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Alachlor 50% EC induced biochemical alterations in *Clarias batrachus* during and after cessation of exposure

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Abstract
The sub lethal effect of the Alachlor 50% EC on certain enzymes in tissue such as total protein, triglyceride, AST (Aspartate transaminase), ALT (Alanine transaminase) and ALP (Alkaline phosphatase) of the freshwater fish, *Clarias batrachus* during and after the cessation of the exposure were observed. Ten fish were exposed chronically to two sublethal concentrations for a period of 21 days and on completion of exposure five fish were sacrificed and tissues such as gill, liver and muscle were analysed for biochemical parameters and remaining five fish were maintained in clean well water for a period of seven days without test substance being spiked in the water and its tissue were collected after reversal period for biochemical analysis. Marked changes were observed in the biochemical parameters of the fish exposed and after the cessation of the exposure.

Keywords: Alachlor toxicity; Total protein; Triglycerides; Aspartate transaminase; Alanine transaminase; Alkaline phosphatase.

1. Introduction
Agrochemicals are extensively used to improve crop yields and as a result they get accumulated in the environment at different strata. Aquatic environment acts as a sink and receives numerous chemical xenobiotics of different moieties like herbicides through the agricultural runoff and pose jeopardize to organisms occupying different trophic levels. It is prone to get affected much due to the runoff from the treated areas and herbicide is one of the pesticides which are widely used in the agricultural field for control of weeds. Alachlor (2-chloro-2', 6'-diethyl – N- methoxymethyl acetanilide) is a selective pre-emergence acetanilide systemic herbicide used for controlling weeds, grasses, broad leaf plants, sorghum and soybean among cereals. Alachlor is considered to be the most hazardous due to its persistent, non-biodegradable and capable of bio-magnification in the aquatic food chain [8].
Being anthropogenic, the acetanilide herbicides interfere with several physiological processes including biosynthesis of lipids and proteins in non-target organisms like fishes [9, 22, 14]. It is important to study the pollutant interactions in non-target organisms at different levels of biological organization to explore the mechanism of action [7]. Hematological and biochemical indices appear to be a suitable means of pesticide contamination. Alachlor induced enzymatic and hematological alterations in fishes are not well studied [25]. The effect of alachlor technical grade and its commercial formulation Lasso 50% EC on the biochemical parameters of the freshwater fish, *Channa punctatus* (Bloch) and the impact on glycogen, total proteins and metabolic enzymes Aspartate Amino Transferase (AAT), Alanine Amino Transferase (ALAT), Lactate dehydrogenase (LDH), Deoxyribonucleic acid (DNA) and Ribonucleic Acid (RNA) were studied. The glycogen, total proteins, DNA, RNA were all decreased but the activity of the enzymes AAT, ALAT and LDH were all increased which was due to the toxic stress [23]. *Clarias batrachus* is one of the fish species cultivated in paddy field as a part of the integrated fish culture in several Asian countries and hence chosen as a model system to study the effects of alachlor at sublethal concentrations on certain key enzymes.

2. Materials and Methods
Healthy catfish *Clarias batrachus*, was procured from commercial fish farm and quarantined

for a period of one month in cement tanks. The fish were fed with commercial fish pellets. The body weight and length of the fish was recorded prior to the acclimatization and the mean value of the body weight was 7.9 g and 11.4 cm. The fish was then acclimatized in glass aquaria for 14 days to the laboratory condition. During the acclimation, the fish were fed and the feed was withdrawn 24 hour prior to exposure. The physico-chemical parameters were analyzed.

2.1 Toxicity bio-assay determination

An acute semi-static 96 hour study [1] was conducted with Alachlor 50% EC and LC_{50} 6.5 mg/L was determined. The exposure water was renewed approximately at the end of 24 hour with the test chemical. Ten fish each were exposed to 3, 3.8, 5.0, 6.5, 8.5, 11.0, 14.3 mg/L and the mortality and behavioral abnormalities were recorded. During the exposure the fish were fed on alternate days and the physico-chemical parameters were analysed periodically. Alachlor is reported to be a stable chemical under normal conditions [26]. Significant losses of alachlor were observed only after 30 days [10].

Based on the 96 hour LC_{50}, two sub lethal concentrations $1/10^{th}$ (0.65 mg/l) and $1/20^{th}$ (0.32 mg/l) along with a control group were chosen for chronic exposure of 21 days with *Clarias batrachus*. Each group consisted of ten fish. The effect of Alachlor 50% EC on the biochemical parameters total protein, triglycerides, alanine transaminase, aspartate transaminase and alkaline phosphatase in the fish tissue of gills, liver and muscles were determined.

On 21^{st} day five fish were euthanized with MS-222 (500 mg/L) and remaining five fish in all treatment and control were kept in well water without the herbicide for another seven days to observe the reversal effect and thereafter sacrificed for biochemical analysis. Tissues gill, liver, kidney, muscle were collected and homogenated in 5 ml of ice-cold phosphate buffer and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected for the estimation of total protein, triglycerides, alanine transaminase, aspartate transaminase and alkaline phosphatase. The biochemical determinations were performed using Erba reagent kits on a semi-autoanalyser (Erba chem-5 plus, Transasia, India).

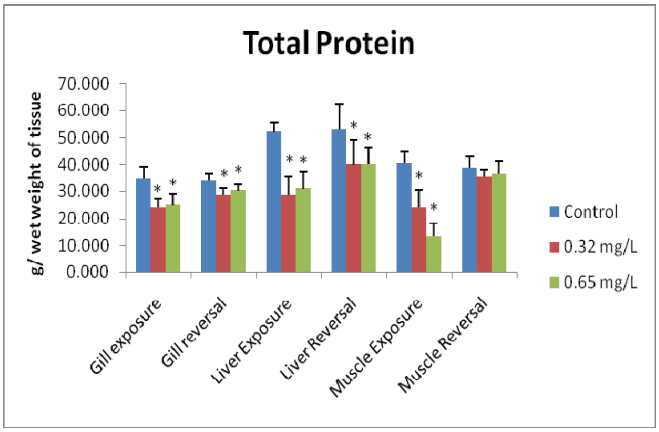
The values were compared using ANOVA (Student-Newman-Keuls test) and the significance was calculated using the statistical package [5].

3. Results and Discussion

Toxic chemicals cause significant changes in the internal organs and perturb biochemical physiology of the exposed fish. The mortality recorded at the end of 96 hour bioassay was 0%, 10%, 30%, 50%, 60%, 100% and 100% in the exposed concentrations. No mortality was observed in the control group of fish throughout the experimental period. Behavioral abnormalities such as loss of equilibrium, rapid opercular movement, and lateral lying at the bottom of the aquaria were observed in fish exposed to high concentrations 11.0 and 14.3 mg/L, whereas, loss of equilibrium and rapid opercular movement was observed in few fish exposed to 6.5 and 8.5 mg/L. Based on the mortality observed, the LC_{50} determined was 6.5 mg/L and the 95% lower confidence limit determined was 5.6 mg/L and higher confidence limit was 7.6 mg/L. The temperature of the exposure water was in the range of 23.8 – 24.2, pH was 7.4 - 7.8, dissolved oxygen was 7.6 - 8.0 mg/l and hardness (APHA, 1998) 265 - 268 mg/l in terms of $CaCO_3$.

Enzymes play an important role in metabolism and changes in the enzyme levels are one of the fundamental steps to assess the effects of toxicants. It is known that the time required for recovery is a function of toxicant concentration and exposure time [13]. The length of time which the herbicides remains in the water column determines the length of exposure. The fate of an aquatic herbicide determines whether aquatic life could be exposed at a later time [6]. In fish it has been observed that the external organ is affected due to the toxic chemicals causes loss of equilibrium, increase in opercular movement, irregular movement and finally leads to death. This may be attributed with significant damage to the internal organs [12].

Significant reduction was observed in total protein estimated in the tissues of gill liver and muscle after 21 days exposure in fish exposed to 0.32 mg/l and 0.65 mg/L when compared to the control. After the reversal period of 7 days in 0.32 mg/l and 0.65 mg/L significant reduction was observed in gill and liver tissue except in muscle tissue the reduction was not significant when compared with the control (Fig. 1).



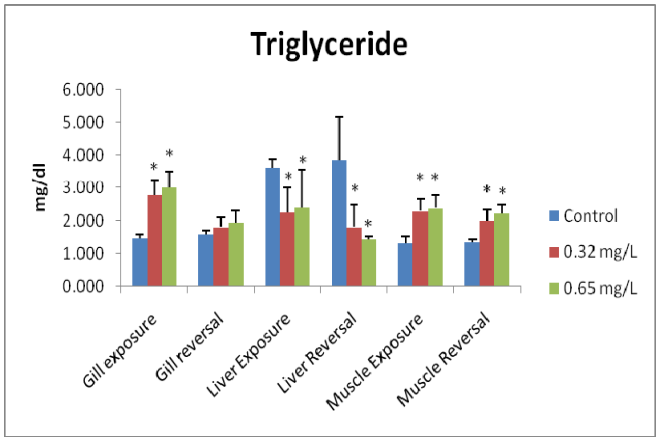
*-Denotes Significant change (P>0.05)

Fig 1: Graphical representation of Total protein levels in tissues of *Clarias batrachus* exposed to Alachlor 50% EC

Acute 96 hr and 15 days effect of sublethal concentrations of carbamate pesticide in *Clarias batrachus* and observed a decrease in the levels of total protein in fish serum [17]. Gradual and significant decrease in proteins was reported in all tissues of *Channa punctatus*, when treated with technical grade Alachlor and 50% EC Lasso [23] under lethal and sublethal concentrations of both the technical grade of alachlor and lasso 50% EC formulation over the control. The variation in distribution suggests difference in metabolic calibers of various tissues. A decrease in liver protein content throughout the exposure and gradual recovery and lesser changes in muscle protein content during exposure of *Clarias batrachus* to carbofuran insecticide exposure was reported. A reduction in protein content of these organs in the exposed fish indicates a physiological adaptability of fish, possibly to compensate for the stress of exposure. The *Clarias batrachus* was transferred to clean water (recovery period) the return in protein content, indicates that these tissues may have recovered to some extent from carbofuran toxicity [7]. Similarly the investigation of fish *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* gill, brain, liver, kidney and muscle exposed to chlorpyrifos showed a decrease in the total protein in the sublethal concentration. The decreased trend of

the protein content in most of the fish tissues is due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose; or due to directing amino acids for the synthesis of necessary proteins [22]. Protein profile decline suggests stress in metabolic process and impairment of protein synthesis in fish; the catabolic process may be proteolysis leading to rapid decline in protein to meet the energy demand in stressful condition.

Significant elevation of triglycerides in gill and muscle tissue and reduction of triglycerides in liver tissue of fish exposed to 0.32 mg/l and 0.65 mg/L was observed when compared with control after the exposure. After reversal period, significant reduction of triglycerides in liver tissue and elevation in muscle tissue was observed in fish exposed to 0.32 mg/l and 0.65 mg/L and elevation in gill tissue was not significant in both the exposed concentrations (Fig. 2).



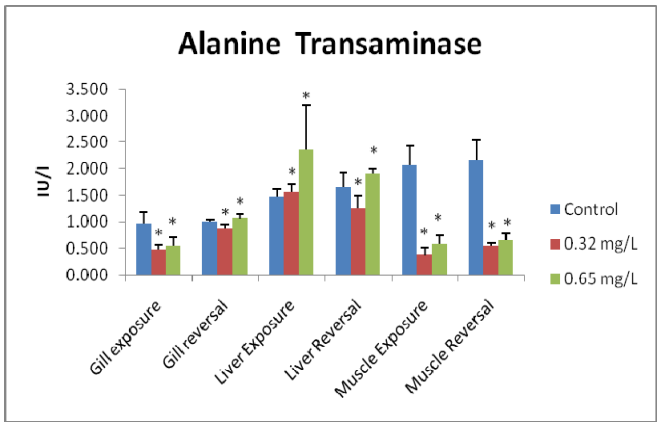
*-Denotes Significant change (P>0.05)

Fig 2: Graphical representation of Triglyceride levels in tissues of *Clarias batrachus* exposed to Alachlor 50% EC

Triglycerides are the form in which fat is stored, as well as transported throughout the body. Herbicides were found to affect the food reserves influencing the reduction of energy resource and hence an overall reduction in protein as well as triglycerides. The decrease is due to proteolysis as well as glycogenesis or lysis [23]. Fish exposed to famfos treatment a decrease in the triglyceride and stated that it may be due to the utilization of cholesterol and other lipid fractions in treated fish to counteract toxic stress and stabilize the molecules of toxicants [20].

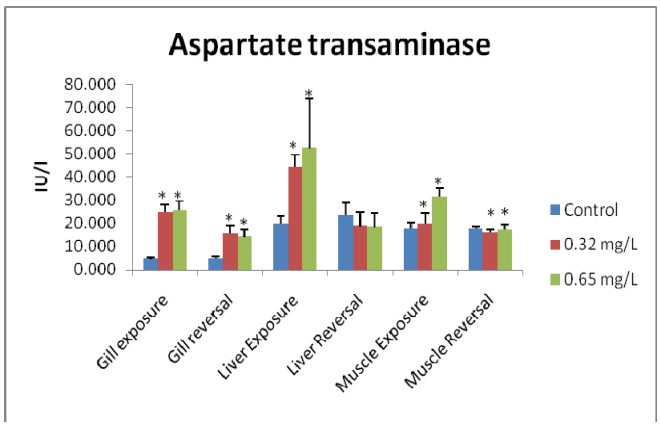
Significant reduction was observed in alanine transaminase (ALT) in gill and muscle tissue and significant elevation in liver tissue after the exposure. After reversal period significant reduction in gill, liver and muscle tissue was observed in fish exposed to 0.32 mg/l and 0.65 mg/l when compared to the control (Fig.3).

Significant elevation was observed in aspartate transaminase (AST) in gill, liver and muscle tissue of fish exposed to 0.32 mg/l and 0.65 mg/l when compared to the control. Significant elevation and reduction was observed in gill and muscle tissue respectively, after the reversal period in fish exposed to 0.32 mg/l and 0.65 mg/l. AST reduction was observed in liver tissue and it was not significant when compared to the control (Fig. 4).



*-Denotes Significant change (P>0.05)

Fig 3: Graphical representation of Alanine transaminase levels in tissues of *Clarias batrachus* exposed to Alachlor 50% EC



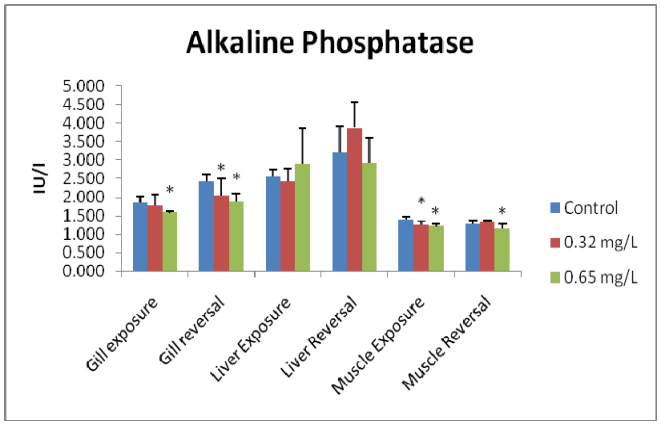
*-Denotes Significant change (P>0.05)

Fig 4: Graphical representation of Aspartate transaminase levels in tissues of Clarias batrachus exposed to Alachlor 50% EC

Aspartate transaminase and Alanine transaminase are located in both mitochondrial and cytosol fractions of the cell. Significant decrease was observed in transaminase levels in most of the tissues in both the fish exposed to both sublethal and lethal concentrations of fenvalerate technical grade. The enhanced activity of the aspartate transaminase was recorded during the exposure and after the reversal period there was a slight decrease. Similarly, Alanine transaminase activity was elevation in the liver and reduction in gill and muscle when compared with the aspartate transaminase which recovered to a great extent, this activity of the transaminases may be due to the supply of oxalacetic acid and pyruvate, alpha ketoglutaric acid to meet the increase energy demand during toxic stress conditions [1].

After exposure, no significance was observed for alkaline phosphatase in gill tissue of fish exposed to 0.32 mg/L whereas, a significant reduction was observed in 0.65 mg/L. In liver tissue the reduction was not significant in 0.32 mg/L whereas, there was slight elevation in 0.65 mg/L when compared with the control. Significant reduction was observed in muscle tissue after the exposure in 0.32 mg/L and 0.65 mg/L. After reversal period significant reduction was observed in gill tissue of fish exposed to 0.32 and 0.65 mg/L when compared to control. In the liver tissue there was

an elevation in fish exposed to 0.32 mg/L and a slight reduction in 0.65 mg/L when compared with the control and it was not significant. In muscle tissue of fish exposed to 0.35 mg/L ALP was not significant; there was slight significant reduction in 0.65 mg/l when compared with the control (Fig 5).



*-Denotes Significant change (P>0.05)

Fig 5: Graphical representation of Alkaline phosphatase levels in tissues of *Clarias batrachus* exposed to Alachlor 50% EC

Alkaline phosphatase is involved in the maintenance of the orthophosphate pool transport of phosphoryl groups and the hydrolysis and esterification of the metabolites through the membrane transport, alteration in alkaline phosphatase activity in the fishes due to the effect of pesticides have been noted by several workers [15]. The enzyme activity of alkaline phosphatase may be due to the herbicide and it suggests enhanced protein catabolism and probable hepatocellular damage in the organism. The effect of sub-lethal concentration of a carbamate pesticide, sevin on acid and alkaline phosphatase enzyme activities of liver and muscle was studied in a freshwater, edible fish, *Sarotherodon mossambicus* (Peters) exposed to 24, 48, 72 and 96 hours. The severe drop in tissue alkaline phosphatase enzyme could be considered adaptive for the fish to meet the energy demand aiding anaerobic breakdown of glycogen under sevin toxicity [16]. Any alteration in the activity of alkaline phosphatase affects the organisms in a variety of ways [18]. The effect of pyrethroid on the fish *Clarias bactrachus* found to reduce alkaline phosphatase in response to the toxicant [4]. Alkaline phosphatase splits various phosphate esters at an alkaline pH and mediates membrane transport [19]. Repeated exposure to alachlor causes hepatotoxicity in rats and tumor formation in rats [24].

4. Conclusion

The present study results it can be inferred that the sub lethal concentrations can be detrimental to the fish exposed. The change in the biochemical parameter is due to induced biotransformation in the exposed fish due to the chemical assault to the physiological system. The estimated biochemical parameters total protein, triglycerides, alanine transaminase and aspartate transaminase and alkaline phosphatase showed a marked difference of induction and inhibition when compared with the control during the exposure and at the end of the reversal period and it serves as an indicator for pesticide stress.

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