Impact of Malathion Toxicity on Acetyl Choline Esterase Activity in ‘*Carassius auratus*’ (Linnaeus, 1758) and *Botia*

***striata* (Narayan Rao, 1920)**

# ABSTRACT

Fishes are considered as the bio-indicator species to monitor water pollution. Malathion, an organophosphate pesticide widely utilized in agriculture across Kerala and other Indian states, is a potent insecticidal agent. This study aims to assess the impact of malathion on acetyl choline esterase (AchE) activity in various tissues of *Carassius auratus* and *Botia striata*. Separately, both fish species were subjected to a sub-lethal concentration of 3.4 ppm malathion for 48, 72, and 96 hours, followed by a recovery period of 15 days. The quantification of AchE activity was done using Ellman’s method. In the control group, highest activity was observed in brain and muscle, with moderate activity in liver, and kidney. In *C. auratus*, following 72 hours of exposure, AchE activity

significantly declined from 7.2 to 1.8 in the brain and in *B.striata*, the value declined from 5.7 to 1.4. In *C. auratus*, there was a gradual recovery with activity reaching 5.26, and in *B.striata* recovery activity reached 4.46 by the end of 15-days. In case of kidney in *C. auratus*, the AchE activity was found to show variation with fluctuating values while in *B.striata* an abnormal AchE activity was found in recovery period. The findings highlight the severe neurotoxic effects of malathion on aquatic organisms and its potential risks to environmental health and biodiversity. Moreover, the persistent use of another organophosphate pesticide, endosulfan in Kasaragod district, Kerala, has led to detrimental effects on human populations, including children. This poses the concern of the prolonged usage of malathion on human population. Reports indicate chromosomal abnormalities and genetic mutations, with the possibility of these defects being transmitted across generations. This study underscores the urgent need for regulatory measures to mitigate the environmental and public health risks posed by malathion.

*Keywords: Acetyl choline esterase; Botia striata; Carassius auratus; malathion pesticide; sublethal toxicity.*

# INTRODUCTION

Pesticides are chemical agents widely used to control insect pests in agricultural fields, particularly in paddy and wheat cultivation. Though pesticides play a critical role in achieving large-scale agricultural yields, their adverse effects on the environment and human health cannot be ignored. One prominent example is the pesticide pollution reported in Kasaragod district, Kerala, where endosulfan exposure has caused severe chromosomal aberrations, leading to cancer and other health abnormalities. The impact extends beyond human populations, as pesticides often contaminate nearby water bodies through rainfall or wind, significantly affecting aquatic biodiversity (Almeida et al., 2005). The harmful consequences of pesticides have led to the banning of certain compounds, such as DDT and endosulfan. However, their prohibition has often resulted in the increased use of alternative chemicals, including organophosphates and carbamates, which remain prevalent in India and globally. Despite their utility in enhancing agricultural productivity, the ecological and health risks associated with these chemicals underscore the need for more sustainable pest control solutions.

Biological markers are measurable cellular, biochemical, or molecular alterations in biological media such as tissues, cells, or fluids. These markers play a crucial role in medicine, environmental health, developmental biology, toxicology and scientific research. Acetylcholinesterase (AchE), a member of the choline esterase family, is a specialized carboxylic ester hydrolase responsible for breaking down esters of choline. AchE is predominantly located at neuromuscular junctions and cholinergic synapses in the central

nervous system, where it terminates synaptic transmission by hydrolyzing the neurotransmitter acetylcholine into choline and acetic acid, preventing continuous nerve firings (Lionetto et al., 2013). Organo phosphorous pesticides exert their toxic effects by inhibiting AchE, leading to excessive stimulation of cholinergic nerves, tremors, convulsions, and, in severe cases, death of aquatic organisms (Walker, 2003; Adedeji, 2011). While many organo phosphates and carbamates degrade rapidly in the environment, their inhibitory effects on esterases can persist for days or weeks (Fulton & Key, 2001).

Malathion (C10H19O6PS2), developed in 1950 and first used by United States Department of Agriculture (USDA), is now regulated by the United States Environmental Protection Agency (Epa, 2009). It is an organophosphorus insecticide and is widely used in pest control in India. Malathion's (chemical name: O, O-dimethyl dithiophosphate of diethyl) International Union of Pure and Applied Chemistry (IUPAC) name is Diethyl 2- dimethoxyphosphinothioylsulfanylbutanedioate (Deka & Mahanta, 2016).

Since fishes are very sensitivity to changes in their aquatic ecosystem they are known to be bio indicators to monitor the water pollution and physical structure of the water bodies. Among ornamental fishes, *Carassius auratus* (goldfish) is widely used in endocrinology, neurobiology, and comparative cardiac studies (Filice et al., 2022). The cypriniformes (cyprinids) are the most diverse fish order consisting of fresh water fishes or teleosts belonging to the class, actinopterygii, and the bony fishes. They are finned fish, with 4400 different species and include true minnows and carps (Tan & Armbruster, 2018). The gold

fish, one of the popular aquarium fish belongs to Cyprinidae family of Cypriniformes order and is seen widely in Asia. Gold fish breeds vary in shape and color and are used as food in East Asia. *Botia striata* commonly called Zebra Loach comes under family Cobitidae under Cypriniformes order. These peaceful fishes, are also suitable for aquariums.

## AchE Inhibition Studies in Fishes

Studies have demonstrated significant AchE inhibition in various fish species. For example, Chandrasekhara & Pathiratne, (2007) reported reduced AchE activity in the brain and liver of *Oreochromis niloticus* exposed to chlorpyrifos and carbosulfan, while Adedeji (2011) observed diazinon-induced AchE inhibition in the brain *of Clarias gariepinus*, which impaired respiratory functions and produced neurological symptoms. Earlier investigations by Kabeer et al., (1981) on *Tilapia mossambica* revealed AchE inhibition in various tissues at 12-hour intervals, with maximum inhibition observed at 36 to 48 hours of malathion exposure. Recovery occurred after 72 hours, likely due to pesticide degradation. Similarly, Halappa & David, (2009) studied the behavioral responses of *Cyprinus carpio* exposed to sub-lethal concentrations of chlorpyrifos for 96 hours, observing irregular swimming patterns, hyper-excitability, and equilibrium loss. AchE activity was analyzed by exposing fishes to different concentrations of malathion, an abnormal fish behavior even at low concentration (0.1 mg/L) of malathion was reported (El-Nahhal, 2018).

## Impact of Malathion on Fishes

Islam et al., (2019) studied the toxic effect of malathion in three fresh-water fishes and analyzed mortality as well as physical behaviors and found that the mortality rate was high at 72 hours of exposure. In another study, histological, biochemical, and haematological changes in different tissues were observed and reported even by sub lethal exposure to malathion (Verma et al., 2024). The impact of malathion brought changes in the histology of gills and kidney in an hybrid of *C.auratus*, *Carassius auratus gibeli* and cytoplasm vacuolization and changes in cell and nuclear volumes were found (Staicu et al., 2008). In another study equilibrium loss, hyper- excitability and medium irreversible toxicity were reported in *C.auratus* (Shahbazi Naserabad et al., 2015). Roopavathy & Sukumaran (2021) studied the effect of malathion toxicity on the histology of fresh water fish *Oreochromis*

*mossambicus* and observed that toxicity of this pesticide in various tissues causes necrosis, lamellar shortening, clubbing in gill, liver, and kidney tissues. The effect of malathion toxicity in the fresh water fish *Ophiocephalus punctatus* was observed, analyzed and showed that histological changes occur in brain, liver, and ovary tissues. The serious changes occur were hypertrophy of the cells and their nuclei, liver tissues on the whole showed distant appearance, vaculation, and fragmentation of ova were also recorded (Pugazhvendan et al., 2009). Alteration in AchE activities indicates malathion-induced stress in fishes enabling the usage of it as aquatic pollution monitors. This toxic agricultural pesticide accumulates in fishes causing various health issues in fishes as well as in human population through food chain (Das et al., 2024).

While numerous studies have been carried out on the impact of malathion on different fish species, few are available *in C.auratus* and no reports are found in *B. striata*. As an essential protein source, fish play a vital role in human diets. However, the widespread use of organophosphate pesticides, such as malathion and methyl parathion, in agriculture poses significant threats to aquatic ecosystems and non-target organisms, causing severe metabolic disturbances. This study investigates the effects of malathion on AchE activity in different tissues of *Carassius auratus* and *Botia striata* shedding light on its toxic impact on aquatic biodiversity.

# MATERIALS AND METHODS

Healthy adult *Carassius auratus* and *Botia striata* were procured from a local aquarium shop and carefully transported to the laboratory. The fishes were acclimatized under laboratory conditions providing feed twice per day with artificial fish food pellets that were halted before testing toxicity. Experiments were conducted in 50 liters capacity glass aquariums with specimens of uniform size, approximately 6.5–7 cm in length and weight, 10–15 gm. The general behavior of fishes was recorded continuously before testing.

## Determination of Lethal Concentration (LC50)

A stock solution of malathion was prepared by dissolving 5 mg of the pesticide (50% EC, manufactured by Kalyani Industries Limited, Mumbai) in 1 litre distilled water. Stock solution converted to different concentrations of malathion such as 3, 4, 5, 6, and 7 parts per million (ppm) solutions were introduced to five

separate tanks with 50 Litre water. To this twenty fishes each were introduced and were observed for 24 hours to identify the LC50. Pesticide-free control fishes were also maintained and observed in separate tanks during these experiments.

## Determination of Sub Lethal Concentration

To determine the sub lethal concentration, above procedure was repeated with different concentrations of Malathion such as 3.2. 3.4, 3.6,

3.8 and 4 ppm. Sub-lethal toxicity analysis was done for 30 days.

## Acetyl Choline Esterase Assay

Twenty acclimatized experimental fishes in four replicates were added into sub lethal concentration of 3. 4 ppm malathion mixed with 50 L water. Fishes were introduced for 48, 72, and 96 hrs for toxicity testing. The control set was also run simultaneously. At different stages of the exposure periods, tissues such as brain, muscle, gill, liver and kidney were carefully dissected and removed for AchE assay analysis. Using mortar and pestle, 100 mg of the dissected tissues were homogenized. This was then centrifuged twice at 6000 rpm for 15 min to obtain the extracts and kept at -20ºC for further analysis. The variation of AchE activity in the tissues dissected from fishes kept for the recovery period of 15 days was also observed.

Total protein in different tissues was determined according to Lowry, (1951) to express the specific AchE activity. AchE activity was determined according to Ellman’s method (1961). This method was conducted by using acetyl thiocholine iodide as substrate and by hydrolyzing acetyl choline to acetic acid and tricholine by the action of AchE. The absorbent was measured in 412 nm. All the AchE values were expressed as m moL/min/mg/protein. At different stages of the exposure periods, the behavioral changes were noticed.

## Statistical Analysis

Statistical analysis of the observation was done using online Jeffreys's Amazing Statistics Program (JASP), a free software provided by the University of Amsterdam (Love et al., 2019).

# RESULTS

## LC50 and Sub Lethal Concentration

In 7, 6, 5, 4, and 3 ppm concentrations of Malathion, the mortality rate observed was 90, 50, 20, 10, and 5%. The LC50 of malathion for C.auratus and B.striata was found to be 6 ppm. For 3.6 ppm and above malathion concentrations, the mortality rate observed 10% while for 3.4 and 3.2 the mortality rate observed was 5%, hence the sub lethal concentration was estimated as 3.4 ppm.

## Fish Behaviour in Malathion Treated

### C. auratus

Normally *C. auratus* are calm fishes without any aggressiveness in behavior, solitary in nature. After the fishes were exposed to malathion, the fishes remained calm with normal behavior for nearly half an hour. After this, the fishes showed abnormal behavior with erratic swimming, jumping, and gulping air showing difficulty in breathing. Hyper activeness was also observed along with the loss of equilibrium. During the later hours, the fishes stopped its movement and started to aggregate at the bottom of the tanks slowing down the breathing process. They were found to undergo hemorrhage, slight color variation from orange to light yellow and was covered with secreted mucus.

These fishes are kept in icepacks and are later dissected. The dissected tissues such as brain, muscle, gill, liver and kidney were carefully removed and AchE assay analysis was carried out. The AchE activity responses on exposure to malathion in different tissues in *Carassius auratus* show variations.

## Malathion Toxicity Effect on AchE in Brain, Muscle, Liver, Gill and Kidney in *C.auratus*

The result of present investigation shows that AchE activities in brain, muscle, gill, liver, and kidney were significantly high at control and at 48 hrs of malathion pesticide exposure the activity declines. Compared to the control a significant reduction of AchE activity was observed in all tissues. The enzyme activity in the brain of the treated fish shows an initial drop from 7.2 to 1.8 at 72 hrs following a gradual increase in the activity reaching 3.4 at 96 hours and reached 5.2 in the recovery stages though below the control level. In muscle the AchE activity dropped to 2.1 at 48 hours and remained in the same stage at

72 hrs and recovering at 96 hrs. In liver and gill tissues, there were only slight variations in the activity, and in kidney there was an instable variation found though the activity was regained in the recovery period. The mean and the standard error of mean of the toxicity effect on AchE of brain, muscle, liver, gill and kidney in *C. auratus* in control, recovery and at various time intervals are given in Table 1.

The mean values of AchE activity observed in brain, muscle, gill, liver and kidney at different time intervals are represented in Fig. 1. From this figure we can see that in brain (Fig. 1a), from the increased AchE activity in the control the activity drops and then increases 96 hrs. In case of muscle (Fig. 1b) the values drops and remains same at 72 hours and then increases. In liver and gill (Fig.1c and Fig. 1e) the values slightly drops then regain the activity while in kidney the activity fluctuates (Fig. 1d). From 72 to 96 hrs of exposure, the effect of malathion decreases due to its degradation.

The mean values of AchE activity occurred at different time intervals including control, and recovery are depicted in Fig. 2. Here we can see

that AchE activity are very high in brain and muscle and are represented by dark blue and red bars, while the activity are lesser in other tissues in the control set. At 48 and 72 hrs, activity decreases and comparatively increases at 96 hrs and in recovery period. Compared to gill liver and kidney, brain and muscle tissues have higher AchE activity. In gill, and liver, AchE activities are low in the control group and shows little variation. The values increase slightly in the recovery period for liver and gill while in the case of kidney, activity reaches control at 72 hrs, then decreases and resumes.

* 1. **ANOVA of *Carassius auratus***

The analysis of variance (ANOVA) of AchE activity in different tissues of *C.auratus* is given in Table 2. F value is calculated to determine difference between the means of multiple groups being compared and a higher F value designates significant differences between groups. P value is the probability and value less than 0.001 is highly significant. There were significant variations found in brain, muscle, gill and liver tissues and no significant variation was observed in kidney.

#### Table 1. Malathion toxicity effect on AchE of brain, muscle, liver, gill and kidney in *C.auratus* in control, recovery and at various time intervals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hrs** | **BRAIN** | **MUSCLE** | **LIVER** | **GILL** | **KIDNEY** |
| **CONTROL** | 7.220±0.037 | 7.160±0.024 | 4.720±0.037 | 4.180±0.037 | 4.260±0.051 |
| **48 HRS** | 2.460±0.024 | 2.180±0.037 | 4.180±0.037 | 3.740±0.024 | 3.840±0.024 |
| **72 HRS** | 1.860±0.024 | 2.180±0.037 | 3.760±0.024 | 3.140±0.024 | 4.260±0.610 |
| **96 HRS** | 3.460±0.024 | 3.400±0.032 | 4.140±0.024 | 3.260±0.024 | 3.680±0.037 |
| **RECOVERY** | 5.260±0.024 | 5.180±0.037 | 4.460±0.024 | 3.860±0.024 | 4.140±0.024 |

*Values given are mean ±standard error at each time intervals for the corresponding tissues.*

## Results of ANOVA on the Malathion Toxicity Effect on AchE in the Brain, Muscle, Liver, Gill and Kidney in *C. auratus*

#### Table 2. ANOVA results of the malathion toxicity effect on AchE of brain, muscle, liver, gill and kidney in *C. auratus* in control, recovery and at various time intervals.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Tissue** | **Cases** | **Sum of Squares** | **df** | **Mean Square** | **F** | **p** |
| Brain | Treated | 86.806 | 3 | 28.935 | 7233.833 | < .001 |
|  | Residuals | 0.064 | 16 | 0.004 |  |  |
| Muscle | Treated | 83.394 | 3 | 27.798 | 5054.182 | < .001 |
|  | Residuals | 0.088 | 16 | 0.006 |  |  |
| Liver | Treated | 2.340 | 3 | 0.780 | 156.000 | < .001 |
|  | Residuals | 0.080 | 16 | 0.005 |  |  |
| Gill | Treated | 3.408 | 3 | 1.136 | 284.000 | < .001 |
|  | Residuals | 0.064 | 16 | 0.004 |  |  |

*Note. Type III Sum of Squares*

*Significance of variations was observed in brain, muscle, gill and liver. In kidney no significant variation is observed*

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| **Malathion toxicity effect on AchE in different tissues in *C. auratus*** |
|  |  |  |
| **Fig. 1a. Malathion toxicity effect on AchE of brain in *C.auratus* in control, and at various time intervals.** | **Fig. 1b. Malathion toxicity effect on AchE of muscle in *C.auratus* in control, and at various time intervals.** | **Fig. 1c. Malathion toxicity effect on AchE of liver in *C.auratus* in control, and at various time intervals.** |
|  |  |  |
| **Fig. 1d. Malathion toxicity effect on AchE of kidney in *C.auratus* in control, and at various time intervals** | **Fig. 1e. Malathion toxicity effect on AchE of gill in *C.auratus* in control, and at various time intervals** |

#### Fig. 1. Mean values of AchE activity observed in brain, muscle, gill, liver and kidney

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**Fig. 2. Malathion toxicity effect on AchE of brain, muscle, liver, gill and kidney in *C.auratus* in control, recovery and at various time intervals. In the X axis, control, time intervals and recovery are given and in Y axis, the mean values of malathion imapct in AchE at each time intervals is expressed in moL/min/mg/protein unit**

## Fish Behaviour in Malathion Treated

### B. striata

*B. striata* are calm fishes showing social behavior and prefer to exist in groups. These fishes are bottom dwellers. Though they are calm, they show aggressiveness while in a tank with other bottom feeders. After malathion exposure the fishes remained calm for nearly half an hour, later showing abnormal behavior in swimming with gulping air. Hyper activeness and loss of equilibrium were also observed. Later stages of the fishes were static in nature with slow breathing. Fishes were also found to be covered with secreted mucus. From the fishes kept in icepacks, tissues were carefully dissected and AchE assay analysis was performed.

## Malathion Toxicity Effect on AchE of Brain, Muscle, Liver, Gill and Kidney in *B. striata*

Compared to the control, a reduction of AchE activity was observed in all tissues. In brain and muscle there was significant decrease while in liver and gill, only slight variation was observed

due to the low AchE activity in the control. In kidney there was an unusual variation found with increased activity at 96 hrs though the activity was slightly dropped in the recovery period. The mean and standard error of mean of the toxicity effect on AchE in different tissues in *B. striata* in the control, recovery period and at different time intervals are given in Table 3.

In all tissues there is significant decrease of AchE levels due to malathion pesticide exposure. As seen in *C. auratus*, the differential inhibition of AchE activity in all tested tissues may be due to presence of isoenzymes. The mean values of AchE activity observed in brain, muscle, gill, liver and kidney at different time intervals are represented separately in Fig. 3. In brain and muscle (Fig. 3a and 3b), the malathion toxicity has a similar effect, the values drops and reaches minimum at 72 hours and then increases slightly at 96 hours. In gill and liver (Fig. 3e and Fig. 3c) the decreased activity is somewhat regained and reaches the value that was at 48 hours while in kidney (Fig. 3d) the activity increases sharply and goes higher at 96 hours than in control.

#### Table 3. Malathion toxicity effect on AchE of brain, muscle, liver, gill and kidney in *B. striata* in control, recovery and at various time intervals

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hrs** | **BRAIN** | **MUSCLE** | **LIVER** | **GILL** | **KIDNEY** |
| **CONTROL** | 5.780±0.058 | 5.660±0.051 | 4.220±0.037 | 3.360±0.075 | 3.220±0.037 |
| **48 HRS** | 2.720±0.037 | 3.460±0.024 | 3.300±0.084 | 3.140±0.024 | 2.680±0.073 |
| **72 HRS** | 1.540±0.024 | 2.220±0.037 | 2.140±0.024 | 2.160±0.024 | 2.160±0.024 |
| **96 HRS** | 2.140±0.024 | 2.860±0.024 | 3.140±0.024 | 3.160±0.024 | 3.320±0.037 |
| **RECOVERY** | 4.460±0.024 | 4.260±0.024 | 3.720±0.037 | 3.180±0.037 | 3.280±0.037 |

*Values given are mean ±standard error at each time intervals for the corresponding tissues.*

|  |
| --- |
| **Malathion toxicity effect on AchE in different tissues in *B.striata*** |
|  |  |  |
| **Fig. 3a Malathion toxicity effect on AchE of brain in *B.striata* in control, and at various time intervals** | **Fig. 3b Malathion toxicity effect on AchE of muscle in *B.striata* in control, and at various time intervals** | **Fig. 3c Malathion toxicity effect on AchE of Liver in *B.striata* in control, and at various time intervals** |
|  |  |  |
| **Fig. 3d Malathion toxicity effect on AchE of Kidney in *B.striata* in control, and at various time intervals** | **Fig. 3e Malathion toxicity effect on AchE of Gill in *B.striata* in control, and at various time intervals** |

#### Fig. 3. Mean values of AchE activity observed in brain, muscle, gill, liver and kidney of *B.striata.* In the X axis, control, time intervals and recovery is given and in Y axis, the mean values of the effect of malathion in AchE at each time intervals is expressed in moL/min/mg/protein unit

The mean values of AchE activity in *B. striata* occurred at different time intervals including control, and recovery are depicted in Fig. 4. The high AchE activity in brain and muscle represented by dark blue and red bars respectively, drops at 48 and 72 hours. The decrease is higher in the brain tissue compared to that of other tissues. At 48 hrs the activity decreases but at 72 hrs, the toxic activity on AchE seems to be reaching similar value in all tissues.

* 1. **ANOVA of *Botia striata***

The analysis of variance (ANOVA) of effect of malathion on AchE activity was carried out and significant variations was observed in all tissues. The Mean square and F value obtained for the activity in all tissues at different time intervals in *B. striata are* given in Table 4.

## ANOVA on the Malathion Toxicity Effect on AchE in the Brain, Muscle, Liver, Gill and Kidney in *B. striata*

#### Table 4. ANOVA results of Malathion toxicity effect on AchE of brain, muscle, liver, gill and kidney in *B.striata* in control, recovery and at various time intervals. Significant results were obtained for all tissues

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Tissue** | **Cases** | **Sum of Squares** | **Degrees of freedom (df)** | **Mean Square** | **F value** | **P value** |
| *Brain* | Treated | 53.349 | 3 | 17.783 | 2371.089 | < .001 |
|  | Residuals | 0.120 | 16 | 0.008 |  |  |
| *Muscle* | Treated | 33.526 | 3 | 11.175 | 1719.282 | < .001 |
|  | Residuals | 0.104 | 16 | 0.007 |  |  |
| *Liver* | Treated | 10.888 | 3 | 3.629 | 302.444 | < .001 |
|  | Residuals | 0.192 | 16 | 0.012 |  |  |
| *Kidney* | Treated | 4.314 | 3 | 1.438 | 130.712 | < .001 |
|  | Residuals | 0.176 | 16 | 0.011 |  |  |
| *Gill* | Treated | 4.362 | 3 | 1.454 | 157.171 | < .001 |
|  | Residuals | 0.148 | 16 | 0.009 |  |  |
| *Note.* Type III Sum of Squares |  |  |  |  |

*Significance in variations was observed in all tissues*

Malathion toxicity effect on AchE in *B.striata*

7

6

5

4

3

2

1

0

CONTROL

48 HRS

72 HRS

96 HRS

RECOVERY

BRAIN MUSCLE LIVER GILL KIDNEY

**Fig. 4. Malathion toxicity effect on AchE of brain, muscle, liver, gill and kidney in *B.striata* in control, recovery and at various time intervals. In the X axis, control, time intervals and recovery is given and in Y axis, mean values of malathion effect in AchE at each time intervals is expressed in moL/min/mg/protein unit**

# DISCUSSION

Acetyl choline esterase are enzymes facilitating neurotransmitter modulation at synapses and are highly active in brain and muscle (Reddy et al., 1992). In the present study, the highest AchE activity was found in the brain tissues of both control fishes followed by muscle. The differential inhibition of AchE activity in the tested tissues may be due to the presence of isoenzymes with different affinities for the substrate and inhibitor that may occur due to the functional specificity and impact of nerve innervations. Brain is the control and commanding center, the seat of millions of neuronal activity requires highest AchE activity. Muscle tissues have high AchE activity than other tested tissues but lesser than brain due to the neuron muscle synapses for muscle contractions concerned with movements.

In both fishes liver has highest activity than gill and kidney, and in *C. auratus* kidney has more AchE activity than in gill and in *B. striata* gill has more AchE activity than in kidney. This may be due to the least neuronal innervations in these tissues compared to that of brain and muscle. Similar results were obtained in same tissues of fresh water teleost *Tilapia mossambica* when exposed to malathion and methyl parathion (Sahib & Rao, 1980).

In cartilaginous fishes urea is the excretory product while in teleosts, it is ammonia. In teleost kidney plays least role in ammonia excretion since major part is excreted through diffusion. In teleosts, kidney plays a major role in regulation of osmotic balance. The effect of malathion pesticide disrupt the normal functioning of kidney leading to the shrinkage of glomerulus and degeneration of renal tubules. The accumulation of waste products in the blood stream disrupts the ultra-filtration process and cause kidney damage. The fluctuating functioning of AchE activity in the kidney of *C. auratus* and the abnormal functioning of kidney in *B. striata* is an indication of this disruption of normal kidney function. Similar observations were obtained in the study of Sivanandan et al., (2021) in fresh water fish *Labeo rohita*. The histological studies of kidney in *Labeo rohita* show shrinkage of glomerulus and increase in the spaces of Bowmans capsule (Uikey, 2015). Nisha & Geetha (2018) studied the effect of malathion in the metabolic and respiratory activities of fresh water fish *Labeo rohita* and observed that high and low concentration of malathion causes alterations in vital organs.

The altered AchE activity caused by malathion pesticide brought in many behavioral changes in fishes. The behavioral changes noticed in these fishes were loss of equilibrium, irregular swimming, and altered sensory process. Such behavioral changes were also observed in a study on malathion toxicity in brain in Labeo rohita (Ullah et al., 2025). Considering the health problems caused by malathion, its effect on mammals including humans are studied immensely. Though it is less toxic than endosulfan, the leading death and disease causing pesticide, widespread use of malathion exposure may cause severe damages. Moreover, toxic effects of malathion in liver, pancreas, kidney, and lungs, are reported in mammals. In addition it is reported as genotoxic and carcinogenic and causing adverse effect on prenatal and postnatal exposure (Badr, 2020).

After recovery period, in *C. auratus* and *B. striata*, the AchE activity shows an increased value showing the degradation of malathion effect on AchE. Similar observations were obtained in the studies on kidney, liver and muscle focusing on mortality and biochemical changes in *Labeo rohita* (Thenmozhi et al., 2011). Malathion, the nonsystemic pesticide is used in various types of fields due to their high insecticidal property, low persistence and degradability in the ecosystem. AchE, is an important biomarker for organophosphate pesticides. The activity of this pesticide inhibits Ach breakdown and blocks synaptic transmission by the accumulation of Ach in synaptic cleft. Hindering impulse transmission can cause muscle fatigue followed by paralysis and death.

# CONCLUSION

The present study deals with the acute toxic effect of an organo phosphorous pesticide malathion. Acetylcholine, the important neuro transmitter in the brain shows highest activity in brain and muscle because of high neural innervations and is least in gill, and kidney. At the time of acute exposure the AchE activity slow down and after 72 hrs its activity increases due to the degradation of these pesticides. The present study focus on the toxic and dangerous effect of these pesticide on the aquatic ecosystem as well as mankind.

The persistent use of endosulfan indicates genetic mutations that can be transmitted across generations. Pesticide toxicity was studied by many researchers and its toxic effects including oxidative damage, histopathological changes and

carcinogenicity are reported. Prolonged exposure to malathion can lead to severe physiological and biochemical disturbances in aquatic organisms. The accumulation of such toxic substances in water bodies poses a significant threat to the entire food chain. Therefore, regular monitoring and strict regulations on pesticide use are essential to prevent irreversible ecological damage. Considering the adverse effects that it causes on fishes and other organisms, there is vital need for stringent rules preventing indiscriminate malathion usage. Unless preventive measures are taken for this dangerous hazard endangering of aquatic biodiversity is inevitable.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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