



Impact of Lambda-Cyhalothrin on tissue lipid in freshwater air breathing fish- *Clarias batrachus*

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Abstract

Lambda-cyhalothrin is a pyrethroid insecticide widely used for the control of insect pests all over the world to increase the production of food grain and many other agricultural products. Synthetic pyrethroids are commonly used because of their rapid bio degradability and non-persistent nature. Pyrethroids are potent neurotoxins that interfere with nerve cell function resulting in repetitive firing of neurons and eventually causing paralysis. The impact of month-long exposure to water borne Lambda-Cyhalothrin on *Clarias batrachus* was evaluated through changes of biochemical parameter. Fish was exposed 0.001, 0.003, 0.005, 0.007, 0.009 μg^{-1} and control for 30 days. The tissue lipid in muscle, liver and gill were measured. There were significant ($p < 0.05$) alterations between the control values and the exposed groups on all the tissues. The alteration was significantly dependent on dosage ($p < 0.05$) and period of exposure.

Keywords-Lambda-Cyhalothrin, Tissue lipids, *Clarias batrachus*.

Introduction

Researchers have proved with evidence that indiscriminate use of insecticides is threatening the very survival of major ecosystems by disrupting the relationship between organisms and environment (Mahdi Banaee, 2013). One such threat is being experienced in conserving freshwater particularly in the agricultural domain throughout the developing countries. Contamination of water by insecticides is mainly due to intensive agriculture combined with surface runoff and subsurface drainage, usually within a few weeks after application of insecticides (Banaee *et al.*, 2011a). Most insecticides ultimately find their way into rivers, lakes and ponds (Vryzas *et al.*, 2009; Arjmandi *et al.*, 2010) and have been found to be highly toxic to non-target organisms that inhabit natural environment close to agriculture fields. Different levels of pesticide concentrations are present in various types of waste waters and numerous studies on such waste waters have found them to be toxic and detrimental to aquatic organisms, especially fish species (Uner *et al.*, 2006).

Pyrethroids are synthetic analogues of the natural pyrethrins, extracts of the ornamental *Chrysanthemum cinerariae folium* and its related species. Pyrethrins had been used for decades for the control of insects. Pyrethroids disrupt the nervous system of the insects and ensures protection of food grains and other agricultural products from pest attacks. They were selective, safe and had short half lives. Although they were acutely toxic to fish, very few accidental poisonings occurred because they were not registered for aquatic use and they seldom have enough persistence to reach water from normal application (DiGiulio and Hinton, 2008). Pyrethroids were also used as ectoparasiticides in veterinary and human medicines (Soderlund *et al.*, 2002 and Waxler *et al.*, 2005). They have

replaced natural pyrethrins especially for their better photo-stability. However, the use of pyrethroids has increased rapidly in the past three decades. It is noteworthy that pyrethroids are thermo-stable and photo-stable, slightly soluble in water and highly soluble in fats. Further the presence of halogens in some pyrethroids contributes to greater persistence and provides better residual activity against insects together with high potential negative effects on the environment (Bradbury and Coats, 1989).

Karate (Lambda-Cyhalothrin) chosen in this study belongs to a class of synthetic pyrethroids which are well known for their insecticidal activity and are being used worldwide for the last forty years (Shafer *et al.*, 2005). The level of pollution due to pesticides of agriculture or pisciculture origin could be assessed using fish as test organisms. Fishes are particularly sensitive to the environmental contamination of water. Hence, pollutants such as insecticides may significantly injure certain physiological and biochemical processes when they enter the organs of fishes (John, 2007; Banaee *et al.*, 2011b).

Methodology

The experiments were conducted for 30 days and once in every 10 days, the fish subjected to various sublethal concentration of the pesticide were removed, stunned with a blow on their head immediately dissected out to separate the whole body tissues. The tissues were washed in fresh water fish ringer solution (Burnstock, 1958) and kept in sterilized containers. Then these tissues were dried in an oven and the dried materials were powdered and used for the bio chemical analysis. Lipid contents of dry tissues were quantified following the standard methods.

Estimation of Lipid

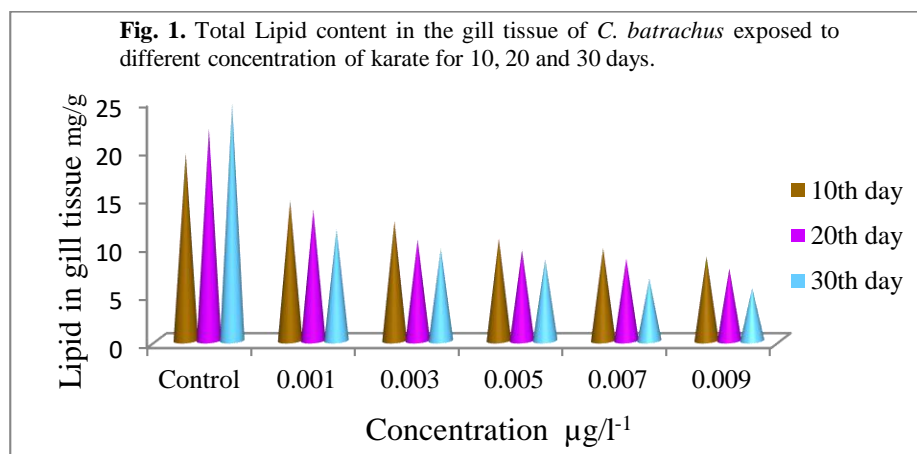
The Method of Bragdon (1951) was followed for the estimation of total lipid. A sample of 5 mg was placed in dry mortar and pestle and ground thoroughly with sufficient amount of chloroform. This was allowed to stand for 48hrs for excretion. This tube is then centrifuged and the chloroform extract was taken in another tube and evaporated to dryness. A 3ml of potassium dichromate reagent (2% potassium dichromate in concentrated sulphuric acid) was added to the sample tube. The tube was shaken well and diluted with equal amount of distilled water. A blank was prepared by adding 3 ml of distilled water to an equal amount of reagent in a clean empty tube. The developed color was read at 450nm in a Spectrophotometer. Lipid content of the tissues was expressed in percentage.

Results

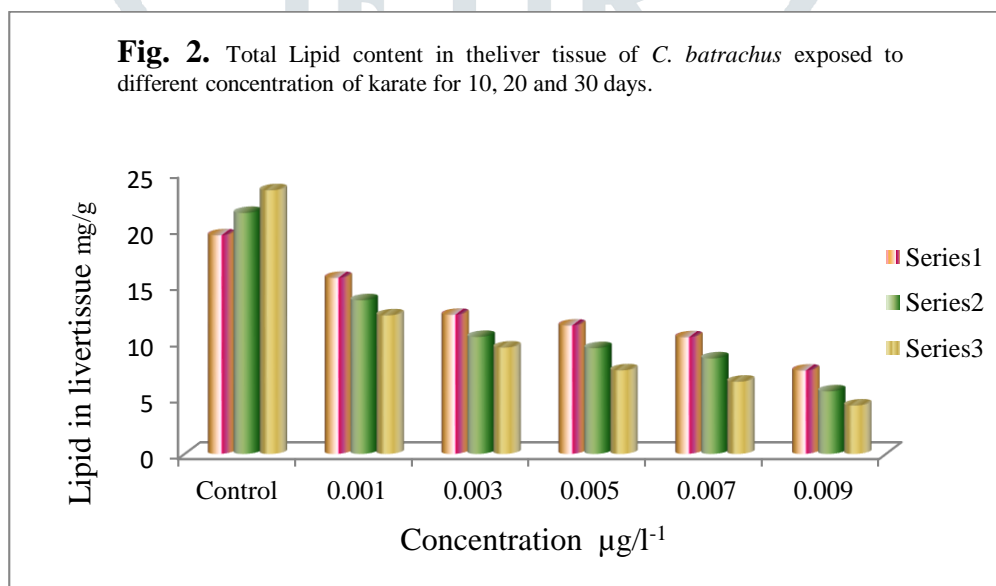
The lipid content in the muscle of the fish was found as 19.31mg/g, 21.31mg/g and 23.21mg/g; in the gill as 19.43mg/g, 21.96 mg/g and 24.56 mg/g; in the liver as 19.43 mg/g, 21.41mg/g and 23.42 mg/g in the control groups at the end of 10th, 20th and 30th day respectively.

Table. 1. Biochemical constituents (mg/g dry tissue) in the chosen tissue of *C. batrachus* exposed to sublethal concentration of karate for 10,20 & 30 days at 28±1°C. Each value represents the average (Mean ± SD) of five estimations.

Tissue	Components	Karate concentration					
		Controls	0.001µg/l ⁻¹	0.003µg/l ⁻¹	0.005 µg/l ⁻¹	0.007 µg/l ⁻¹	0.009 µg/l ⁻¹
Gill	Lipid 10 th day	19.43±0.26	14.49±0.29	12.38±0.16	10.59±0.15	9.59±0.23	8.74±0.26
	Lipid 20 th day	21.96±0.23	13.60±1.44	10.49±0.16	9.41±0.34	8.51±0.21	7.49±0.29
	Lipid 30 th day	24.56±0.26	11.56±0.20	9.56±0.14	8.47±0.22	6.44±0.32	5.45±0.30
Liver	Lipid 10 th day	19.43±0.29	15.66±0.30	12.39±0.29	11.43±0.15	10.39±0.29	7.42±0.21
	Lipid 20 th day	21.41±0.12	13.68±0.16	10.42±0.18	9.42±0.11	8.51±0.31	5.57±0.09
	Lipid 30 th day	23.42±0.20	12.34±0.18	9.47±0.22	7.44±0.26	6.43±0.15	4.31±0.08
Muscle	Lipid 10 th day	19.31±0.18	17.26±0.28	15.44±0.34	14.29±0.34	12.23±0.11	10.26±0.19
	Lipid 20 th day	21.31±0.05	15.37±0.37	14.64±0.19	13.30±0.14	10.40±0.17	7.80±0.18
	Lipid 30 th day	23.21±0.20	14.22±0.18	13.34±0.07	11.46±0.23	9.67±0.18	6.34±0.11



After toxicant exposure, the lipid content in the muscle significantly decreased (17.26 mg/g to 10.26 mg/g), (15.37mg/g to 7.80mg/g) and from (14.22 mg/g to 6.34mg/g) at the end of 10th, 20th and 30th day at the lower and higher concentration respectively. The decreases were at 10.61%, 20.04%, 25.99%, 36.66%, 46.86% in the muscle tissue on the 10th day. On the 20th day the decreases were at 27.87%, 31.29%, 37.58%, 51.19% and 63.39%. The reduction on the 30th day was recorded as 38.73%, 42.52%, 50.62%, 58.29% and 72.68% in 0.001µg/l⁻¹, 0.003µg/l⁻¹, 0.005µg/l⁻¹, 0.007µg/l⁻¹, 0.009µg/l⁻¹ concentrations of insecticide respectively (Table 1 Fig 2k).

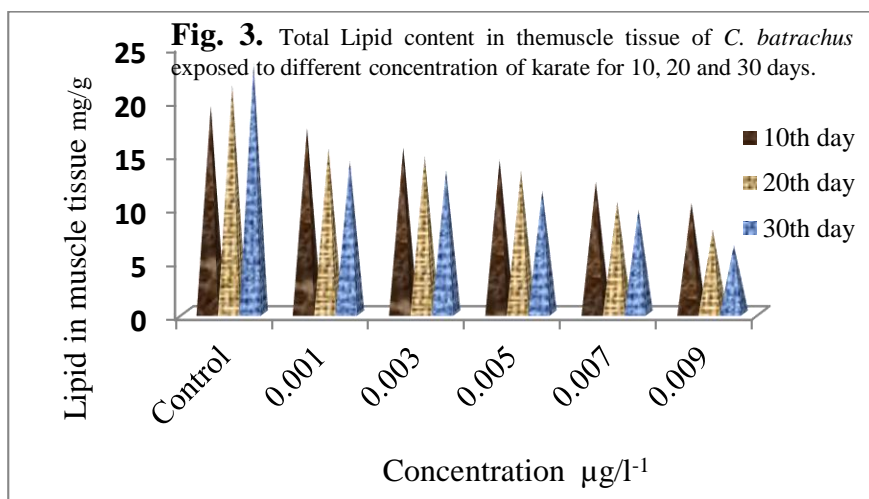


After toxicant exposure the lipid content in the gill tissue decreased (14.49mg/g to 8.74mg/g), (13.60mg/g to 7.49mg/g) and (11.56 mg/g to 5.45mg/g) at the end of 10th, 20th and 30th day at the lower and higher concentration respectively. The decreases were at 25.42%, 36.28%, 45.49%, 50.64% and 55.01% in the gill tissue on the 10th day. On the 20th day, the decreases were at 38.06%, 25.39%, 57.14%, 61.24% and 65.89%.a (Table ;2 Fig 3 P<0.05.) The reduction on the 30th day was recorded as 52.93%, 61.07% ,65.51%, 73.77%, and 77.80% at 0.001µg/l⁻¹, 0.003µg/l⁻¹, 0.005µg/l⁻¹, 0.007µg/l⁻¹, 0.009µg/l⁻¹ concentrations of insecticide respectively.

Table. 2. Biochemical constituent values of different tissues subjected to F-test analysis to confirm the level of significance. The readings were found to be significant at P<0.05 by F-test.

Biochemical constituents	Sources of variation	F-Value	P-Value
Muscle tissue	Lipid	2.25*	P<0.05
Gill tissue	Lipid	1.28*	P<0.05
Liver tissue	Lipid	0.08*	P<0.05

* P< 0.05 is statistically significant



After intoxication, the lipid content in the liver tissue decreased from (19.43mg/g to 7.42mg/g), (21.41mg/g to 5.57mg/g) and (23.42mg/g to 4.31mg/g) at the end of 10th, 20th and 30th day at the lower and higher concentration respectively. The decreases were 19.40%, 36.23%, 41.17%, 46.52% and 61.81% in the liver tissue on the 10th day. On the 20th day the decreases were 36.10%, 51.33%, 56.00%, 60.25% , 73.98%. The reduction on the 30th day was 47.30%, 59.56%, 68.23%, 72.54% and 81.59% at 0.001 $\mu\text{g/l}^{-1}$, 0.003 $\mu\text{g/l}^{-1}$, 0.005 $\mu\text{g/l}^{-1}$, 0.007 $\mu\text{g/l}^{-1}$, 0.009 $\mu\text{g/l}^{-1}$ concentrations of insecticide respectively. (Table 1; Fig 2). A two way ANOVA test indicated significant decrease in lipid content in all the selected tissues exposed to different time periods of exposure in the test media (Table 2; $F = 2.25; P < 0.05$).

Discussion

Lipids play very important role in the transport mechanism across cell membrane. Any stress is bound to change the course of events associated with the lipid synthesis. Lipids also contribute to energy production as they are having high calorific values (Guyton, 2006) and play a vital role in the biochemical adaptations of animals to stress conditions (Tayyabhe *et al.*, 1981). Extensive literature is available on the effects of different pesticides on tissue lipid fraction of various animals (Chetty and Indira, 1994; Govindan *et al.*, 1994, Martin *et al.*, 2007).

Some of the authors who have reported a decreasing trend in lipid are stated here for emphasis. Srinivas *et al.* (1991) reported decreased lipid content in *Tilapia mossambica* exposed to atrazine; Leela Shivaparvathi *et al.* (2002) also have reported a decreased level due to the effect of phosalone on total lipid and triglycerides in freshwater fish *Tilapia mossambica*. Under sublethal exposure to cypermethrin in *Ophiocephalus punctatus* an overall decrease in lipid levels has been observed by Shruti *et al.*,(2011). This result of cypermethrin suggests that lipid might have been channeled for energy production for other metabolic function in which these products play a vital role during stress condition. Lipids serve as energy reserves to meet the metabolic demand for more energy to mitigate toxic stress. Since lipids form the rich energy reserves whose calorific value was reported to be twice to that of an equivalent weight of carbohydrate or protein. The same results have been observed by Amudha *et al.*, (2002) in the freshwater teleost fish *Oreochromis mossambicus*.

In the present investigation, lipid profile of liver tissue has decreased through the whole exposure period. Present findings are supported by the findings of Awasthi (1982), Murthy and Devi, (1982) and Rathore and Singh, (2000). This lipid decreasing trends may be attributed to abnormalities in fat deposit cells of liver and also changes in lipid content was due to inhibition of uptake of lipid compound by the tissue for utilization of cellular level increase lipolysis of mitochondrial injury which impaired the function of citric acid toxic stress. Jha, (1999) supported the findings of the present study related pesticide inhibited lipid synthesis and mobilizing the stored lipid molecules.

Various authors have studied similar reduction of lipids in different tissues. Ram and Sathyanesan (1984) observed a decreased lipid level in the liver of *Channa punctuatus* exposed to toxicants. Srinivas *et al.*, (1991) have showed decreased lipid content in *T. mossambica* on exposure to atrazine. Gradual depletion in lipid content of liver and muscle of *Anabas testudineus* when exposed to malathion was analysed by Mishra *et al.*, (2004). Arockia

and Mitton (2006) have showed declining trend of lipid content in the tissues like brain, gill, kidney, liver and muscles upon exposure to lannate in the fish *Oreochromis mossambicus*. Saradhamani and Selvarani (2009) have showed significant decrease in cholesterol content in the tissues of the freshwater fish, *Tilapia mossambica* exposed to metribuzin.

Lipids play an important role as sources of energy for fish. Since most insecticides are lipophilic compounds they can easily pass through biological barriers as they contain lipids and accumulated in fat tissue. Lipid molecules are highly susceptible to oxidative reactions. Due to cell membrane lipid peroxidation of unsaturated fatty acids, short chain fatty acids with R-COOH, R-CHO and R-OH bases are created which seriously affect the cellular membrane functions such as the activity of hormone receptors and neural mediators, ion transport channels and the activity of membrane enzymes and the transportation of specific molecules. On the other hand, the formation of malon-di-aldehyde (MAD) during peroxidation process of fatty acids having double bonds can create covalent bonds and polymerize cellular membrane components (Sureda *et al.*, 2006; Tejada *et al.*, 2007). In addition, accumulation of fatty acids in the cytosol increased peroxidation of fatty acids in perxisomes and the endothelial reticulum resulting in overproduction of ROS and further damage.

The results of the present investigation revealed that the pesticides significantly affect the levels of the major organic constituents i.e., lipid of whole body as well as the tested tissues of *Clarias batrachus*. Sublethal concentration of Karate at different time intervals caused notable effects on the lipid content of muscle, gill and liver of *Clarias batrachus*.

Gill has come to direct contact with ingredients of the pesticides through respiratory water current and diet respectively and hence these organs are subjected directly to the toxic effects of the pesticide. Moreover, due to hypoxic condition produced by the pesticide, gills have to work hard to extract oxygen from the medium, which results in the increased energy cost of the respiratory process. Thus the significant depletion of lipid, followed by protein and carbohydrate in the gill could be attributed to the direct and continued access of the organ to the pesticides as well as the increased energy cost of its functioning.

The energy cost of liver function in connection with detoxification process is increased due to the exposure of *Clarias bartachus* to pesticides, and hence the lipid components of the liver are subjected to severe depletion to meet the increased energy demand of the organ. Increased levels of stress hormones such as cortisol in blood of fish exposed to various insecticides stimulates lipid breakdown in adipose tissue. Both elevated and reduced free fatty acids level in plasma have been observed in different fish species exposed to insecticides. Changes in cholesterol and triglyceride levels in the blood and other tissues such as muscles and liver of fish treated with insecticides indicate the effect of these compounds on lipid metabolism. Depletion of lipid content may be due to lipolysis or the mitochondrial injury which impair the function of TCA cycle and the fatty acid oxidation mechanism (Shruti *et al.*, 2011).

Conclusion

In conclusion it can be said that these results can be used by environmentalists to conserve fish biodiversity. It can also be inferred that the significant downfall in this major organic constituent of *Clarias bartachus* resulted in the deterioration of the nutritive value of the fish for human consumption.

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