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Glycogen and protein levels of *Ctenopharygodon Idella* exposed to Lambda (λ) Cyhalothrin

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Abstract
The freshwater fish *Ctenopharygodon idella* was exposed to sub lethal concentration of synthetic pyrethroid insecticide λ- cyhalothrin for a period of 1, 4, 8 and 12 days. The biochemical parameters of various organs such as gill, muscle, liver, kidney and brain were isolated after stipulated time. The glycogen content and total protein content were observed in test fish. In sub lethal concentrations of λ-cyhalothrin, the glycogen content and total protein content were decreased in all the tissues to compared with controls. In the present study on day 12, the maximum decrease of glycogen content was observed in liver (-36.11%) and minimum decrease was in brain (-17.33%), whereas the maximum decrease of total protein content was observed in brain (-64.13%) and minimum decrease was in gill (-26.57%) respectively.

Keywords: λ- cyhalothrin, biochemical changes, Ctenopharygodon idella, sub lethal concentrations, etc.

Introduction
Pesticides, being indispensable in modern agriculture, are the main harmful pollutants among the chemicals released by man into environment. In an ideal situation, a pesticide should fall exactly on the target and get degraded completely too harmless compounds. Ever since aquatic ecosystems have been considered potential sources of contamination, fish have been used as bioindicators and as biomonitor organisms for the monitoring or environments (Viana *et al.*, 2013) [1]. The use of biomarkers in environmental monitoring provides information on not only the intensity, tolerance limits and effects of pollutants on the organisms but also the process of transfer of these substances within the trophic levels. In fact, these markers serve as a 'warning signs' of environmental integrity. They may be used to guide the development of effective bioremediation measures before the environment would undergo irreversible damage (De La Torre *et al.*, 2005) [2]. Surface water contamination by pesticides usually depends on the agricultural season, while ground water contamination has a stronger persistence, which may cause continuous toxicological effects for human health if, they are used for public consumption (Bakouri *et al.*, 2008) [3]. Several studies reported that synthetic pyrethroids are highly toxic to a number of non-target organisms (Gowri *et al.*, 2013) [4] and these pyrethroids are readily absorbed by the gills of fish even at very low concentration (Edwards *et al.*, 1986; Clark *et al.,* 1975) [6]. Among the synthetic pyrethroids, λ- cyhalothrin is one of the world wide insecticide used in agriculture, home pest control, protection of food stuff and disease vector control (Fetoui *et al.*, 2010) [7]. Hence, this study is aimed to find out the biochemical changes, if any, in *Ctenopharygodon idella* exposed to sub lethal concentration of λ-cyhalothrin.

Materials and Methods
The freshwater fish of Indian major carp *Ctenopharygodon idella* were collected from ponds in around Kuchipudi, Guntur District brought to the laboratory, then acclimatized to the laboratory conditions in large plastic tanks with un chlorinated ground water for two weeks at a room temperature of $28\underline{+}20\ ^0$C prior to experimentation. LC_{50} for 96h was found out by using Probit method (Finney, 1971). For biochemical studies fishes were reared in sub lethal concentration for a period of 1, 4, 8 and 12 days. The glycogen content was estimated by the method of Kemp *et al.,* (1954) [8] and total protein content was estimated by Lowry *et al.*, (1951) [10].

Results and Discussion
The calculated values for *Ctenopharyngodon idella* exposed to sub lethal concentrations of λ-cyhalothrin, the glycogen content and total protein content along with standard deviation and

percent change is given. Present study the glycogen content and total protein content was depleted in all the tissues of test fish *Ctenopharygodon idella* exposed to λ -Cyhalothrin in sublethal concentrations for 1st, 4th, 8th and 12 days to compare with controls. In the control fish *Ctenopharygodon idella* the glycogen content was in the order of Liver>Kidney>Gill>Muscle>Brain.

Under sublethal exposure to λ-cyhalothrin for day 1, maximum percentage of depletion was in kidney (-16.36%) followed by liver, brain, muscle and minimum depletion in gill (-3.57%) whereas day 4 sublethal exposure, maximum percentage of depletion was in kidney (-21.82%) followed by liver, muscle, brain and minimum depletion in gill (-7.14%); for day 8 sublethal exposure, maximum percentage of depletion was in liver (-32.64%) followed by kidney, brain, muscle and minimum depletion in gill (-10.71%) and for day 12 sublethal exposure, the maximum percentage of depletion was in liver (-36.11%) followed by kidney, muscle, gill and minimum depletion in brain (-17.33%) were observed in test fish *Ctenopharygodon idella*. In the present study glycogen content showed marked decrease in the exposure periods increased λ- cyhalothrin. The results indicated that in almost all the tissues of brain, muscle, gills, liver and kidney were affected by this synthetic pyrethroid at sub lethal exposures.

In the control fish *Ctenopharygodon idella* the total protein content was in the order of Liver>Brain>Kidney>Muscle>Gill. Under sublethal exposures of λ-cyhalothrin for day 1, maximum percentage of depletion was in Kidney (-15.34%) followed by other tissues like brain, muscle, liver and minimum depletion in gill (-3.61%); for day 4 maximum percentage of depletion was in brain (-33.25%) followed by other tissues like kidney, muscle, liver and minimum depletion in gill (-13.38%): for day 8 maximum percentage of depletion was in brain (-51.45%) followed by kidney, muscle, liver and minimum depletion in gill (-19.86%) and for day 12 the maximum percentage of depletion was in brain (-64.13%)followed by kidney, muscle, liver and minimum depletion in gill (-26.57%) were observed in test fish *Ctenopharygodon idella*. In the present study total protein content showed marked decrease in the exposure periods increased λ- cyhalothrin. The results indicated that in almost all the tissues of brain, muscle, gill, liver and kidney tissues were affected by this synthetic pyrethroid at sub lethal exposures.

In the present investigations the test species, *Ctenopharygodon idella* has shown decreased glycogen content when exposed to sub lethal concentrations of λ-cyhalothrin for 1, 4, 8 and 12 days. The significant decrease was observed in liver tissue, because the liver is the site of carbohydrate metabolism and the decrease is deleterious for the survival of fish. Glycogen is the major storage form of carbohydrate in animals, 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, 2012; Nagaraju and Rathnamma, 2013) [11, 12]. The fall in glycogen content in the body tissues of the fish indicates its rapid utilization by the respective tissue because of toxic stress of the pesticide. Present study, depletion of glycogen might be due to direct utilization of the compound for energy generation, a demand caused by pesticide-induced hypoxia. Under hypoxic condition, the fish derives its energy from anaerobic breakdown of glucose which is available to the cells. Though gill 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 (Lehninger 2008) [13]. A reduction of brain glycogen of fish *Ctenopharygodon idella* exposed to λ-cyhalothrin the sub acute doses points to deranged intermediary metabolism primary to ATP production.

Pallavi Srivastava *et al.*, (2016) [14]; Kumar and Pandey, (2014) [15], the glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Muscle glycogen can be exhausted during vigorous activity and in stress conditions. Tiwari and Singh, (2009) [16], liver tissue is a reservoir of glucose for other tissues when dietary glucose is not available and liver being a detoxificative organ it is expected that toxicant could reach there for detoxification and disposal. Anitha susan *et al.*, (2010) [17], reported that envisages the variations of glycogen and protein contents in major tissues like liver, muscle, kidney, brain and gill of the two carps *Labeo rohita* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of pyrethroid, Fenvalerate.

Oluah *et al*., (2014) [18], liver glycogen and serum glucose concentrations decreased with increasing **λ-** cyhalothrin pesticide concentration and duration of exposure was observed in African cat fish *Clarias gariepinus*. Muthukumaravel *et al.*, (2013) [19] the total glycogen content in liver was depleted by the effect of sub lethal concentrations of λ-cyhalothrin in *Oreochromis mossambicus*. Suneetha, (2012) [20] decline in total glycogen was observed in all the tissues brain, gill, kidney, liver and muscle of fresh water fish *Labeo rohita* when exposed to fenvelerate for 24h and 15 days. Decrease of glycogen content in liver and muscle tissue in *Clarias batrachus* was observed under sublethal exposure of arsenic (Kumara and Ahsan, 2011) [21]. *Cirrhinus mrigala*, treated with Fenthion and Avaunt showed decreased glycogen content (Israel Stalin *et al.*, 2012) [22]. Jimena *et al.*, (2011) [23] observed reduced glycogen level in different tissues in fresh water cray fish *Cherax quadricarinatus* when exposed to glyphosate acid and polyoxyethylen amine.

Proteins are ubiquitous components of all living tissues of an organism. They serve indispensable functions in cellular architecture, catalysing metabolic regulation and contractile processes and acts as an important weapon in defense arsenal of many higher organisms. N. Padmavathi *et al.*, (2017) [24] the decrease in total protein content in freshwater fish *Mysstus vittatus* was exposed to sub lethal concentrations of the cypermethrin. Pallavi Srivastava *et al.*, (2016) [14], protein is the alternative source of energy and reduction in level of protein in experimental fish under pesticides influence indicates hepatic insufficiency and probably malnutrition. Protein reduction might observe due to high-energy demand in TCA cycle. Mastan and Rammayya, (2010) [31], decrease in total protein content was observed in *Channa gachua* on exposed to sublethal concentrations of dichlorvos. Patil and David, (2009) the decrement in the proteins of freshwater fish *Labeo rohita* treated with sublethal concentrations of malathion suggests the existence of high proteolytic activity and impairment in the protein biosynthesis and degradation is due to oxidative stress, decreased protein levels may be attributed to the destruction or necrosis of cells and consequent impairment in protein synthesis (Satyavardhan, 2010) [33]. The liver serum protein content was depleted in African cat fish *Clarias gariepinus* (Burchell), on exposure to sub lethal concentrations 0.01 and 0.04mg/L of **λ-** cyhalothrin for 21 days (Oluah *et al*., 2014) [18].

Nagaraju and Rathnamma, 2013 [12], decreased proteins were observed in liver, kidney and followed by other tissues of freshwater fish *Labeo rohita* after 8 days exposure of dimethoate relative to control. In the present study maximum decrease in protein content is observed at sub-lethal exposure in all tissues. There is no storage form of protein and the reserve protein appears to be drawn from the tissues themselves and organs such as liver, kidney and blood during the time of fasting. Veeraiah *et al.,* (2013) [34] observed that decreased levels of proteins in gill, liver, muscle and kidney of freshwater fish *Labeo rohita* exposed to sub lethal concentrations of indoxacarb. The levels of protein decreased significantly in liver, brain, gill, kidney and muscle *Ctenopharygodon idella* treated with malathion (Satyavardhan, 2013) [33].

Exposure to carbryl for 4 and 21 days, decreased total protein content in fish *Mugil cephalus,* Shivanagouda *et al.,* (2013) [36]. The decreased trend of the protein content as observed in the present study in most of the fish tissues 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) [37]. Pallavi Srivastava *et al.,* (2016) [14]; Lakshmanan *et al.,* (2013) [38], dichlorvos showed significant impact in total protein content in muscles, liver and kidney of *Oreochromis mossambicus.* Neeraja and Giridhar, (2014) [39] the levels of total protein declined relative to control in all organs of fish Labeo rohita on exposure of deltamethrin upto a period of 15 days.

The present study under sublethal exposure, the total protein content was found to decrease in all the tissues of *Ctenopharygodon idella* evidenced by other researchers. M.K Ahmad *et al.,* (2012) [40], studied the effect of λ- cyhalothrin and Neemgold to 96 h LC_{10}, LC_{20}, and LC_{40} in the gill, liver and ovary of zebra fish *Danio rerio.* It was found that the total protein content was reduced to 38, 46 and 45% in gill, liver and ovary respectively on exposure to LC_{40} dose for 21 days. In the present study, decrement was more apparent in sub lethal concentrations, might be due to detoxification of enzymes or mechanism, which is apparently slow. Further the insecticides probably acts continuously on the animal system for longer periods, thereby rendering detoxification mechanism less efficient and thus making recovery slow at sub lethal concentrations. Khare. A and Singh. S (2002) [41] observed that the sub lethal concentrations of malathion showed 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.

All these investigations support the present study of decreasing trend of proteins in the tissues of the fish *Ctenopharygodon idella* exposed to sub lethal concentrations of λ- cyhalothrin. Several other investigations also revealed a decrease in protein profiles with synthetic pyrethroids. Cypermethrin and malathion decreased protein contents in *Labeo rohita* (Thenmozhi *et al.*, 2011) [25]. Thiamethoxan affected liver total protein of *Oreochromis niloticus* (Bose *et al.*, 2011) [26] while propiconazole and mancozeb induced changes in protein content in *Clarias batrachus* (Srivasatava and Singh, 2013a) [27]. The total protein was decreased in *Tilapia mossambica* on exposure to synthetic pyrethroid cypermethrin (Jipsa *et al.*, 2014) [28]. Same result was also observed in fresh water fish *Alburnus mossulensis*, exposed to fenpropathrin at the concentrations of 2.75, 5.50 and 12.60µg/L for 15 days (Mahdi Banaee *et al*., 2014) [29]. Fry of *Cyprinus carpio* were exposed to Karate an commercial formulation of **λ-** cyhalothrin at different concentrations (Bibi *et al*., 2014) [30].

Cypermethrin exposure resulted in significant decrease in protein contents in *Tor putitora* (Ullah *et al.*, 2014) [42]. **λ-** cyhalothrin exposure resulted in significant decrease in protein contents of *Oreochromis mossambicus* and *Cyprinus carpio* (Muthukumaravel *et al.*, 2013; Bibi *et al.*, 2014) [19, 30]. The biochemical analysis on fresh water teleost fish *Colisa fasciatus* on exposure to LC_{50} of cypermethrin was studied, (Shailendra Kumar Singh *et al.*, 2010) [43] significant dose dependent decrease in total protein level in both muscle and liver tissues of fish. Padmini and Rajaram (2016) [44] studied decreased levels of glycogen and protein on exposure of chlorpyrifos in liver and kidney of *Channa gachua*.

Table 1: Changes in the glycogen (mg/g wet weight of tissue) in different tissues of *Ctenopharygodon idella* on exposure to sub lethal concentrations of λ- cyhalothrin

Tissue	Control	Exposure Periods (days)							
		1		4		8		12	
		Sub lethal	% change	Sub lethal	% change	Sub lethal	% change	Sub lethal	% change
Gill	0.84 ± 0.02	0.81 ±0.02	-3.57	0.78 ±0.01	-7.14	0.75 ±0.01	-10.71	0.69 ±0.01	-17.86
Muscle	0.77 ± 0.02	0.74 ± 0.02	-3.9	0.70 ± 0.01	-9.09	0.67 ± 0.01	-12.99	0.62 ± 0.01	-19.48
Liver	1.44 ± 0.02	1.38 ± 0.01	-4.16	1.13 ± 0.01	-21.53	0.97 ± 0.01	-32.64	0.92 ± 0.01	-36.11
Kidney	1.10 ± 0.05	0.92 ± 0.01	-16.36	0.86 ± 0.01	-21.82	0.81 ± 0.01	-26.36	0.78 ± 0.01	-29.09
Brain	0.75 ± 0.02	0.72 ± 0.01	-4	0.69 ± 0.02	-8	0.64 ± 0.01	-14.63	0.62 ± 0.02	-17.33

Results are the mean values if five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant ( )

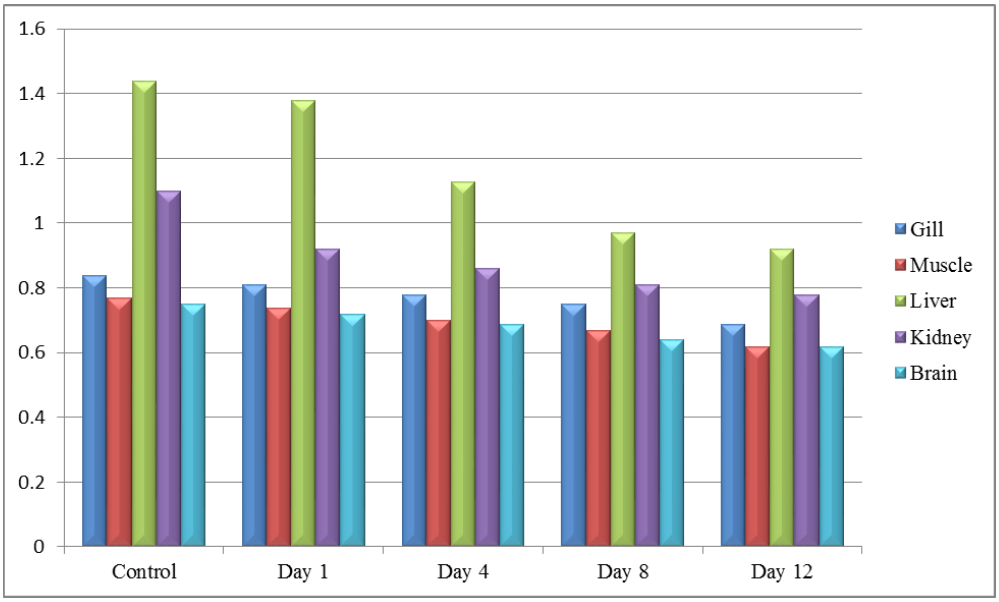

Fig 1: Change in Glycogen content (mg/g wet weight of tissue) in different tissues of *Ctenopharygodon idella* on exposure to sublethal concentration of λ- cyhalothrin (mean ± S.D, n=5 observations, $p<0.05$)

Table 2: Changes in the Total protein (mg/g wet weight of tissue) in different tissues of *Ctenopharygodon idella* on exposure to sub lethal concentrations of λ- cyhalothrin

Tissue	Control	Exposure Periods (days)							
		1		4		8		12	
		Sub lethal	% change	Sub lethal	% change	Sub lethal	% change	Sub lethal	% change
Gill	60.82 ± 0.05	58.63 ±0.17	-3.61	52.68 ±0.17	-13.38	48.74 ±0.12	-19.86	44.66 ±0.48	-26.57
Muscle	72.36 ± 0.45	65.42 ± 0.09	-9.6	57.14 ± 1.15	-21.03	43.36 ± 0.61	-40.07	35.04 ± 0.61	-51.57
Liver	101.81 ± 0.03	92.41 ± 0.48	-9.23	84.45 ± 0.27	-17.05	75.91 ± 0.37	-25.43	62.03 ± 0.24	-39.07
Kidney	77.94 ± 0.82	65.98 ± 0.56	-15.34	54.86 ± 1.31	-29.61	40.96 ± 0.70	-47.44	28.45 ± 0.18	-63.13
Brain	98.99 ± 0.79	86.49 ± 0.56	-12.63	66.07 ± 0.80	-33.26	48.05 ± 1.22	-51.46	35.50 ± 0.33	-64.14

Results are the mean values if five observations and the Standard Deviation is indicated as ± and figures in % change over control and sub lethal respectively. Values are significant ( )

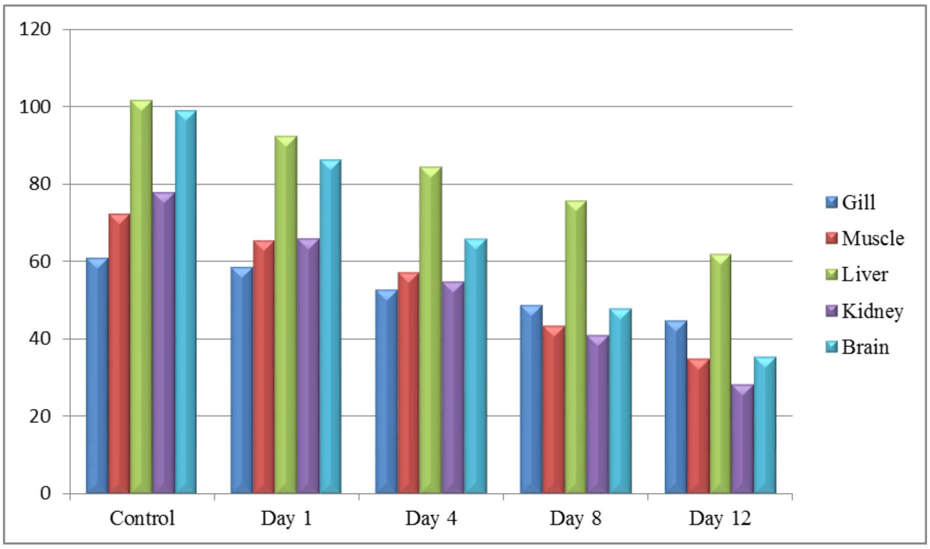

Fig 2: Change in Total protein content (mg/g wet weight of tissue) in different tissues of *Ctenopharygodon idella* on exposure to sublethal concentration of λ- cyhalothrin (mean ± S.D, n=5 observations, $p<0.05$)

Conclusion

The present study revealed that the pesticide λ-cyhalothrin is potent cause toxic responses, even biochemical alterations, in aquatic organisms like fish *Ctenopharygodon idella* at different sub lethal exposure periods, confirms that this species can be used as bioindicator in polluted areas. Pesticides especially the non-degradable ones, even in minute levels, are causing a stress to aquatic organisms. But an agricultural effort reducing the use of pesticides and implementing natural remedies for pest encroachment can become one solution for pesticide pollution. Therefore extensive use of these pesticides should be avoided near water bodies and the applications should be judicious and rationalized. So that cultured fish with high nutritive value could safely be utilized for human consumption.

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