_{50} values were higher, compared to the continuous flow-through values. The toxicity can be influenced by exposure conditions, formulation, source and size of fish and water quality. Borthwick *et al*. (1985) and Mayer (1987) reported that LC_{50} values were 2-5 times higher in static systems in marine fish. The size of the fish had been reported to influence toxicity in static tests, possibly because absorption of exposure concentration by fish (Barron , 1993). The continuous flow-through system LC_{50} values were relatively less compared to the static values. This may be due to the constant maintenance of concentration in flow-through system. The static values were higher because of bioaccumulation, absorption to toxicant chamber walls and degradation of pesticide effect of the compound.

Imidacloprid is a relatively new, systemic chloro-nicotinyl insecticide (Caroline Cox, 2001). It is used as a crop and structural pest insecticide, a seed treatement and a flea control treatement chemical. It is used for the control of sucking insects including rice hoppers, aphids, thrips, white flies, termites, soil insects and some beetles. It is most commonly used on rice, cereal, maize, potatoes, vegetables, sugarbeet, fruit, cotton. Based on the available aquatic toxicity profile, imidacloprid had variable toxicity (LC_{50} Values) to aquatic vertibrates and invertebrates (EPA, 1998a; Stark and Banks, 2001; Cleveland et al., 2001).

The toxicity of imidacloprid to fish is moderately less. The 96-hour LC_{50} of imidacloprid is 211 mg/L for rainbow trout, 280 mg/L for carp, and 237 mg/L for golden orfe. In a test with the aquatic invertebrate Daphnia, the 48- hour EC_{50}(effective concentration to cause toxicity in 50% of the test organisms) was 85 mg/l Kidd, and James 1994). Products containing imidacloprid may be very toxic to aquatic invertebrates. In the present study, it was observed that the static LC_{50} values were higher, compared to the continuous flow-through values. The toxicity can be influenced by exposure conditions, formulation, source and size of fish and water quality.

In the present investigation, when the fresh water fish, *Labeo rohita* were exposed to sub-lethal concentration of imidacloprid for 96 hours, several behavioral changes were observed. The control fish behaved in a natural manner i.e., they were active with well-coordinated movements. They were alert to the slightest disturbance. In the toxic environment fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium. They slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies. Opercular movements increased initially in all exposure periods but decreased steadily later in the lethal as compared to the sub-lethal exposure periods. The surfacing phenomenon was more in fish exposed to lethal concentration than sub-lethal concentration compared to the control fish. Behavioral changes are the most sensitive indication of potential toxic effects. Impact of different pesticides on the behavior of *Labeo rohita* have been studied by various workers (Marigoudar *et al*., 2009; Anita *et al*., 2010; Dube and Hosetti, 2010; Nagaraju *et al*., 2011; Imtiyaz Ahmad Bhat *et al*., 2012; Walia *et al.*, 2013; Ghazala *et al*., 2014; Selvam *et al.*, 2014).

Glycogen:

The results of the present study along with calculated values for glycogen in control and exposed fish along with per cent change over control, with and standard deviations after 96 h exposure were presented in Table.1 and graphically represented in Figures. 1.

The total glycogen levels of brain, liver, gill, kidney and muscle were more or less stable in control fish during the experiment. The total glycogen levels decreased on exposure to sub-lethal concentration of imidacloprid for 96 h.

The maximum level of total glycogen was found in liver and minimum in brain. The following was the order of total glycogen content in different tissue of control fish.
Liver > Gill > Muscle > Kidney> Brain

Table 1. Changes in Glycogen content (mg/gram wet weight of the tissue) and % change over the control in different tissue of the fish, *Labeo rohita* under exposure to sub-lethal concentration of imidacloprid for 96 h.

Tissue	Control	Sub-lethal	Percent change
Liver	111.160.53	86.550.57	22.1393
Muscle	54.490.55	48.360.59	11.2498
Gill	64.990.70	55.140.76	15.1562
Kidney	84.040.64	64.240.50	23.5602
Brain	86.040.73	82.140.98	4.5328

Results are the mean values of five observations
Standard Deviation was indicated as (±)
Values are significant at p<0.05

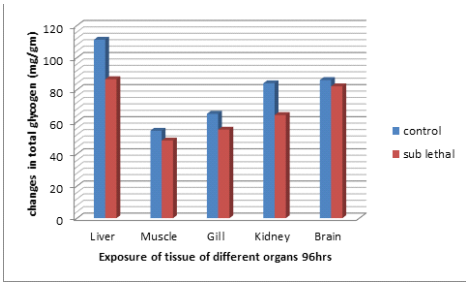

Fig 1. Changes in Glycogen content (mg/gram wet weight of the tissue in different tissue of the fish, Labeo rohita on exposure to sub-lethal conc of imidacloprid for 96 h.

The glycogen content, after exposure to sub-lethal dose of imidacloprid in, Labeo rohita at 96 hrs, was found to decrease highest in kidney and liver followed by gill,muscle and brain and lowest in brain. The depletion of glycogen may be due to utilization of stored carbohydrates in liver for energy production as a result of pesticide-induced hypoxia.

The results indicated that the liver, a vital organ of carbohydrate metabolism, was drastically affected by imidacloprid. The glycogen content in liver of the exposed fish for sub-lethal concentration was reduced. Fish liver is a primary organ for detoxification (Kabeer et al., 1979). Hence, it might be due to the presence of the toxicant in the liver, through hepatic portal system in abundance for detoxification and disposal. The impairment in the glycogen content of liver has also influenced the glycogen content in the gills, muscle, kidney and brain. Decrease in tissue carbohydrate content might also be due to either decreased synthesis as a consequence of toxic stress or breakdown (Dezwaan and Zendee, 1972). The carbohydrates were considered to be the first among the organic nutrients to decrease under any physiological stress conditions imposed on the animal (Clarke, 1987). The earlier observations on the effect of pesticide on carbohydrate metabolism in various species indicated an attenuation of the energy reserve under pesticide stress (Rama Murthy, 1988; Radaiah, 1988; Kabeer *et al.*, 1979; Holden, 1974).

The reduction of carbohydrates suggests the possibility of active glycogenolysis and glycolytic pathway to provide excess energy in stress condition (Remia et al., 2008). Saravanan et al. (2010) reported that the neem leaf extract of biopesticide, Azadiracta indica caused significant alteration in glycogen and protein content of liver and muscle of freshwater fish, Labeo rohita. Anita Susan et al., (2010) observed that the liver, a vital organ of carbohydrate metabolism was drastically affected by fenvalerate in C.mrigala and Labeo rohita. She also stated that a highly significant decrease in glycogen content was noticed in sub-lethal concentrations of technical grade fenvalerate in most of the tissues in both the experimental fish. The decrease in glycogen content was higher in sub-lethal concentrations than in lethal concentrations. Anthony Reddy, *et al*. (2015) observed that the liver a vital organ of carbohydrate metabolism was drastically affected by confidor. Fish liver is the primary organ for

detoxification. Hence it might be due to this stress, the glycogen levels were decreased more in liver of fish, *Labeo rohita*.

In the present study, it was observed that exposure to sub-lethal dose of imidacloprid, in the fish Labeo rohita caused moderately changes in the total glycogen level which is due to toxic stress, resulting in the disruption of enzymes associated with carbohydrate metabolism.

Total Proteins

The calculated values of total proteins and per cent change over control along with standard deviation and error bars with standard error were presented in. Table.2. and graphically represented in Figures 2.for 96 hrs.

In control fish, the total protein levels of brain, liver, gill, kidney and muscle of control fish were more or less stable during the 96 h cycle of the experiment. The maximum level of total protein content was found in liver and minimum in brain. The following was the order of total protein levels in different tissues of control fish.

Liver > Kidney > Gill > Muscle > Brain

The variation in distribution suggests the gradual difference in metabolic calibers of various tissue. The present trend in the tissue is justifiable in the wake of mechanical tissue of muscle intended for mobility and it does not participate in metabolism. Liver is the seat for the synthesis of various proteins, and also the regulating centre of metabolism.

Table.2. Changes in protein content (mg/gram wet weight of the tissue) and % change over the control in different tissue of the fish, Labeo rohita on exposure to sub-lethal conc of imidacloprid for 96 h.

Tissue	Control	Exposure	Percent Change
Liver	93.440.65	65.856.41	29.5270
Muscle	26.130.47	23.45	10.2564
Gill	23.710.54	18.72±0.37	21.0460
Kidney	54.740.57	45.360.66	17.1356
Brain	34.54±0.68	21.140.60	33.7956

Results are the mean values of five observations
Standard Deviation is indicated as (±)
Values are significant at p<0.05

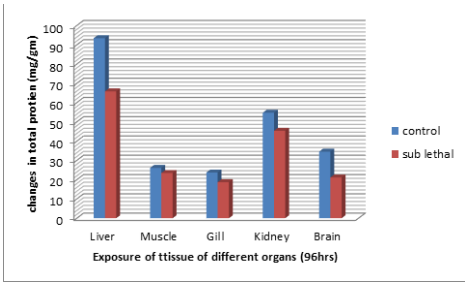

Fig.2. Changes in protein content (mg/gram wet weight of the tissue) in different tissue of the fish, *Labeo rohita* on exposure to sub-lethal conc of imidacloprid for 96 h.

Under exposure to sub-lethal dose of imidacloprid the total protein content was found to be decreased in all the tissues at 24, 48, 72 and 96 h. Maximum decrease was noticed in liver and muscle. The minimum decrease was almost equal in brain and kidney tissues. The per cent changes over controls at four test periods were in the order of Brain > Liver > Gill > kidney > Muscle

In the present study, liver and muscle tissue of the fish *Labeo rohita* evidenced a significant per cent change in the protein content under sub-lethal concentration of imidacloprid.

The decreased trend of the protein content as observed in the present study in most of the tissue may be due to metabolic utilization of the ketoacids in 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. The liver is the prime location for detoxifying pesticides in fish. The protein, one of the main source of energy for the fish, helps in the body tissue building, muscle glycogen and protein response appear particularly suitable for measuring stressful level of pollutants and have been used as indicators of stress in fish. The changes and decrease in protein level might be due to inhibition or induction of metabolizing enzymes by administration of toxicants (Narayan swamy, 1995).

The protein content was decreased after *Labeo rohita* exposed to cypermethrin (Veeraiah and Durgaprasad, 1996). Rao (2002) reported that the levels of protein decreased significantly in liver, kidney and muscle of *Catla catla* treated with endosulfan. Aruna Khare *et al*. (2000) observed a significant increase in total protein content in kidney of exposed fish Clarias batrachus during the first week and thereafter a gradual decrease in protein content was observed in the later periods of exposure to sub-lethal concentrations of malathion. Anita Susan et al. (2010) reported that there was a significant decrease of total proteins in all tissues of *L. rohita* and *C. mrigala*. Changes in brain, gill and kidney in both the fish were relatively less affected than hepatic tissue under fenvalerate toxicity. The protein, glycogen and free amino acids were decreased in exposed fish with the increase in period of exposure gradually. The depletion of protein might also be attributed to spontaneous utilization of amino acids in various catabolic reactions inside the organism in order to combat the stress condition (Borah and yadav, 1996a). Thenmozhi et al. (2011) observed a gradual decrease in the protein content of treated fish, Labio rohita which was due to the disruption of carbohydrate metabolism, destruction of protein and protein synthesis machinery and inhibition of ATP synthesis.

Rathod (2013) observed that the biopesticide, *Azadirachta indica* exposure to freshwater cat fish, *Heteropneustes fossilis* showed decreased trend in total protein in liver and muscle tissue. A significant decrease was observed in muscle and liver tissues due to the disturbance of biochemical and physiological activity of those organs and proteolysis in freshwater cat fish. Anisuddin Siddiqui et al. (2013) observed that the quantity of total protein in the liver of experimental fish *Clarias batrachus* exposed to neem (*Azadirachta indica A. Juss*) after 12, 24, 48, 72 and 96 h exposure were found to be decreased.

In the present investigation, the decrease of proteins under exposure to imidacloprid was more apparent in sub-lethal concentrations. The maximum decrease (per cent change) of proteins was observed in brain, kidney and liver during all the time period of 96 h,. All these investigations support the present study, of decreasing trend of proteins in the fish *Labeo rohita*, exposed to sub-lethal concentration of imidacloprid.

Lipids :

Lipids were heterogenesis group of complex macro molecules. They were the components of living system, insoluble in water but soluble in polar solvents. They form energy rich reserves whose calorific values are twice that of carbohydrates or proteins. The mobilization of lipid reserve in an organism testifies the imposition of high energy demands. Swami *et al*.,(1983) observed on the involvement of lipid metabolism exposed to pesticides are many.; Tiwari et *et al*.,(1987) ; and Singh *et al*.,(2011) stated that the shifts in lipid metabolism takes place when animals are exposed to toxicants.

The present study indicates the involvement of lipid metabolism in the fish Labeo rohita) reports on exposure to sub lethal concentration of imidachloprid the levels of total lipids declined, Whereas the levels of lipase activity and free fatty acids elevated on first day of exposure. The decline in total lipid levels was followed by elevation in the levels of lipase activity and free fatty acids elevation on first day of exposure. The decline in the total lipid levels or lipase activity and free fatty acids indicates the high energy demand associated with imposed imidachloprid stress. Some of the observations also supports the present study.

The decline of total lipid levels Anita Susan *et al.*,(1999) reported decreases in the total lipid content when *Catla catla* exposed to

pyrethroid,fenvalerare. Senthil kumar et al., (2007) found the reduction in lipid content of the fresh water crab Spiralathelphusa hydrodroma ofter exposure to pesticide, chlorpyrifos. Frontera et al.,(2011) observed decline in lipid content in hepatopancreas of fresh water crayfish cherax quadricarinatus on exposure to glyphosate

Lipase activity was initially elevated on day 1 exposure followed by its inhibition on 8 days exposure periods. This is clearly indicates the drastic decline in free fatty acids elevation in total lipids upto 8 days exposure period. Thus maximum percent inhibition in lipase activity and free fatty acids were seen at 8days exposure period. Kamble *et al*., (2000). Swami *et al*.,(1983) observed metabolic shift from carbohydrate to lipid metabolism through acetyl-CoA barrier leading to an increment in lipid content in the organs of fish water mussel lamellidens marginalis under pesticide toxicity.

The lipase activity and free fatty acid levels came nearer to control on 8 day exposure period, Where as levels of total lipids followed an opposite trend Metabolic compensation involves break down and synthesis of products necessary to cope up with altered situations. In conclusion the shifts in lipid metabolism may be to compensate with the altered situation shown by the fish for its survival.

IV.4.3.1 Triglycerides:

The calculated values of total Triglycerides and per cent change over control along with standard deviation and error bars with standard error were presented in. Table.3 and graphically represented in Figures.3. for 96 h.

In control fish, the total Triglycerides levels of brain, liver, gill, kidney and muscle of control fish were more or less stable during the 96 h cycle of the experiment. The maximum level of total Triglycerides content was found in liver and minimum in brain. The following was the order of total Triglycerides levels in different tissues of control fish.
Liver > Muscle > Gill > Kidney> Brain

Under exposure to sub-lethal concentration of imidacloprid the total Triglycerides content was found to be decreased in all the tissues at 96 h. Maximum decrease was noticed in liver and muscle. The minimum decrease or almost equal in both control and exposed brain and kidney tissues. The per cent changes over controls at four test periods were in the order of
kidney > Liver > Muscle > Gill > Brain

In the present study, liver and muscle tissue of the fish *Labeo rohita* evidenced a significant per cent change in the Triglycerides content under sub-lethal concentration of imidacloprid.

Table.3. Changes in Triglyceride content (mg/g wet weight of the tissue) and % change over the control in different tissue of the fish, *Labeo rohita* on exposure to sub-lethal concentration of imidacloprid for 96 h.

Tissue	control	Test	Percent change
Liver	30.2±0.13	21.1±0.2	30.1325
Muscle	28.9±0.22	22.4±0.28	22.4914
Gill	22.3±0.21	19.4±0.21	13.0045
Kidney	20.4±0.14	13.2±0.14	35.2941
Brain	15.2±0.18	13.2±0.18	13.1579

Results are the mean values of five observations
Standard Deviation is indicated as (±)
Values are significant at p<0.05

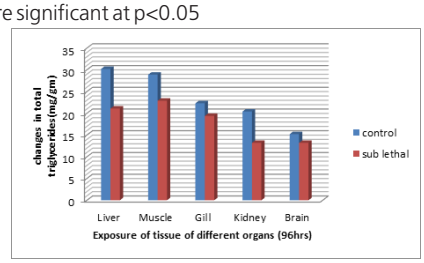

Fig .3. Changes in Triglyceride content (mg/g wet weight of the tissue) in different tissue of the fish, labeo, rohita on exposure to sub-lethal concentration of imidacloprid for 96 h.

The decreased trend of the Triglycerides content as observed in the present study in most of the tissue may be due to metabolic utilization of the ketoacids in 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. Tont (1979) stated that the fish is an important source of food for human nutrition.. The liver is the prime location for detoxifying pesticides in fish. The Triglycerides one of the main source of energy for the fish, helps in the body tissue building, muscle glycogen and protein response appear particularly suitable for measuring stressful level of pollutants and have been used as indicators of stress in fish.

Cholesterol:

The calculated values of total Cholesterol and per cent change over control along with standard deviation and error bars with standard error were presented in. Table.4 and graphically represented in Figure 4 for 96 hrs.

In control fish, the total Cholesterol levels of brain, liver, gill, kidney and muscle of control fish were more or less stable during the 96 h cycle of the experiment. The maximum level of total Triglycerides content was found in liver and minimum in brain. The following was the order of total Cholesterol levels in different tissues of control fish.
Gill > Liver > Muscle > Kidney > Brain

The variation in distribution suggests the gradual difference in metabolic calibers of various tissue. The present trend in the tissue is justifiable in the wake of mechanical tissue of muscle intended for mobility and it does not participate in metabolism. Liver is the seat for the synthesis of various Cholesterol and also the regulating centre of metabolism.

Under exposure to sub-lethal concentration of imidacloprid the total Cholesterol content was found to be decreased in all the tissues at 96 h. Maximum decrease was noticed in liver and muscle. The minimum decrease was almost equal in brain and kidney tissues. The per cent changes over controls at four test periods were in the order of
Gill > Brain > Muscle > Kidney>liver

In the present study, liver and muscle tissue of the fish Labeo rohita evidenced a significant per cent change in the Cholesterol content under sub-lethal concentration of imidacloprid Neeraja and Giridhar (2014) reported that the effect of sub-lethal concentration of deltamethrin (0.01ug/l) on total lipids, lipase activity and free fatty acids of the fish *Labeo rohita* and reported that the total lipids decline on 1stday exposure and gradually elevated on 7th day and 15th day on wards their levels gradually declined and came nearer to control 30th day exposure period. Cholesterol serves as energy source for fish metabolism and hence reveals their importance during stress condition (Jezreiska *et al*., 1982). The disturbance of fat metabolism is an indication impaired pancreatic functions (Jayantharao et al., 1984).

The decreased trend of the Cholesterol content as observed in the present study in most of the tissue may be due to metabolic utilization of the ketoacids in 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. Tont (1977) stated that the fish is an important source of food for human nutrition.. The liver is the prime location for detoxifying pesticides in fish. The Cholesterol one of the main source of energy for the fish, helps in the body tissue building, muscle glycogen and protein response appear particularly suitable for measuring stressful level of pollutants and have been used as indicators of stress in fish.

Table.4. Changes in Cholestrol content (mg/g wet weight of the tissue) and % change over the control in different tissue of the fish, Labeo rohita on exposure to sub-lethal concentration of imidacloprid for 96 h.

Tissue	control	Test	Percent change
Liver	28.0±0.37	22.4±0.25	20
Muscle	25.5±0.21	18.2±0.37	28.6275
Gill	26.2±0.41	16.4±0.14	37.4046
Kidney	24.5±0.14	19.3±0.18	21.2245
Brain	16.4±0.2	10.4±0.17	36.5858

Results are the mean values of five observations
Standard Deviation is indicated as (±)
Values are significant at p<0.05

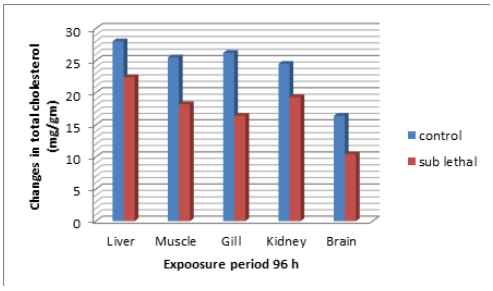

Fig.4. Changes in Cholestrol content (mg/g wet weight of the tissue) in different tissue of the fish, *Labeo rohita* on exposure to sub-lethal concentration of imidacloprid for 96 h.

Binu kumarai et,al (2013) reported that the effect of pesticide Endosulfan 35% EC on the Lipid content of the fresh water fish *Catla catla*. Padmabhushan et al, (2012) reported that the effect of Dimethoate on the level of cholesterol in fresh water *puntius ticto* (Ham) exposed to lethal and sub-lethal concentration of Dimethoate and reported that the cholesterol content decreased during exposure period in ovary, testis, intestine, muscles, gills, kidney and brain.

Neeraja and Giridhar (2014), observation on the involvement of lipid metabolism exposed to pesticide are (Singh and Singh 2011). The studies shows that shifts in lipid metabolism when animals are exposed to toxicants. The decline in total lipid level of lipase activity and free fatty acids on first day exposure indicates the high energy demand associated with imposed deltamethrin stress.

CONCLUSION:

The inhibition or activation of physiological activities by pesticides is due to the interaction between the animal and the chemical nature of the pesticide (Yun Peisun, 1970). The stress induced biochemical changes are described as secondary responses of the fish. The pesticides are causing untold damage to the nutritive value of the fish and the residues of many pesticides are lipophilic in nature they are accumulated in fat and transferred to higher carnivores via food chain causing damage to all the higher level organisms of the food chain.

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