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ACUTE TOXICITY STUDIES ON THE FRESH WATER FISH *CLARIAS BATRACHUS* EXPOSED TO PESTICIDE ROGORIN

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ABSTRACT

Pesticides find a perennial application in agriculture, combating pest and enhancing productivity. But on the rear end, these pesticides find avenues to enter water bodies thereby affecting aquatic flora and fauna. These pesticides cause significant variation in structural, histological and biochemical changes in the aquatic fauna, particularly in fish. Present study focus on the effect of Rogorin induced biochemical changes, protein profiling and histological alteration in the tissues of freshwater fish *Clarias batrachus*. Biochemical, protein profile and histological studies were carried out on the various tissues such as kidney, liver and gills of *Clarias batrachus* treated with Rogorin for 96hr at 27ppm (LC_{50}). Biochemical analysis resulted in prominent decrease in protein, carbohydrate and lipids levels, whereas increased in level of free amino acid was observed. SDS-PAGE analysis revealed decreased in intensity and the formation/deletion of bands of the treated tissues with Rogorin when compared to control. Various alterations in the tissues such as such as kidney, liver and gills were observed in the exposed fish were due to pesticidal stress.

Keywords: *Clarias batrachus*, Pesticide, Dimethoate, Biochemical alterations, Histological alteration.

INTRODUCTION

Current agricultural practice compelled the utilization of pesticides in a large scale with the hope of high crop yield, resulting in pushiness of these organics in the food chain causing detrimental effects on biomarkers of aquatic fauna (Yu Zhang *et al.,* 2012). Pesticide dimethoate is widely used in India (Ganeshwade, 2011). Uncontrolled utilization of dimethoate, lead to bio-accumulation of pesticide in nearby lentic and lotic systems and greatly influences the non-target organisms. Among aquatic fauna, fish are very sensitive to wide variety of toxicants especially pesticides, which cause deleterious effects through accumulation (Herger *et al.,* 1995).

Pesticides accumulated in tissues leads to many physiological and biochemical changes thereby influencing the activities of several enzymes and metabolites and finally causes the entire metabolic process disturbed (Scott, 1967; Jackson, 1968; Mukesh, 2013). Examinations of tissues from organisms after toxic stress may possibly reveal the damage extensively and is a tool to assess the environmental pollution. The degree of toxicity depends on the concentration of the toxicants. The objective of the study is to analyze and comprehend the acute toxic effect in Lethal Concentration$_{50}$ (LC_{50}) of commercial grade organophosphate pesticide Rogorin on biochemical, protein profiling and histological alterations in the freshwater fish *Clarias batrachus*.

MATERIALS AND METHODS

Live fish *Clarias batrachus* were collected from Bharat fish farm, Poondy, Tamil Nadu, India and brought to laboratory in well aerated plastic bags. They were transferred to aquarium of 500 L capacity for two weeks of acclimatization in the laboratory conditions. During acclimatization, fish were fed daily with live *Tubifex tubifex* worm. Fish with uniform length (7.4 ± 0.54 cm) and weight (15.14 ± 1.95 g) were selected for bioassay. The stock solution was prepared by dissolving Rogorin (30% dimethoate) in double distilled water. Lethal concentartion$_{50}$ (LC_{50}-27ppm) was calculated (Finney, 1954) and fish were treated for 96 hours in the same.

Fish were sacrificed immediately at the end of exposure period according to Dybem (1983) and organs were taken out immediately. The various tissues such as gill, kidney and liver of control and treated fish were subjected to biochemical studies such as protein (Lowry *et al.,* 1951), carbohydrate (Gerhardt *et al.,* 2004), lipid (Folch *et al.,* 1957) and free amino acid (Kaiser *et al.,* 1970). SDS-PAGE (Shapiro *et al.,* 1967) for both control and treated tissues such as gill, kidney and liver were performed to analyze the protein profile. Histological studies were performed by following modified method of Culling (1974) for the organs such as gills, liver and kidney of fish exposed to the lethal concentration of Rogorin along with control.

RESULTS AND DISCUSSION

In the present study fish exhibited altered behavioral responses such as restlessness, hyperactivity, occasionally jerky swimming and rapid opercular movement when exposed to the pesticide rogorin (27ppm). It might be due to the toxic stress of the pesticide which inhibits the acetylcholine esterase in the brain and

neuromuscular junctions (Rao *et al.,* 2005; Pandey *et al.,* 2005; Agrahari *et al.,* 2006). As a defense mechanism to neutralize the effect of pesticide toxicity, fish secreted copious amount of mucus, which gradually covers all over the body, gills and the buccal cavity (Bisht *et al.,* 2007).

Biochemical Assay:

Exposure of fish to lethal concentrations of Rogorin showed decreased protein, carbohydrate and lipid content, with increased free amino acid content in the gill, kidney and liver after 96hr when compared to control respectively (Table-1). The decreased level of carbohydrate content in the organs of the fish after 96 hours exposure were observed in all tissues when compared to control. Decrement in the carbohydrate level indicates its rapid utilization to meet the enhanced energy demands under dimethoate toxicity through glycolysis or hexose monophosphate pathway (Cappon *et al.,* 1975). During stress, an organism needs sufficient energy which usually supplied from reserve materials like protein, glycogen and cholesterol (Ganeshwade, 2012). Carbohydrate depletion is more prevalent under hypoxic conditions due to toxic stress (Dezwaan *et al.,* 1972, Chandrawathy and Reddy, 1995).

In the present investigation, the level of protein was observed to be decreased in all organs after the treatment with pesticide when compared to the control. Decrease in protein might be due to inhibition of protein synthesis or increase in the rate of degradation of amino acids (Ganeshwade 2011, Binukumari and Vasanthi 2013) which may be entered into tricarboxylic acid (TCA) cycle through aminotransferases probably to cope up with high energy demands in order to meet the stress condition. The fall in protein level during exposure might be due to increased catabolism and decreased anabolism of proteins. Similar kinds of observations were reported for various toxic stresses (James *et al.,* 1979; Natarajan, 1981).

Lipids are an important source of nutrition that provides a significant amount of energy and structural components for reproductive growth (Sargent, 1995). Reduced level of lipid content in the organs of the fish after 96 hours exposure were found to be 1.56±0.13mg/100mg wet tissue, 1.90±0.04 mg/100mg wet tissue and 1.23±0.19 mg/100mg wet tissue in gills, liver and kidney respectively in the current study. It might be due to the reduction in absorption of carbohydrate and protein, resulting in the depletion of energy during toxic stress, which leads to the degradation of lipid to combat the required energy. As the level of the protein and carbohydrate absorption decreases the lipid level also decreases due to lipid metabolism to meet the required energy during the stress condition (Binukumari andVasanthi, 2013).

Increased level of free amino acid was observed in the organs (Liver>Kidney>Gill) of treated fish. This rapid increase in level of free amino acid is probably attributed to step up proteolysis or increased synthesis of free amino acid by transaminase reaction (James *et al.,* 1979; Malla *et al.,* 1988).

Protein Profiling:

SDS-PAGE analysis was performed to analyze the protein profile (Figure-1) for both control and treated fish tissues such as kidney, liver and gill of *Clarias batrachus*. Protein profiling revealed variation in the intensity and protein band width. Electrophoretogram for the tissues of gill, kidney and liver of *Clarias batrachus* showed decrease in intensity and some protein band were disappeared/appeared. The variations in protein band patterns may be due to change in the turnover of various proteins. The inhibition in synthesis of proteins might be due to tissue necrosis which leads to loss of intracellular enzymes or other proteins (Jyothirmayee *et al.,* 2005). Inhibition/activation of genes by the pesticides also might result in synthesis of stress induced proteins (Suneetha *et al.,* 2010).

Histology:

Histological studies reported despair among control and treated tissues. In control gill, kidney and liver exhibited normal structural arrangement and alterations were observed in the tissues of treated fish respectively. In gill tissue at lethal concentration of Rogorin histological alterations such as congestion, shortened swelling lamellae, lifting of lamellar epithelium and broadened secondary lamellae and the mucus depositions on the gills were found (Figure-2). Gill is an important organ for respiration and has direct contact with water, which allows the pesticides to enter through it and get accumulated in the fish body. The increase in mucus deposition on the gills and damage caused to gill lamellae by the toxicant would reduce gaseous exchange (De Silva *et al.,* 2002, Al-Ghanim *et al.,* 2008).

Treated kidney tissues exhibited tubular epithelial cell degeneration, increase in Bowman's capsule space, shrinkage of glomeruli and decreased haematopoietic activity (Figure-3). Kidney plays the major role in producing large quantities of urine to eliminate nitrogenous waste products. Although kidney does not possess high levels of xenobiotic metabolizing enzymes as does the liver, many of the enzymatic reactions occurring in liver have been

shown to occur in the kidney to certain extent (Mohssen, 2001; Durmaz *et al.*, 2006). The shrinkage in renal corpuscles clearly indicates that treated fish adopt some other route of nitrogen excretion while the dilation of the renal corpuscles might be due to an increase in the filtration rate and consequently increases in urine volume which may be a mechanism used by fish to overcome the toxic effect of the pesticide (Roy and Bhattacharya, 2006, Aliaa *et al.*, 2011).

Similarly, treated liver tissue show signs of cell wall rupture, necrosis, parenchymal cells leading to appear smaller in size, cytoplasm become granulated and vacuolated and damaged vacuolar degeneration of hepatocytes (Figure-4). The liver is an important vital organ through which most of the important metabolic functions are occurring and the entry of toxicants primarily affects the liver. Alteration in its structure could be a significant in the evaluation of fish health and exhibit the effects of variety of environmental pollutants (Cough et al., 1975).

Table.1.Change in the biochemical constituents *Clarias batrachus* exposed to LC_{50} of pesticide Rogorin for 96 hours

Biochemical parameters	Organs (mg/100mg wet tissue)	Control (mg/100mg wet tissue)	After 96hours (mg/100mg wet tissue)
Carbohydrate	Liver	8.12±0.44	2.63±0.26
	Kidney	4.02±0.13	1.83±0.04
	Gill	4.98±0.13	2.22±0.15
Protein	Liver	29.32±0.67	15.32±0.48
	Kidney	20.21±0.51	7.42±0.51
	Gill	34.83±0.38	17.22±0.48
Lipid	Liver	6.7±0.13	1.90±0.04
	Kidney	5.55±0.49	1.23±0.19
	Gill	3.32±0.26	1.56±0.13
Free Amino Acid	Liver	4.32±0.24	12.41±0.87
	Kidney	3.26±0.23	7.95±0.42
	Gill	5.42±0.34	10.12±0.43

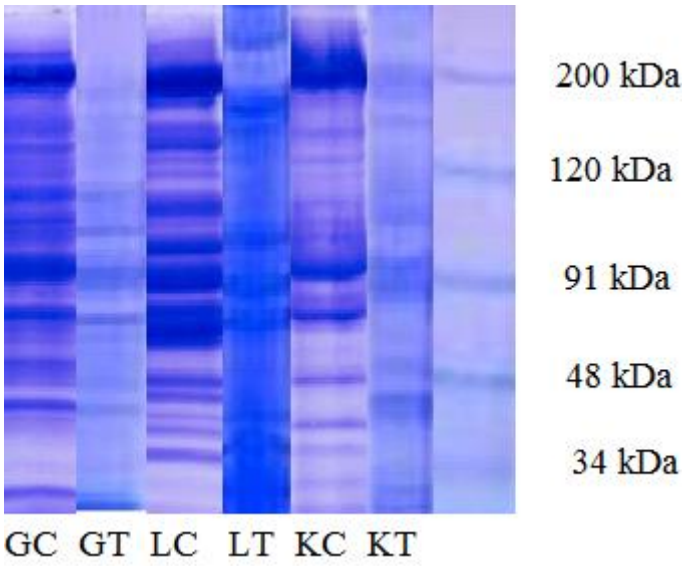

Figure.1.Electrophoretogram of tissues of *Clarius batrachus* treated with Lethal Concentration$_{50}$ of Rogorin for 96 hours

GC-Gill control; GT-Gill treated; LC-Liver control; LT-Liver treated; KC-Kidney control; KT-Kidney Treated

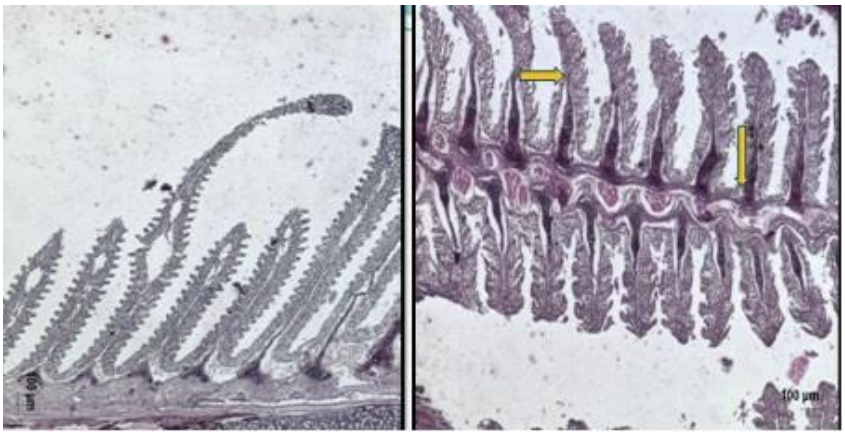

Figure.2.Photomorphograph of control and treated gill tissue of *Clarius batrachus* with Lethal Concentration$_{50}$ of Rogorin for 96 hours showing congestion, shortened and broadened secondary lamellae and mucus deposition

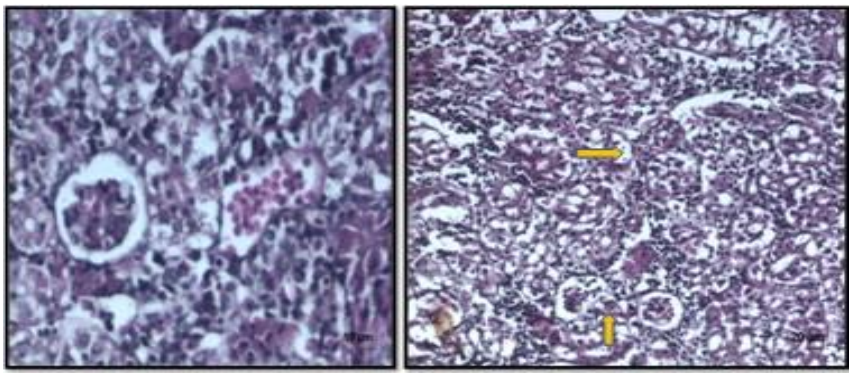

Figure.3.Photomorphograph of control and treated kidney tissue of *Clarius batrachus* with Lethal Concentration$_{50}$ of Rogorin for 96 hours showing tubular epithelial cell degeneration and decreased haematopoietic activity

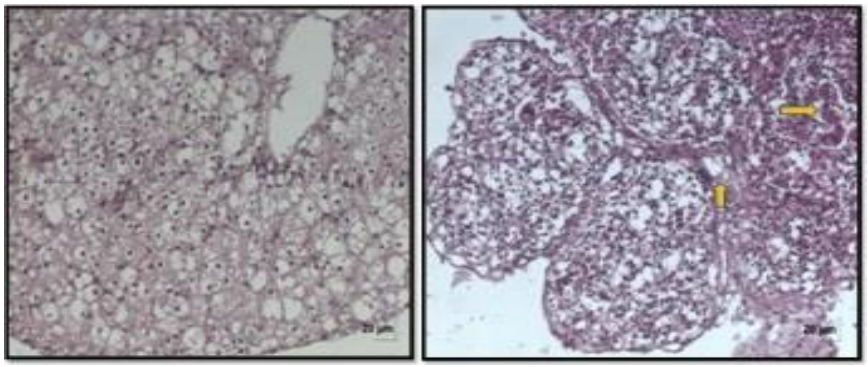

Figure.4.Photomorphograph of control and treated kidney tissue of *Clarius batrachus* with Lethal Concentration$_{50}$ of Rogorin for 96 hours showing vacuolar degeneration of hepatocytes and congestion

CONCLUSION

The present investigation on the fresh water fish *Clarias batrachus* treated with rogorin a commercial organophosphate pesticide revealed the susceptibility of the fish to the toxic stress (LC_{50} – 27ppm). The variations in biochemical parameters and protein profile serve as a tool to monitor the pathological status of the treated fish. Histological alterations also could be used as meaningful indicators of pesticide pollution. Accumulation of pesticides in the water body primarily affects the non-target organism especially fish and get deposited. These fish through food chain affects humans and causes deleterious effects. Hence, the usage of the pesticide should be restricted to have a healthy ecology.

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