

# “Effects of Sublethal Dimethoate Exposure on Acetyl Cholinesterase (AChE) Activity in *Clarias batrachus* (Linn.)”

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## ABSTRACT

The widespread use of organophosphorus insecticides in agriculture poses a serious threat to aquatic ecosystems, particularly to non-target organisms such as fish. Dimethoate is one of the commonly used pesticides that can enter freshwater bodies through agricultural runoff, leading to sublethal toxic effects. The present study investigates the impact of sublethal concentrations of Dimethoate on the specific activity of Acetyl Cholinesterase (AChE) in the freshwater edible fish *Clarias batrachus* (Linn.).

Healthy specimens of *Clarias batrachus* were exposed to predetermined sublethal concentrations of dimethoate for a specific experimental period under controlled laboratory conditions. The activity of AChE enzyme was assayed in vital tissues such as brain, gill, and muscle using standard biochemical methods. The results revealed a significant inhibition of AChE activity in all tissues, with the highest inhibition observed in the brain, followed by gill and muscle tissues. The degree of enzyme inhibition was found to be both concentration- and exposure-dependent.

The inhibition of AChE activity indicates disruption in normal neurotransmission due to accumulation of acetylcholine at synaptic junctions, leading to neurotoxicity. Behavioral alterations such as erratic swimming, loss of equilibrium, and reduced responsiveness were also observed in treated fish. These findings suggest that even sublethal levels of Dimethoate can induce significant biochemical and physiological stress in aquatic organisms. A significant elevation in Acetyl Choline content and inhibition in the activity of the Acetyl cholinesterase was observed in fishes with insecticide Dimethoate for 48 hrs. 72 hrs. and 96 hrs.

**Keywords:** Dimethoate, Acetyl Cholinesterase, *Clarias batrachus*, Organophosphorus insecticides, Sublethal toxicity

## INTRODUCTION AND REVIEW OF LITERATURE

The extensive use of pesticides in modern agriculture has significantly increased crop productivity, but it has also led to serious environmental concerns, particularly in aquatic ecosystems. Among various classes of pesticides, organophosphorus compounds are widely used due to their high insecticidal efficiency and relatively rapid degradation. However, their continuous application results in contamination of nearby water bodies through agricultural runoff, posing a threat to non-target aquatic organisms, especially fish.

Dimethoate is a commonly used systemic organophosphorus insecticide employed to control a wide range of pests in agricultural fields. Despite its effectiveness, dimethoate is highly toxic to aquatic fauna and can induce both acute and chronic effects even at low (sublethal) concentrations. Once introduced into aquatic environments, it can persist long enough to affect fish physiology, behavior, and survival. Fish are considered excellent bioindicators of environmental pollution due to their sensitivity to toxic substances and their ecological relevance in aquatic food chains. The freshwater edible fish *Clarias batrachus* (Linn.), commonly known as walking catfish, is widely distributed in Indian freshwater systems and holds significant economic importance.

Its hardy nature and tolerance to varying environmental conditions make it a suitable model organism for toxicological studies.

One of the most important biochemical targets of organophosphorus insecticides is the enzyme Acetyl Cholinesterase (AChE), which plays a crucial role in nerve impulse transmission by hydrolyzing the neurotransmitter acetylcholine at synaptic junctions. Inhibition of AChE activity leads to accumulation of acetylcholine, resulting in continuous stimulation of nerves, muscle paralysis, and ultimately physiological dysfunction. Therefore, AChE activity is widely used as a reliable biomarker for assessing neurotoxicity in aquatic organisms.

Previous studies have reported that exposure to organophosphorus pesticides results in significant alterations in enzymatic activities, behavioral responses, and metabolic processes in fish. However, most research has focused on lethal concentrations, whereas the effects of sublethal exposure are equally important as they can cause long-term physiological stress and ecological imbalance without immediate mortality. In this context, the present study aims to evaluate the impact of sublethal concentrations of dimethoate on the specific activity of Acetyl Cholinesterase in different tissues of *Clarias batrachus*. Understanding these biochemical changes will provide valuable insights into the toxic effects of pesticide exposure and help in developing effective strategies for monitoring and managing aquatic pollution.

The benefits due to use of Organophosphorus insecticides are numerous, but at the same time they show considerable harm to biosphere, pesticides through agricultural run off to the streams, lakes and ponds during rainy season adversely affect the non-target aquatic fauna. Zooplankton, insects, worms, mollusks and fishes suffer from various ill effects, like mass mortality, chronic changes in behavior, low survival rates and morphological changes in different organ system, by altering biochemical constituents of fish and other animals (Singh *et al.*: 2004; Setha and Saxena, 2003, Mahboob and Siddique, 2002, Barcavolli and Martinez, 2004, Thangavel *et al.*: 2004). Among the aquatic fauna fish are most sensitive to pesticides especially organophosphorus insecticides. The wide range of application of Organophosphorus (OP) insecticide in agriculture has resulted due to the ban on organochlorine (OC) compounds. The use of OP insecticides is now increasing at a fast rate because of their high efficiency and biodegradability. Pollution of biosphere by insecticides is characterized by the presence of OP residues in food products and their circulation along the food chains. As the fish constitute an important link in the food chain its pollution causes threat to the entire aquatic environment. In the present investigation an attempt has been made to study the toxic effects of technical grade OP insecticides i.e. **Dimethoate** on target enzyme Acetyl cholinesterase and neurotransmitter Acetylcholine were studied in different tissues of normal and exposed fish for 48 hours 72 hours and 96 hours of time period. The action of OP insecticides is generally neuro toxic and the inhibitors of Acetyl Cholinesterase enzyme.

## MATERIALS AND METHODS

Healthy fresh water fish *Clarias batrachus* weighing around 30-40 gm and 18-20 cm in length were collected from the local market and were acclimatized to laboratory conditions for 15 days in a cement tank under running tap water. The fish were fed with groundnut cake and white of the egg, but starved one day before the day of experimentation. The LC<sub>50</sub> was determined by the method of Finney (1964) and Bayne *et al.*: (1977). The fish were exposed to a sublethal concentration of Dimethoate Organophosphorus (OP) insecticide for 48 hrs, 72 hrs, and 96 hrs. Liver, brain, muscle and gill tissues were isolated from the normal and exposed fish.

### Estimation Of Acetylcholine

Method.: Hestrin (1949) modified by Augustinson (1957) Tissues weighing 50-100 mg were taken into test tubes containing 2.0ml of double distilled water and boiled for 5 minutes to release the bound acetylcholine (Ach) by inactivating the AChE. The tubes were cooled and the homogenates were prepared in 2.0 ml of double distilled water. The contents were centrifuged at 3000 rpm for 15 minutes and to 1.0 ml of the supernatant, 2.0 ml of 2M alkaline hydroxylamine hydrochloride reagent was added followed by 1.0 ml HCl (HCl: H<sub>2</sub>O). The contents were centrifuged at 3000 rpm for 10 minutes. 2.0 ml of the clear filtrate 1.0 ml of 0.37 N Ferric Chloride

was added and the color developed was react at 540 nm against the reagent blank. Acetylcholine chloride was used as standard. Units: micro moles/g wet weight of the tissue.

### Acetyl Cholinesterase Enzyme (Ache).

Method. Metcalf (1951) described by Augustinson (1957)

The percent (w/v) tissue homogenates were prepared in 0.25 m sucrose solution which form the enzyme source. The reaction mixture in a total volume of 2 ml contained 100 micro moles of phosphate buffer (pH 7). 8 micro moles of acetylcholine chloride and 0.3 ml of enzyme source. The reaction mixture was incubated for 30 minutes at 29°C and the reaction was stopped by adding 2 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 6N HCl. The content were shaken well and centrifuged. 2.5 ml of supernatant. 5 ml of 10% ferric chloride was added and the color developed was read at 540 nm against the reagent blank in a colorimeter.

Units;  $\mu$  moles of acetylcholine hydrolyzed/mg protein/h.

## RESULTS AND DISCUSSION

During the exposure of fish to different concentrations of of organophosphorus insecticides. Few changes were observed in the behavior of fish. there were no casualties in the controls. The test fish lies irregularly often coming to the surface followed by loss of equilibrium. The color of the fish skin gradually became very dark during the time of death.

**Table.1:** Changes in Acetylcholine content ( $\mu$  moles/gm wet weight of the tissue) intissues of control and insecticide exposed fish *Clarias batrachus* .

Tissue	Control	48 hrs.	72 hrs.	96 hrs
<b>BRAIN</b>	47.43 $\pm$ 3.92	73.29 $\pm$ 2.15 PC = + 54.52	68.42 $\pm$ 2.25 PC = + 44.25	62.34 $\pm$ 1.84 PC = + 31.43
<b>MUSCLE</b>	33.89 $\pm$ 2.73	52.21 $\pm$ 2.39 PC = + 54.08	50.75 $\pm$ 2.11 PC = + 49.75	47.34 $\pm$ 2.18 PC = + 39.68
<b>GILL</b>	25.73 $\pm$ 1.62	48.62 $\pm$ 1.38 PC = + 89.18	2.73 $\pm$ 0.96 PC = + 66.07	39.82 $\pm$ 2.24 PC = + 54.76
<b>LIVER</b>	21.79 $\pm$ 3.26	40.69 $\pm$ 1.05 PC = + 86.73	38.46 $\pm$ 1.96 PC = + 76.50	38.89 $\pm$ 1.96 PC = + 60.12

Each value is mean  $\pm$  S.D. of 6 individual observations. Values are significant at 1% level (P<0.01).

The levels of Acetylcholine in tissues of control and insecticide exposed fish are presented in table-1. The Acetylcholine content in control tissues viz., **Brain, Muscle, Gill** and **Liver** is 47.43, 33.89, 25.73, and 21.79 micro moles/gm wet weight of the tissues respectively.

The percent changes in treated fish by exposed with Dimethoate at 48 , 72 and 96 hours, are in **Brain** +54.52 ,+44.25 and +31.43 in **Muscle**, +54.08, +49.75, and + 39.68, in **Gill**, +89.18, +66.07 and + 54.76, finally in **Liver** +86.73, +76.50, and +60.12 respectively.

A significant elevation in Acetylcholine content and inhibition in the activity of the neuro transmitter enzyme AChE was observed in Fish on treatment with OP insecticide. The serine hydroxylgroup is blocked by the OP compound leading to the inhibition of AChE which result in the excessive accumulation of Acetylcholine at the synaptic cleft disrupting the transfer of nerve impulses leading to paralysis and death of the fish. (O. Brien,1967).

**Table .2:** Changes in Acetyl Cholinesterase activity ( $\mu$  moles of Acetylcholine Hydrolyzed/mg protein/h) in tissues of control and insecticide exposed fish *Clarias batrachus*.

Tissue	Control	48 hrs.	72 hrs.	96 hrs
<b>BRAIN</b>	7.21 $\pm$ 0.39	3.11 $\pm$ 0.40 PC = - 56.86	3.89 $\pm$ 0.49 PC = - 46.05	4.97 $\pm$ 0.51 PC = - 31.06
<b>MUSCLE</b>	4.72 $\pm$ 0.16	2.37 $\pm$ 0.44 PC = - 49.79	2.95 $\pm$ 0.52 PC = - 37.50	3.28 $\pm$ 0.40 PC = - 30.51
<b>GILL</b>	3.27 $\pm$ 0.17	1.98 $\pm$ 0.37 PC = - 39.45	2.14 $\pm$ 0.39 PC = -34.55	2.22 $\pm$ 0.37 PC = -32.11
<b>LIVER</b>	2.12 $\pm$ 0.15	0.99 $\pm$ 0.17 PC = - 53.30	1.18 $\pm$ 0.16 PC = - 44.33	1.38 $\pm$ 0.15 PC = - 34.90

Each value is mean  $\pm$  S.D. of 6 individual observations. Values are significant at 1% level (P<0.01).

The Activity of Acetyl Cholinesterase Enzyme (AChE) in tissues of control and insecticide exposed fish are presented in table-2. The AChE in control tissues viz., **Brain, Muscle, Gill and Liver** is 7.21, 4.72, 3.27 and 2.12, respectively. The interaction of OP insecticides with AChE indicate that their inhibitory action is reversible. The OP compound inhibits the activity of enzyme AChE and by doing this they disrupt the transmission of nerve impulses due to the enormous accumulation of ACh at the synaptic cleft. The ACh significantly increased whereas the enzyme AChE involved in terminating the action of AChE was inhibited in brain, muscle, liver and gill of fish (O. Brien, 1967, Shobha Rani 1990, Venkateshwarlu *et al*: 1992, Chitra *et al*: 1993, Sivakumari *et al*: 1997, Sridevi *et al*: 1998, Ganesh *et al*: 1999, Janaiah *et al*: 2006).

Fresh waterfish *Clarias batrachus* exposed to Dimethoate treated at different time periods. The Percent Changes at 48 hrs, 72 and 96 hrs. of time period are -56.86, -46.05, and -31.06 in **Brain**. -49.79, -37.50, and -30.51, in **Muscle**, -39.45, -34.55, and -32.11, in **Gill** and in **Liver** -53.30, -44.33, and -34.90 respectively.

## CONCLUSION

In the present investigation The toxic property of Organophosphate insecticides and inhibiting their target enzyme AchE was well studied, A significant elevation in acetylcholine content and inhibition in the activity of the neurotransmitter enzyme was observed in fresh water fish *Clarias batrachus* on treatment with OP insecticide Dimethoate for 48,72, and 96 hrs. Activity of Acetyl Cholinesterase disrupted the transmission of nerve impulse due to the enormous accumulation of Acetylcholine at the synaptic cleft.

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