EVALUATION OF BIOCHEMICAL PROFILE IN LIVER TISSUES OF TWO FISH *CHANNA PUNCTATUS AND CHANNA GACHUA* EXPOSED TO DELTAMETHRIN

Dubey J.K., Mishra D.B. and Tripathi V.K.

Department of Zoology, Tilakdhari Post Graduate College Jaunpur-222001

e-mail: devbrat1003@gmail.com

Introdcution

Water is a precious gift of nature to us. Only less than 2% water is available as fresh water and rest of the water is sea water which is considered as ultimate sink of the pollutants. Water provides a good medium for growth of aquatic flora and fauna with great biodiversity. Major problem associated with the natural and manmade water bodies across the globe is its pollution. Substances which kill or repel, attract and mitigate the insect or pest is known as insecticide and pesticide respectively. Now days a collective term Integrated Pest Management (IPM) introduced defining not only insect and pest but weeds also.

A variety of organophosphate, organochlorine, organometallic and carbamide pesticides are extensively used in agriculture for the control of pests. It has been established that the toxic action of these pesticides has specific toxicity for a particular organism. The toxicity levels were influenced by the sex and the nutrient supply (Arunachalam, 1980 & Mathivanan, 2004 & Sharma & Ansari, 2010). The effects of several insecticides and pesticides on various physiological responses of fishes were reported by several workers time to time (Keith *et al.*, 2010, Prashanth *et al.*, 2011; Amin *et al.*, 2012; Murthy *et al.*, 2013 and Prusty, 2015, Mishra. et al., 2008-09)

Pesticides contamination of surface water from agricultural runoff is a global and serious problem. Bioaccumulation of pesticides in aquatic species is increasing through progression of trophic level and reached up to alarming condition posing a threat to aquatic life (Ron *et al.*, 2003; Heger *et al.*, 2008 & Alani *et al.*, 2013). Fish constitutes one of the important target organisms in any aquatic systems and are one of the major sources of cheap protein for human beings. A variety of fish species showed uptake and accumulation of many contaminants such as pesticides and these are used as an indicator organisms to assess the quality of water because they accumulate the higher concentration of these compounds present in original concentration in water bodies (Uysal *et al.*, 2008; 2009; Metian *et al.*, 2013; Yilmaz *et al.*, 2007 and Bilandzic *et al.*, 2011, Singh et al., 2009) The deleterious effect of chemical pollutants on aquatic ecosystem may result in to changes in physiology and metabolism ultimately leading to death of an organism.

Deltamethrin belongs to synthetic pyrethroids that are the most popular and widely used insecticides all over the world. Deltamethrin is commonly known by its trade name as Decis and ButoxR as shown in Fig.1.

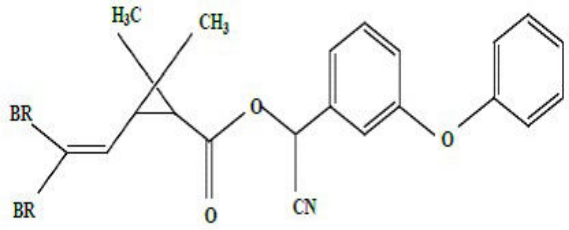


Fig. 1: Chemical formula of deltamethrin

MATERIALS AND METHODS

Glasswares used in present investigation were of 'Borosil make'. Washing of glasswares was done by emerging them in chromic sulphuric acid mixture for 24 hrs, followed by through washing in flowing tap water, then were cleansed by detergent washing with several changes of tap water. Finally, the glassware were rinsed with double distilled water and dried in a hot air oven at 100^{0}C for overnight. Most of the chemicals used were from Hi Media, Sisco Research laboratory, SRL (India). All the general chemicals used were Anal AR grade. The pesticide deltamethrin was of commercial grade purchased from local market. Only deionized water has been used for the preparation of the reagents. Deltamethrin was dissolved in acetone and the required concentrations were maintained by adding them in fresh tap water to avoid toxicity caused by the excretory materials. Glassware were sterilized by wet heat in an autoclave at 121^{0}C, 15 lb per square inch for 18 minutes.

Water Analysis of Experimental Site

The estimation of physico-chemical characteristics of water is carried out by standard methods as described by American Public Health Association (APHA), 2005.

Temperature - The temperature of pond water recorded with the help of "Celsius Thermometer" at the time of sampling on the sites and expressed in degree centigrade (°C).

Hydrogen Ion Concentration (pH) - pH was measured in the laboratory with the help of systronic pH Meter (Model 324) with combination electrode having a precision of 0.05. pH meter was standardized with standard buffers before each reading.

Dissolved Oxygen (DO) - Modified Winkler's method was used for the estimation of dissolved oxygen in water. Dissolved oxygen of water sample is

measured by precipitating as manganic basic oxide which is dissolve by concentrated sulphuric acid forming manganic sulphate. It immediately reacts with potassium iodide, already present liberating iodine which is determined by titration with sodium thiosulphate (0.025 N). The quantity of iodine liberated during the reactions is equivalent to the quantity of oxygen present in the sample. The DO value was calculated with the help of following formula-

$$DO\ (mg/L) = \frac{\text{Volume of titrant (V) X Normality of titrant (N) X 8}}{\text{Volume of water sample (ml) X 1000}}$$

where V and N are volume and normality of the titrant respectively.

Total Alaklinity - The alkalinity of water can be determined by titrating water sample with sulphuric acid of known values of pH, volume and concentrations. Based on stoichiometry of the reaction and number of moles of Sulphuric acid needed to reach the end point, the concentration of alkalinity in water is calculated.

Collection and Acclimatization of fish - The healthy adult fishes, irrespective of sex (17.80 ± 0.50 cm length and 47.85± 0.75gm weight) of *Channa punctatus* were collected from the Guzartal, situated in Shahganj Subdivision of District Jaunpur. It is a fish cultivator centre under Uttar Pradesh State Fishery Department. The collected fishes were air-breathing teleost cat fish (medicinally important). The fish were transported in plastic containers to the fisheries laboratory and washed with 0.1% $KMnO_4$ solution. Prior to the start of the experiment the fish were acclimatized to the laboratory conditions for 15 days in dechlorinated tap water. They were maintained in glass aquaria containing dechlorinated tap water. The commercial grade pesticide Deltamethrin was dissolved in acetone and the required volume of the desired concentration of pesticides is added in each aquarium. The water of aquarium was replaced every 24 hrs with fresh water in control and deltamethrin solution, were mixed into the water of aquariums. The experiment was conducted under natural light and ambient temperature. Feeding of fish was stopped 48 hrs prior to the commencement of the experiment with a view to avoid any possible change *in situ* in the toxicity of pesticide.

Determination of LC_{50} - Bioassay or toxicity tests were carried out for the determination of LC_{50} values by following FAO procedure for short term bioassays (Reish and Oshida, 1987). The duration of the test was 96 hours. Fishes were exposed to 50 litres of test solution that contained graded, series of concentrations of the toxicants. Fibre glass tanks, inner coated. with chemical resistant epoxy resin, were used for the toxicant exposure. Ten animals were used for each test concentration of the toxicant. Appropriate duplicates and controls were invariably maintained for all the experiments. The test media were

replenished totally every 24 hour. The animals were inspected at regular intervals, and were considered dead if it did not respond to mechanical stimulation, and the opercular movements ceased. The dead animals were removed and the percentage mortality at every 12 hour recorded. The LC_{50} values and their 5% confidence limits were calculated.

All the experiments were carried out in duplicate for control group of organism standard deviation was found to be statistically insignificant at 5% confidence level. Hence, no standard error bar were showm in entire graphical presentation

Bio-Chemical Studies:

Tissue preparation - The specimens were sacrificed, the liver, brain and gills removed, cleaned and weighed rapidly, a ten percent (w/v) homogenate of different tissues were prepared in cold fish saline with the aid of Potter Elvehjem type homogenizer fitted with a teflon pestle. The homogenate was first centrifuged at 2000 rpm for 15 minutes in a refrigerated centrifuge, the pellet consisting of a nuclear fraction and cell debris is discarded. The supernatant again centrifuge at 8000 rpm for 20 minutes for the post mitochondrial supernatant, now this clear supernatant was taken for the biochemical studies and crude enzyme studies.

Total carohydrate estimation - Added 4 ml of the anthrone reagent to 1 ml of a protein-free carbohydrate solution and rapidly mixed. Kept the tubes in a boiling water bath for 10 min with a marble on top to prevent loss of water by evaporation then cooled and read out the extinction at 620 nm against a blank. Prepared the standard curves using glucose and compared it with test sample and deduced the concentration.

Protein estimation - Protein estimation in post mitochondrial and cytosolic supernatant was carried out by the method of Lowry *et al.*, (1951). In 0.5 ml of sample, 0.5 ml of TCA (10%) was added. The content was kept in cold for 4 hours and protein precipitate was recovered by centrifugation. The proteins were dissolved in 1.0 ml of 0.1N NaOH, 5 ml. of alkaline copper reagent was added to the diluted sample and incubated at room temperature. After 30 minutes 0.5 ml of Folin's reagent was added at the same temperature. Optical density of blue colour developed was read at 660 nM exactly after 30 minutes, Standard solution (BSA, 20 - 100 µg) and blank were also run simultaneously. Standard curve of BSA (20µg-100 µg) was plotted and concentration of protein in the sample was calculated, The results were expressed in mg protein/mg tissue fresh weight.

Lipid content estimation - Briefly, lipid samples and standards are placed in a heating block set at 100°C to allow the solvent to evaporate. Once the solvent is gone (about 10 min), 0.1 ml of concentrated sulfuric acid is added to each tube, vortexed, and then heated at 100°C for 10 min. Samples are then removed from

the heat block and allowed to cool to room temperature before adding 2.4 ml of vanillin reagent (600 mg of vanillin, 100 ml of hot water, 400 ml of 85% phosphoric acid) and vortexed. The pink color is allowed to develop for 5 min, and then 0.2 ml of standards and samples are read at 490 nm. Since the color continues to develop over time, the standards should be re-read at 5 min intervals if a standard spectophotometer is used.

RESULTS AND DISCUSSIONS:

The physical and chemical conditions of a water bodies gives it to living and setting the hydrodynamics of the water bodies. Several parameters are taken into account and considered to be prime and are temperature, change in acid & base strength, dissolved oxygen concentration which play critical role and directly affect the aquatic flora and fauna and its diversity. Physico-chemical parameters of water was recorded and presented in following Table 1.

Table1: Physico-chemical properties of the water body

S.No.	Tempertaure	26.5^{0}C	
1	pH	7.2	
2	Dissolved Oxygen (DO)	5.8 ppm	
3	Total Alkalinity	Methyl Orange	256 ppm
4		Phenolphthalein	Nil

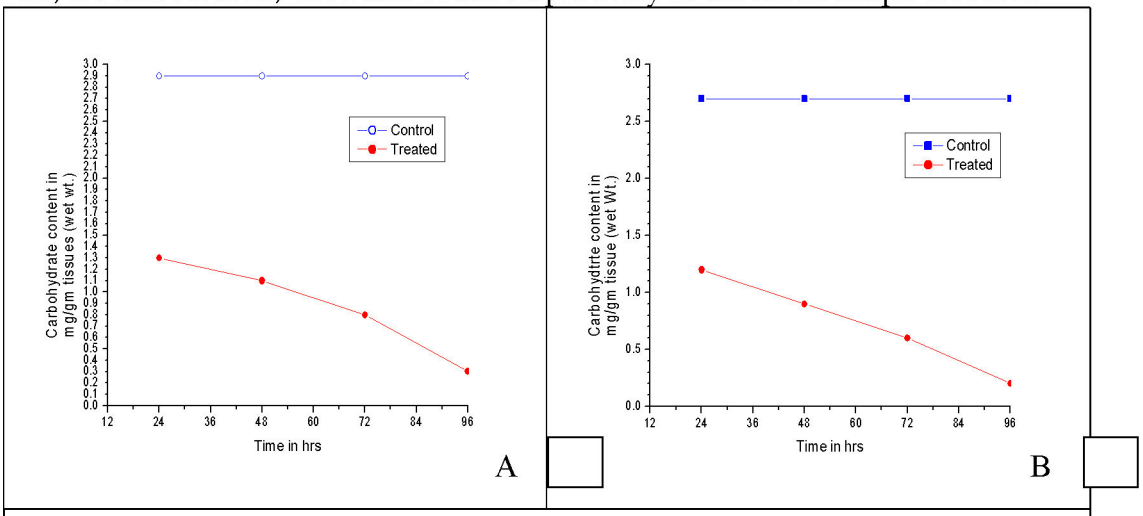
Bioassay or toxicity tests were carried out for the determination of LC_{50} values by following FAO procedure for short term bioassays (Reish and Oshida, 1987). The duration of the test was 96 hours. Stock solution of deltamethrin 10% Effective Concentration (EC) was prepared by diluting 1ml insecticide in 100 ml of distilled water, and was diluted to different concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.08 ppm, which were used as experimental waters for toxicity study of selected model animals *Channa*. The experiment was set in duplicate and healthy fishes (n=10) fishes were maintained in 10 litre of experimental water having different concentrations of deltamethrin. Similarly a control was set up with water devoid of deltamethrin. Feeding was stopped one day prior to the experiment and also during the experimental period, as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). The LC_{50} values were calculated as average from the two replicates for each experimental concentration of water by arithmetic graph method as shown in graph 4.1 (Reish and Oshida, 1987). LC_{50} at 96 hrs were found at 1.3 and 1.4 ppm for *Channa punctatus* and *Channa gachua* respectively.

Behavioral Response - The main behavioral changes observed as result of deltamethrin exposure are represented by respiratory and neurological

manifestations in both the moderl organisms showed almost similar response. Rapid gill movement, erratic swimming, swimming at the water surface and gulping for air and prolonged and motionless laying down on the bottom were observed in each of observation depending on concentration of deltamethrin toxicity. At lower concentration insignificant changes were observed while increasing time and concentration impart marked changes.

Carbohydrate Content:

Carbohydrate content in different tissue are different and showing progressive decreasing profile. In liver tissues control was found to be 2.9 and 2.7 mg/gm of wet weights of liver tissue in *Channa punctatus* and *Channa gachua* respectively. While treated with deltamethrin was found to be 1.3, 1.1, 0.8 and 0.3 mg/gm of tissue in *Channa punctatus* and in *Channa gachua* it was observed as 1.2, 0.9, 0.6 and 0.2 at 24, 48 72 and 96 hrs respectively as shown in Graph 1. A & B.



Graph 1: Carbohydrate content in Liver tissues

***C. punctatus* (A) and *C. gachua* (B)**

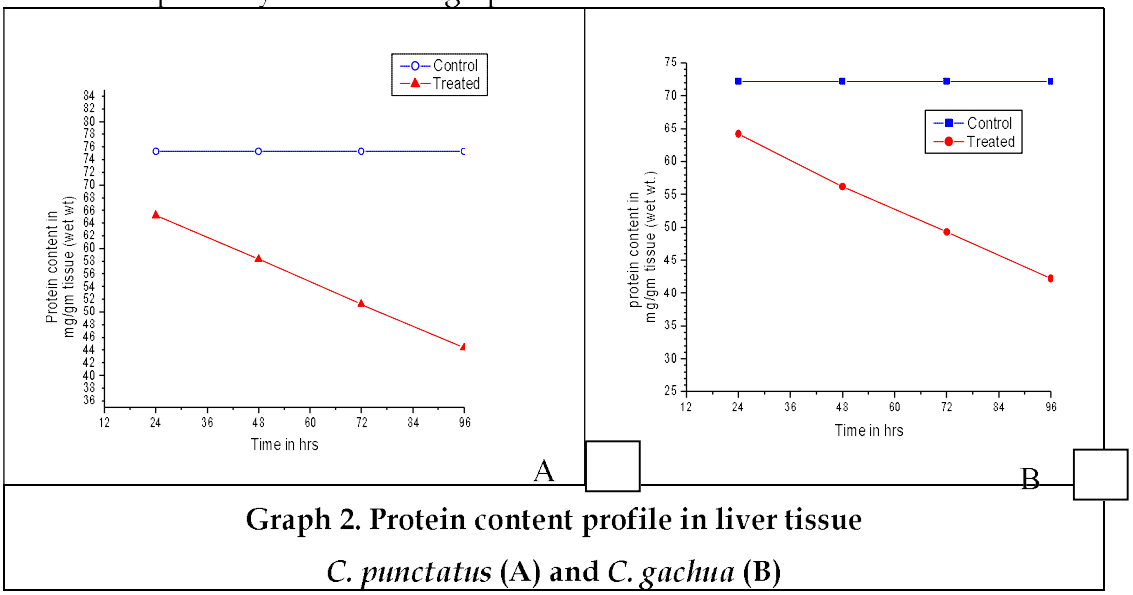
The decreased level of carbohydrate contents in organs of the fish after 96 hrs exposure with deltamethrin in all tissues when compared to control. Decresment in carbohydrate level indicates its rapid utilization to meet and the increased energy demand to cape up with stress due to deltamethrin toxicity. This demand is channelized either viz glycolytic pathway or oxidative reaction of pentose phosphate pathway (Cappon *et.al.* 1975; Prusty *et al.*, 2015 & Amin *et al*,, 2012, Singh et. al., 2009-10).

During stress the energy demand is supplied usually from reserve food materials in order of carbohydrate, lipid and protein, a final class of biomolecules which serves as important source of biofuel., Ela Carbohydrate content reduction

is more prevalent under hypxic conditions due to stress produced by a pesticides or heavy metals (Dezwaan *et.al.* 1972 Chandrawathy & Reddy, 1995, Branislav *et al.*, 2013 and Younus *et al.*, 2015).

Proteins Contents:

Proteins are very important biomolecules and building block of organisms and considered as final classs of biomolecules as far as energy requirement is concerned. In case of liver tissues protein contents were found to be 65.2 at 24 hrs, Protein content in brain tissue were found to be 60.3, 53.4, 48.3 and 39.2 mg/gm of we weight of tissue as compared with control 68.6 mg/gm of tissue at 24, 48, 72 and 96 hrs respectively in *Channa punctatus* as shown in graph 2 A. In case of *Channa gachua* it was found to be 58.2, 52.4, 46.2 and 37.2 mg/gm of we weight of tissue as compared with control 65.5 mg/gm of tissue at 24, 48, 72 and 96 hrs respectively as shown in graph 2 B.

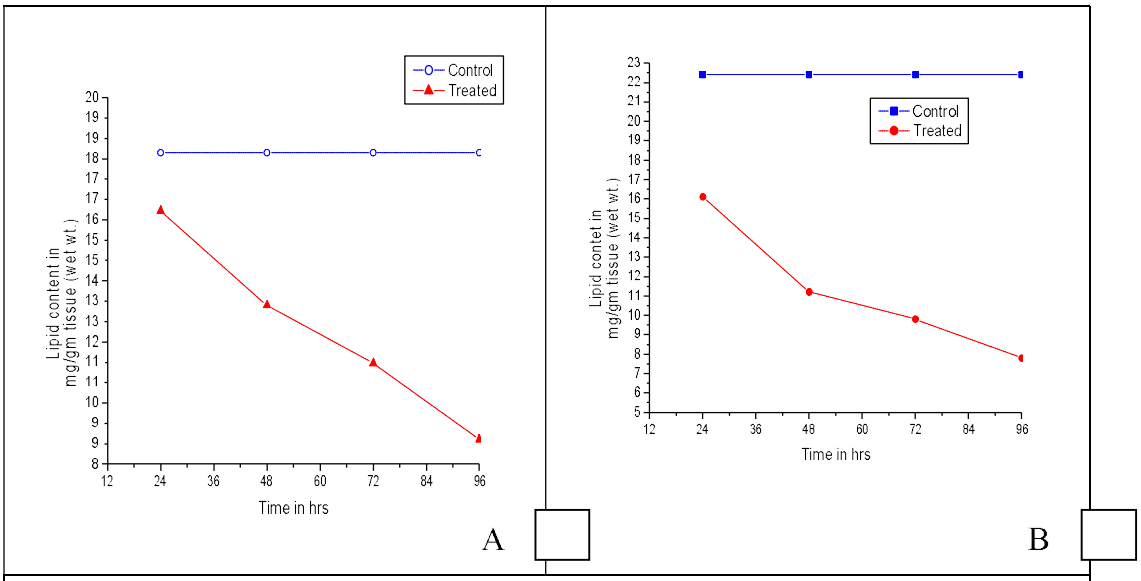


Graph 2. Protein content profile in liver tissue *C. punctatus* (A) and *C. gachua* (B)

In present study the protein content was found to be decreased in all organs after the treatment with deltamethrin upto 96 hrs when compared with the control. This decreased level of proteins might be due to inhibition of translational proces are increased rate of catabolism of proteins which may be entered into Krebs cycle via transamination process, carried out by aminotransferases and it might be due to meet out the organism high energy demand due to deltamethrin stress (Ganeswade 2011, Binu Kumari & Vasanthi, 2013).

Lipid Contents

Lipid are important constituent of cell membrane and it also provide buoncy to aquatic organism. Lipid content in liver tissue of *C. punctatus* was found to be 17.8, 12.3, 10.2 and 8.4 mg/gm of tissue as compared with control (23.4 mg/gm tissue) at 24, 48, 72 and 96 hrs respectively. While in case of *C. gachua* it was found to be 16.1, 11.2, 9.8 and 7.8 mg/gm of tissue as compared with control (22.4 mg/gm tissue) at 24, 48, 72 and 96 hrs respectively as depicted in graph 3. A & B.



Graph 3. Lipid content in liver tissues

***C. punctatus* (A) and *C. gachua* (B)**

Lipids are important biochemical constituent and upon oxidation contributes a significant amount of energy via β-oxidation and structural components for reproductive growth (Surgent, 1995, Kylie *et al.*, 2014). Dunning Reduced level of lipid content in the organs selected in presented investigation i.e. brain, liver are gills after 96 hrs exposure of deltamethrin. This reduction in absorption of carbohydrate and proteins which in turn resulting into exhaust of energy during stress leading to oxidation of lipids to meet out the organisms high energy demands.

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