International Journal of Advanced Educational Research
ISSN: 2455-6157, Impact Factor: RJIF 5.22
www.newresearchjournal.com/educational
Volume 1; Issue 2; March 2016; Page No. 18-21

The impacts of Triazophos pesticide on muscle, liver and intestinal protein levels of fish *Oreochromis mossambicus*

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Abstract
Physiological functions of animals get disturbed on exposure to pollution stress. The better understanding of this mechanism can help us to predict the harmful effects of various chemicals on environment. This study estimates the chronic effect of this pesticide on biochemical composition of protein. The sublethal concentration of Triazophos treated with *Oreochromis mossambicus* protein level at different time interval and observed that in the liver, muscle and intestine protein level showed declined trend. The changes in the level of protein contents in muscle, liver and intestine of the fish *O .mossambicus.* The decrease in protein content of Triazophos in toxicated fish in the present study also indicates the physiological adaptability of the fish to compensate for pesticide stress.

Keywords: IgY technology, antibody, microorganisms, medicine

1. Introduction
Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition, which is affected by the water pollution (Prado *et al*., 2009) [10]. Alterations in biochemical components as response to environment sites of application and becomes an aquatic pollutant (Tatjana and Nevenka, 2006) [15]. In addition Fish, generally accumulate contaminants from aquatic environments and have been largely used in food safety studies. Environmental and chemical stress can interfere with physiological and biochemical functions such as growth, development, reproduction and circulatory system in fish. The circulatory system is greatly affected by the water quality and external environmental factors but there is a lack of information about cardiac muscle metabolites of aquatic animals. Aquaculture plays an important role in country's economy in augmenting food supply generating employment, rising nutritional levels and earning foreign exchange. In India per capita consumption of fish was 3.2 kg against the requirement of 11 kg. Inland fish production has been catching up fast with marine fish production and by the end of the eighth plan period (1994-95), the production of inland fish would probably equally that of marine fish production of the country.

The pesticides used in pest control programmes also produce many physiological and biochemical changes in freshwater organism particularly the fish (Girija 1984) [4]. Presence of pesticides in ecosystem constitutes an immense environmental stress leading to the development of various types of adjustments/adaptations such as morphological, physiological, biochemical and behavioural at various levels of organisation in the organisms to suit their environment (Rakesh *et al.*, 2009) [12]. In contrast to other man-made chemicals that cause local pollution, pesticides are deliberately spread over vast areas, and thus have global distribution.

Due to repeated application of pesticides may get into human body directly from water or indirectly through plants, animals feeding on plants or fishes and such other animals developing in polluted water. Circulation of these pesticides in a water body may lead to accumulation in fish and other aquatic plants (Natarajan, 1993) [8]. Firdous *et al*., (2012) [3] studied the assessment of Total Protein concentration in liver of fresh water fish, *Channa punctatus* with special reference to an organophosphate insecticide, Triazophos. Muzaffar Ahmed *et al.,* (2012) [7] studied the effect of dietary protein, lipid and carbohydrate contents on the liver composition and enzyme activity of *Cyprinus carpio*. Qadir *et al.,* (2014) [11] studied the effects of Imidacloprid on the hematological and serum biochemical profile of *Labeo rohita*. Reddy *et al.,* (2015) [13] reported some biochemical changes in the tissues of the fresh water fish *Labeo rohita* (Hamilton) exposed to Confidor.

2. Materials and methods
Selection of pesticide
In order to enrich the present knowledge on pesticide toxicity and to explore the extent of toxicity of pesticides, which areas are unexamined in relation to fish relation to fish toxicity, the present study was chosen. Further, in the study area during every season huge amount of selected organophosphate pesticides (Triazophos 20 % EC) are used to raise various crops like paddy, vegetables, sugarcane etc.

Experimental fish *Oreochromis mossambicus*
For the present study, the live *Oreochromis mossambicus* (8±5g) were collected from local fish pond, Thiruvithancode, Kanyakumari district. Care was taken to reduce hyperactivity and physical injuries to the fish. They were screened for any possible pathological symptoms and washed with 0.1 percentage potassium permanganate ($KMnO_4$) solution. They were then stocked and maintained in large cement tank containing chlorine free bore well water for 10 days under normal temperature. Before stocking, the tank was washed with 1 percentage $KMnO_4$ to avoid the fungal infection. Water was changed in alternate days. The fishes were fed *ad libitum*

on the formulated fish diet prepared from ground oil cake and rice bran in the laboratory.

LC_{50}

Well acclimatized *Oreochromis mossambicus* approximately (8±5 g) were selected from the stock and exposed to different concentrations of Triazophos individually for the static bioassay test. The experiments were conducted in 10 liter tanks with 10 fishes each, starved for 24 hours prior to the experiment, for the maintenance of bioassay. The experimental medium was renewed daily till the end of the experiment. The mortality of fishes in different concentrations was noted at 12, 24, 48, 72 and 96 hours, and the dead animals were removed immediately. Fishes showing no respiratory movement and response to tactile stimuli were considered dead. Then 12, 24, 48, 72and 96hrs LC_{50} values of Triazophos were computed using software by transforming mortalities (percentage values) into probit scale. The experiment was repeated three times and the mean values recorded separately for each test fish. Simultaneously ten fishes were reared in pesticide-free medium and are treated as control for each pesticide experiment.

Estimation of protein (Lowry *et al*., 1951) [5]

The protein content of liver, muscles and intestine of each fish was estimated using the method of Lowry *et al*., (1951) [5]. 10 mg of each of the wet tissue sample was used for the protein estimation. The protein was precipitated with 100 ml of 10% TCA and the precipitate was dissolved in 1 ml of 1N sodium hydroxide solution. 1 ml of this extract was made into 3 ml with distilled water. The colour was developed by adding 1 ml of reagent containing 1 ml of 0.5% $CuSo_4$ in 1 % sodium tartrate and 5 ml of 2% sodium carbonate followed by 0.1 ml of folin phenol reagent. The developed colour was read at 550 nm. The protein content was expressed in mg/100 mg wet tissue.

3. Results

Acute toxicity

The percentage mortality of *Oreochromis mossambicus* exposed to various concentrations of the pesticide Triazophos for 12, 24, 48, 72 and 96 h hours are given in the Fig 1. The data clearly shows the relationship between the concentration of the pesticide and the percentage mortality. Toxicity curve constructed by plotting LC_{50} values against time was given in Fig 1. The peak LC_{50} values for Triazophos was at 1.004 mg/l (12 hours LC_{50}) and the LC_{50} values decreased with increased exposure hours. The minimum LC_{50} values 0.679 mg/l at 96 hours.

Toxicity level

The sublethal concentration of Triazophos treated with *Oreochromis mossambicus* protein level at different time interval and observed that in the liver, muscle and intestine protein level showed declined trend. The changes in the level of protein contents in muscle, liver and intestine of the fish, *Oreochromis mossambicus*, were given in the Figure 4.1-4.3.

Muscle protein level

In the experimental fish, the muscle protein contents registered were 7.55, 6.83 and 5.23 mg/100mg wet tissue at 0.084mg/l and 6.65, 6.11 and 5.13 mg/100mg wet tissue at 0.169 mg/l for 10, 20 and 30 days of exposure respectively. The percentage of decrease was 20.42, 37.84 and 52.16 in 0.084mg/l and 31.59, 44.40 and 54.06 in 0.169 mg/l after 10, 20 and 30 days of exposure respectively.

Liver protein level

Protein content in liver showed a similar trend as that of muscle, reduced to 8.97 mg/100mg wet tissue at (0.084mg/l) on 10^{th} day. It was found to have been further decreased to 5.89 mg/100mg wet tissue after 30 days of exposure period at the same concentration, which was equivalent to 50.71 % reduced from the control value. In the experimental fish, the liver protein contents registered were 8.97, 7.76 and 5.89 mg/100mg wet tissue at 0.084mg/l and 7.44, 6.68 and 4.51 mg/100mg wet tissue at 0.169 mg/l for 10, 20 and 30 days of exposure respectively. The percentage of reduction in liver protein over the control was 31.76, 41.57 mg/l and 62.65 mg/l after 10, 20 and 30 days of exposure respectively.

Intestinal protein level

The intestine protein content also showed the same decline trend. The lowest protein content was observed in the intestine (9.15 mg/100mg wet tissue) after 30 days in the control fish. In the exposed fish, the intestine protein level reduced to 3.41cmg/100mg wet tissue in 0.169 mg/l concentration after 30 days. In the experimental fish, the intestine protein contents registered were 6.54, 4.75 and 4.51 mg/100mg wet tissue at 0.084mg/l and 5.61, 4.07 and 3.41mg/100mg wet tissue at 0.169 mg/l for 10, 20 and 30 days of exposure respectively. The percentage of decrease at 0.084mg/l was 21.69, 44.32 and 51.98 and at 0.169 mg/l it was 39.16, 53.48 and 60.45 after 10, 20 and 30 days respectively.

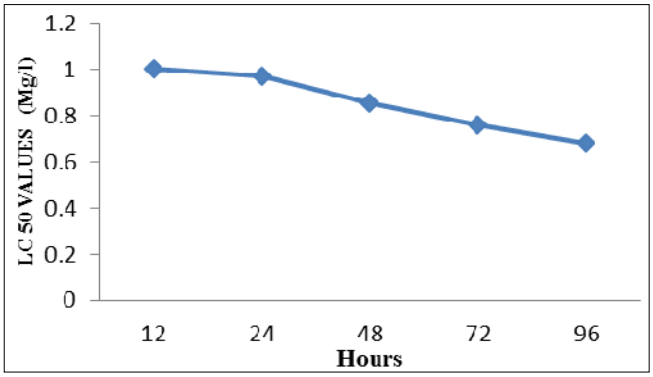

Fig 1: Toxicity curve

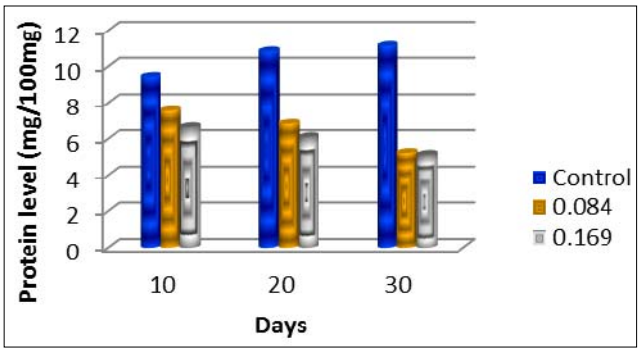

Fig 2: Changes in the level of Protein content in the muscle tissue of *Oreochromis mossambicus* exposed to Triazophos

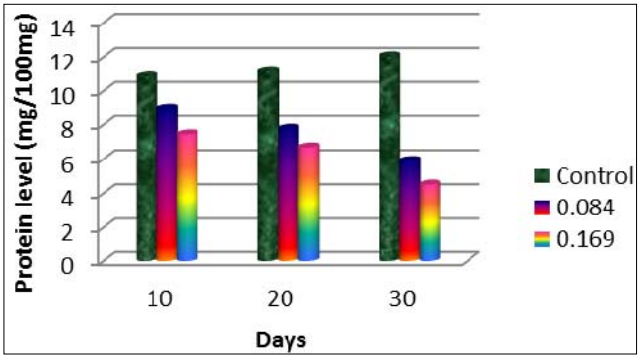

Fig 3: Changes in the level of Protein content in the liver tissue of *Oreochromis mossambicus* exposed to Triazophos

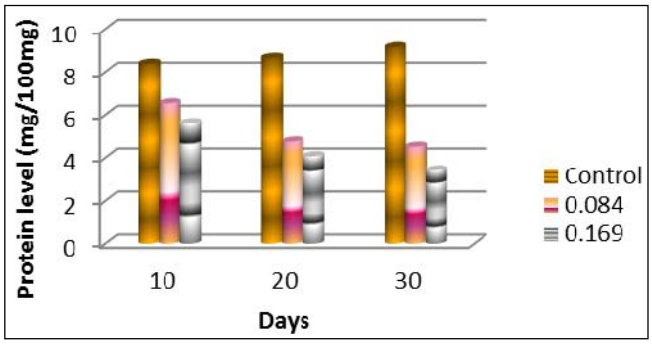

Fig 4: Changes in the level of Protein content in the Intestinal tissue of *Oreochromis mossambicus* exposed to Triazophos

4. Discussion

Toxicity studies have long played an important role in man's efforts to monitor and modify the effect of his activities on the biota. They are highly useful in identifying the sensitive species of an ecosystem that can be used as indicator species for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration LC_{50} or median tolerance limit (TLm). In the present study the computed LC_{50} values for 12, 24, 48, 72 and 96 hours (Table 4.1. to 4.5) were found to be 1.004, 0.972, 0.854, 0.760 and 0.679 mg/l respectively.

Mortality rate increased with the increasing exposure period. A dose dependent mortality was observed. The increase of exposure duration was associated with decrease of LC_{50}. At 12^{th} hour of exposure the LC_{50} dose was 1.004 mg/l but at 96 hours of exposure the LC_{50} dose was 0.679 mg/l. Such type of correlation between the LC_{50} dose and hours of exposure had been reported by Tilak *et al*., (2005) [16]. The pesticides in the aquatic medium lead to massive killing of fish (Shea, 1970). To avoid such damage to aquatic and terrestrial organisms, it is important to test the toxicity of the pesticides before they are applied to the agricultural field on a large scale (Duffus, 1980) [2].

Protein is an important constituent of animal tissue which plays an important role in cellular metabolism and regulates the process of interactions between intra and extra cellular media as constituent of cell membrane. But the protein metabolism has been reported to alter due to the stress of various contaminants. This depletion of tissue protein may reflect a prior increased energy cost of homeostasis, tissue repair and detoxification during stress (Nimmi, 1990) [9]. Mali (2005) [6] opened that depletion in protein level would be due to the diversification of energy to meet the impending energy demands when an animal was under toxic stress.

The decrease in protein content of Triazophos in toxicated fish in the present study also indicates the physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress the animals require high energy. This energy demand might have led to the stimulation of protein catabolism. The present analysis also coincides with the findings of Sastry and Siddiqui (1984) [14] who reported that the protein content was decreased in liver, muscle, kidney, intestine, brain and gill when *C. punctatus* treated with quinalphos. Similar reports of Tilak *et al*., (2005) [16] support the present data. The changes and decrease in protein level might also be due to inhibition of metabolizing enzymes by administration of toxicants.

Sudden induction of the fish to toxic environment should have triggered the protective and defensive mechanism and geared up to energy resources to encounter the adverse environment. As a result, the available carbohydrate resources were quickly exhausted (Borach and yadav, 1985) [1], and to maintain the uninterrupted and increasing energy requirement, the protein breakdown should have commenced to supply necessary precursor to carry on carbohydrate metabolism by TCA pathway, to release the much needed energy. The fall in protein level during toxicant exposure may be due to increased catabolism and decreased anabolism of proteins.

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