Pak. J. Chem. 15(4):121-131, 2025 Research article
ISSN(Print):2220-2625
ISSN(Online):2222-307X DOI:10.15228/2025.v15.i4.p19

An investigation of Comparative Effects of Cypermethrin and Methamidophos on Acetylcholinesterase Activity in Ducklings of Mallard Duck (*Anas platyrhynchos*)

*R. Kanwal[1], H. Ali[1], A. Rehmani[1], S. Siddiqui[2], and R. Naseem[1]
[1]Department of Zoology, University of Karachi, Karachi, Pakistan
[2]Department of Zoology, Federal Urdu University of Arts, Science & Technology, Karachi, Pakistan
***Corresponding Author email**: roohia2z@hotmail.com

Abstract
The current study aimed to compare acetylcholinesterase (AChE) activity in liver, kidney, and muscle tissues of ducklings (*Anas platyrhynchos*) following treatment with Cypermethrin (a pyrethroid) and Methamidophos (an organophosphate). Results showed a dose-dependent response (250 µg, 500 µg, and 1000 µg) in AChE activity at 30, 60, and 90 seconds. It was observed that cypermethrin displays a biphasic response, with initial inhibition of AChE followed by recovery or activation at higher doses (46.6-47.1 µg at 250 µg to 99-99.3 µg at 1000 µg). It may be related to enzyme induction, metabolic adaptation, or detoxification mechanisms in hepatic and muscular tissues. The pattern suggests moderate neurotoxicity, with potential for physiological compensation, as evidenced by the weight changes in the studied birds. Methamidophos exposure led to marked inhibition of AChE, consistent with organophosphate toxicity. Systemic enzyme suppression in liver (177–182%) and kidney tissues showed potent inhibition at 1000 µg (47%), whereas in muscle tissues, a striking hyperactivation(177–182%) was observed. The marked elevation of muscle AChE at the high dose may indicate neuromuscular hyperactivity or secondary enzymatic overexpression resulting from prolonged inhibition. The research concluded that it provided a more comprehensive insight into the neurotoxic actions and physiological responses of birds to pyrethroid and organophosphate compounds, which would help evaluate their potential ecological and environmental hazards.

Keywords: Pyrethroids, Organophosphates, Toxicology, Acetylcholinesterase

1. INTRODUCTION

Pesticides refer to chemical compounds that are extensively applied to curb pests that affect agricultural productivity, health, and the environment [1]. Although pesticides have been beneficial in enhancing crop yields and managing disease, their widespread, largely uncontrolled use has led to serious environmental and ecological issues. These chemicals not only harm the target pests but may also harm other non-target organisms, such as beneficial insects, amphibians, and birds. Ecologists and toxicologists have shown a growing interest in analyzing the effects of pesticides on natural communities and ecosystem equilibrium over the years [2].

Birds are among the organisms most threatened by pesticides due to their wide distribution, feeding habits, and their position in the food chain. They may come into direct contact by eating contaminated food and water or indirectly by collecting pesticide residues in the area where they live. Farmers have also been reported to deliberately poison birds to reduce crop destruction, although such cases are rarely documented in detail [3].

Pesticide poisoning is usually ignored or unreported in developing nations such as Pakistan because of illiteracy, ignorance, poor monitoring mechanisms, and low laboratory diagnostic centers. As a result, we have limited knowledge of the toxicity of pesticides on wildlife, particularly on avian species. One of the most effective biochemical indicators for identifying and measuring pesticide intoxication is the estimation of cholinesterase activity [4]. The cholinesterase enzymes that are mainly acetylcholinesterase (AChE) are essential in the operation of nerves as they break down the neurotransmitter acetylcholine into acetate and choline, thus ending nerve impulses. The blockage of AChE results in the accumulation of acetylcholine at the synaptic junctions, which results in constant nervous stimulation, muscle paralysis, respiratory failure, and eventual death, which is lethal in severe cases. Birds naturally have lower erythrocyte cholinesterase activity than mammals; therefore, the diagnosis of pesticide exposure in birds is mainly based on the estimation of cholinesterase activity in serum, plasma, and brain tissues [5-11]. Acetylcholinesterase (AChE) is a biomarker that has been widely used in toxicological studies, as it is vital to the normal functioning of the nervous system and highly sensitive to exposure to pesticides (especially organophosphate and carbamate compounds) [12-13]. AChE plays a role in the hydrolysis of the neurotransmitter acetylcholine (equation # 1) at synaptic junctions, as shown in the following biochemical pathway.

$$CH_3COOCH_2CH_2N(CH_3)_3^+ \xrightarrow{AChE/H_2O} CH_3COOH + HOCH_2CH_2N(CH_3)_3^+ \quad \text{..... Equation (1)}$$

It is crucial for the standard transmission of nerve impulses and the control of muscular contraction [14]. Blockage of AChE causes excessive acetylcholine buildup in the synaptic cleft, leading to constant stimulation of nerve cells, which may result in neuromuscular paralysis, abnormal behavior, and, in extreme situations, death [15].

Organophosphates ($C_2H_8NO_2PS$) and pyrethroids ($C_{22}H_{19}Cl_2NO_3$) are two chemical groups of pesticides that are among the most widely used in agriculture, are harmful to the health of the population, and are considered the most common pesticides that disrupt AChE activity and alter neural transmission [16-17]. The two types of pesticides

are thus highly neurotoxic, and they interfere with the normal functioning of the nervous system in organisms exposed to them [18]. Methamidophos is an organophosphate that is a potent inhibitor of acetylcholinesterase, causing neurotoxicity by inducing prolonged nerve excitation. On the other hand, cypermethrin, a synthetic pyrethroid, interacts with sodium ion channels (equation # 2) on nerve membranes, making them hyperexcitable and slowing nerve conduction via the following biochemical pathway.

$$\text{Cypermethrin} + Na^{+} \rightleftharpoons [\text{Cypermethrin}\cdots Na]^{+} \quad \ldots\ldots \text{Equation (2)}$$

Sodium can transiently associate with electron-rich sites on the cypermethrin molecule, forming a complex stabilized by electrostatic attraction and partial charge redistribution; it is reversible and solvent-dependent. Though the two compounds act on the nervous system, their actions and toxicological reactions are quite different.

Organophosphates and pyrethroids, among the most widely used pesticides, can bioaccumulate in the bodies of birds used in agricultural practices, potentially interfering with key biochemical and physiological pathways [19, 18]. Consequently, studies on the use of these pesticides in avian species are imperative to understanding their ecological and toxicological effects. *Anas platyrhynchos,* or the Mallard duck (also called Batakh), is a valuable bioindicator species, feeds in a particular way, and is extremely sensitive to pollutants in the human-made environment[20-21]. The Mallard ducklings were used in this research as an experimental model because they best demonstrate the effects of environmental pollution, particularly from pesticides. Mallards are commonly exposed to pesticide residues through contaminated water, sediments, and food, as they are widespread waterfowl that live in both agricultural and aquatic environments [22]. Their omnivorous feeding habits, which include the consumption of seeds, insects, and water invertebrates, render them especially susceptible to the bioaccumulation and biomagnification of harmful chemicals across trophic levels [15,13]. The Mallard duck is therefore a beneficial model organism for assessing the ecotoxicology of pesticides and monitoring ecological health. Ducklings are also at an early stage of development and hence more physiologically sensitive to toxic agents than adult birds. The study will provide a better opportunity to detect biochemical and physiological alterations, such as changes in cholinesterase activity. Additionally, the Mallard duck is a well-studied species in ecotoxicology, with reference data on enzyme activity, making it relatively easier to compare and interpret the results. The fact that they can be handled, their size is manageable, and they are adaptable to laboratory conditions also contributes to their appropriateness as a model organism for performing controlled toxicological studies.

Recent research in Pakistan has shown that brain acetylcholinesterase activity is significantly lower in birds from agricultural cropland than in those from protected areas, indicating that exposure to pesticides poses a severe environmental hazard [23]. In addition, the native bird species in Australia have been characterized by their plasma cholinesterase activity, which reveals species-specific baseline variations crucial for monitoring exposure [24].

Thus, the current research work was undertaken to investigate the induced effects of cypermethrin (pyrethroid) and methamidophos (Organophosphate) on the cytotoxic acetylcholinesterase activity in the liver, kidney, and muscle tissues of the Mallard duck duckling. The comparative toxicity and enzyme-inhibitory potential of these pesticides were discussed in the relevant section of the article, thereby enhancing understanding of their probable neurotoxic and ecological risks in avian populations.

2. MATERIALS AND METHODS

The current experiment aimed to compare the effects of Cypermethrin (a pyrethroid) and Methamidophos (an organophosphate) on cholinesterase activity in Mallard ducklings (*Anas platyrhynchos*), locally known as Jangli Batakh. Experimental ducklings were picked as healthy four-week-old ducklings of uniform size and weight.

Three concentrations of each pesticide 250 µg, 500 µg, and 1000 µg per individual, were prepared, and the experimental groups received each concentration. The exposure effects of pesticides were examined on three tissues, including muscle, kidney, and liver.Another control group was kept in the same condition without pesticide treatment. The ducklings were then humanely sacrificed and dissected to extract the required organs (Liver, kidney, and muscle) after a duration of 24 hours. A tissue grinder was used to weigh and homogenize each sample of the organs with 2 mL of distilled water. The homogenates were further centrifuged at 3500 rpm for 15 minutes to obtain a clear supernatant. The supernatant fractions thus obtained were stored at low temperature (frozen) and used subsequently for biochemical estimation of cholinesterase activity.

2.1. Estimation Activity of Cholinesterase

The activity of cholinesterase was determined by a spectrophotometer UV-160 and the kit No. CE 190, prepared by Randox, was used for this purpose.

Formula:

The enzyme activity was calculated according to the following equation:

$$\mu\text{mol/L/min at } 37°C = 23460 \times \Delta A_{405\ nm}/\text{min}$$

Whereas; $\mathbf{\Delta A_{405}nm/min}$ = Change in absorbance per minute at 405 nm,

23460 = Conversion factor derived from the molar extinction coefficient of DTNB ($13{,}600 M^{-1}cm^{-1}$) and the reaction parameters under assay conditions.

The level of enzyme activity was measured in µmol of substrate hydrolyzed per minute per liter at 37℃ and compared among the treatments to determine the inhibitory properties of cypermethrin and methamidophos.

2.2. Preparation of working solution

The reagent contents were dissolved in 30 mL of double-distilled water to make the buffer solution (R1), and the substrate solution was prepared by dissolving the contents in 1 mL of double-distilled water (R2). In the case of the enzyme assay, 22 test tubes were set and labeled as per the experimental groups:

Control (CT) - untreated samples,

Cypermethrin (C) 250 µg/individual, 500 µg/individual, and 1000 µg/individual,

Methamidophos (M) 250 µg/individual, 500 µg/individual, and 1000 µg/individual.

1.5 mL of buffer solution (R1), 50 uL of substrate solution (R2), and 100 µL of tissue supernatant sample (Liver, kidney, or muscle) were added to each test tube in turn. The mixture was swirled, then spectrophotometrically measured at the beginning of the reaction. The first absorbance was measured at 30 s and then (0, 30, 60, and 90sec) at 405 nm in a UV-visible spectrophotometer [6]. The acetylcholinesterase activity was calculated with the help of the rate of change of the absorbance per minute (ΔA/min) using the following formula:

$$\mu\text{mol/L/min at } 37°\text{C} = 23460 \times \Delta A_{405\ \text{nm}}/\text{min}$$

3. RESULTS AND DISCUSSION

Initially, the effects of the treatments Cypermethrin and Methamidophos on the physiology of the ducklings were observed and reported in Tables 1 & 2.

3.1. Weight of the Ducklings under treatment with cypermethrin

Results in Table 1 report the data on the change in weight and the mortality of ducklings (*Anas platyrhynchos*) after exposure to cypermethrin, a synthetic pyrethroid insecticide, at various concentrations (Control, 250 µg, 500 µg, and 1000 µg/kg/duckling) and a duration of 36 days. The Table (1) contains data on weights at different time points, and the abbreviation 'd' indicates that a duckling died at that point.

The ducklings in the control group exhibited normal development throughout the study, with steady increases in body weight and minimal mortality. This trend indicates normal physiological growth. Conversely, when ducklings were exposed to 250 µg of cypermethrin, they exhibited slightly slower growth relative to their controls, and some died (denoted by d). Growth inhibition was more pronounced at 500 µg; some ducklings had stunted weight and reduced body mass, and the mortality rate increased. Survival and weight loss indicate metabolic and physiological derangements that may have resulted from cypermethrin toxicity. The most acute effects were observed in the 1000 µg treatment group, where some ducklings either retained their original weight or lost it, and death was common during the observation period. This implies that increased levels of cypermethrin brought about acute toxicity, which impacted badly on neuromuscular coordination, feeding habits, and metabolism.

In general, the evidence suggests that cypermethrin exhibits dose-dependent toxicity in ducklings, affecting growth and survival. In addition to the neurotoxic and metabolic effects, the concentration that enhanced growth retardation and increased mortality increased with increasing insecticide concentration. These findings align with previous research, which has shown that exposure to pyrethroids causes ion channel dysfunction, neuromuscular paralysis, and death in birds [17, 25, 22].

Table 1: Weight of Ducklings (*Anas platyrhynchos*) after Cypermethrin (Pyrethroid*)* treatment

Conc. (µg/ind)	No. of ducks	No. of Days															
		1st	3rd	5th	8th	9th	10th	11th	13th	15th	17th	20th	25th	30th	31st	32nd	34th	37th
Control	1st	80g		75g[d]														
	2nd	63g					75g					104g		101g		84g[d]		
	3rd	39g					52g				54g[d]							
	4th	67g					84g		128g[d]									
	5th	38g		36g[d]														
	6th	41g		38g[d]														
250	1st	53g					66g					81g		87g	74g[d]			
	2nd	48g					47g	45g[d]										
	3rd	70g	54g[d]															
500	1st	84g					87g					122g		158g				150g[d]
	2nd	53g		41g[d]														
	3rd	52g					46g			42g[d]								
	4th	56g					62g					71g	65g[d]					
	5th	66g					60g	47g[d]										
	6th	60g			50g[d]													
1000	1st	59g				58g[d]												
	2nd	84g				89g[d]												
	3rd	53g					59g					75g		91g			74g[d]	
	4th	46g					67g	75g[d]										

	5th	63g		70gd										
	6th	54g				53gd								

"d" Shows the death of ducks

3.2. Weight of the Ducklings under Treatment with Methamidophos

Results reported in Table 2 show changes in the body weight of ducklings (*Anas platyrhynchos*) treated with methamidophos, an organophosphate, at varying concentrations over multiple observation days. They were compared in four categories: a control group (unexposed) and three treatment groups assigned dosages of 250 µg, 500 µg, and 1000 µg of methamidophos per individual. Masses were measured at various times, from the 1st day through the 36th day following exposure, to track changes in growth.

The control group of ducklings exhibited healthy growth, characterized by normal weight gain and development over time. Contrastingly, ducklings exposed to 250 µg of methamidophos were slightly depressed. Still, some of them experienced a low or stagnant weight gain, indicating that toxic stress was already apparent even at low levels of exposure. The growth rate in the 500 µg treatment group decreased; some ducklings showed variable weights, while others exhibited a definite decrease, indicating moderate toxicity that likely affects metabolic and physiological activities. The worst effects were observed in the 1000 µg group, where some ducklings were losing weight or exhibiting minimal growth during observation, indicating a high level of toxicity and potentially interfering with feed intake, enzyme activity, or energy metabolism.

In general, the data indicate that the toxic effect of methamidophos on ducklings increases with concentration, with higher concentrations resulting in greater growth inhibition. The results suggest that methamidophos disrupts normal physiological functions, leading to reduced weight gain and growth retardation. The trend suggests that organophosphate pesticides are toxic and can impact metabolic processes and overall growth in ducklings.

Table 2: Weight of Ducklings (*Anas platyrhynchos*) after Methamidophos (organophosphate)treatment

Conc. (µg/ind)	No. of ducks	No. of Days												
		1st	3rd	5th	9th	10th	11th	13th	16th	17th	20th	21st	30th	32nd	36th
Control	1st			75gd											
	2nd					75g					104gd		101g	84gd	
	3rd					52g				54gd					
	4th					84g		128gd							
	5th			36gd											
	6th			38gd											
250	1st		38gd												
	2nd					94g		77gd							
	3rd					96g			84gd						
500	1st					66g					106g		137g		91gd
	2nd					61g					75gd				
	3rd					71g				84gd					
	4th				45gd										
	5th					74g		59gd							
	6th			52gd											
1000	1st	42gd													
	2nd					60gd									
	3rd					73g					85g	85gd			
	4th														
	5th					66g	60gd								
	6th					53gd									

"d" Shows the death of ducks

3.3. Acetylcholinesterase Activity in Liver after Treatment with Cypermethrin

Acetylcholinesterase Activity in the Liver, as reported in Table 3, under several doses of cypermethrin, revealed that acetylcholinesterase activity in liver tissue decreased considerably at lower doses of cypermethrin (250 µg/individual) to around 46% of the control activity. Higher concentrations (500 µg/individual with a display of about 69 percent) had partial recovery of enzyme activity, and almost normal (about 99 percent) levels were found at an even higher dose (1000 µg/individual), indicating that there could be an adaptive or compensatory response in enzyme activity at higher doses.

Table 3: Acetylcholinesterase Activity in Liver after Treatment with Cypermethrin (Pyrethroid)

Conc. (µg/ind)	Time (Sec.)	Mean Enzyme AA	% ACTIVITY
Control	0	10838.52	100
	30	10932.36	100
	60	11026.2	100
	90	11096.58	100
250 µg/individual	0	5028.26	46.4
	30	5090.82	46.6
	60	5145.56	46.7
	90	5215.94	47.1
500 µg/individual	0	7522.84	69.5
	30	7616.68	69.7
	60	7663.6	69.6
	90	7687.06	69.3
1000 µg/individual	0	10689.94	98.6
	30	10838.52	99.1
	60	10924.54	99
	90	11018.22	99.3

3.4. Acetylcholinesterase Activity in the Kidney after Treatment with Cypermethrin

Acetylcholinesterase (AChE) activity in liver tissue from *Anas platyrhynchos* at several cypermethrin doses, reported in Table 4, illustrates the impact of cypermethrin on AChE activity. Acetylcholinesterase activity in the kidney after treatment with cypermethrin at a dose of 250 µg/ind was 71.5%, 71.4%, and 71.6%, respectively. At doses of 500 µg/ind, the percentages were 104.9%, 104.8%, and 104.9%, respectively. At a dose of 1000 µg/ind, the percentages were 63.3%, 62.6%, and 62.4% at 30, 60, and 90 seconds, respectively (Table 4). The AChE activity remained constant over the experimental period in the control group (5778.98-5904.10 AA/min), representing 100% enzyme activity. The enzyme activity at the low dose of 250 µg/individual was reduced by 7172% compared to the control, indicating that cypermethrin significantly inhibited renal cholinesterase at lower concentrations. Interestingly, at 500 µg/individual, enzyme activity was slightly higher than control levels (104-105% activity), suggesting that enzyme activity may have been stimulated or compensated for at moderate concentrations. Nevertheless, a sharp decrease was again observed at 1000 µg/individual, where enzyme activity was reduced to 62-63% of the control, and higher doses of cypermethrin strongly inhibited the enzyme, potentially causing toxic stress in kidney tissues. In general, AChE activity in kidney tissue exhibited a biphasic response to cypermethrin, where inhibition was observed at low doses, increased at intermediate doses, and decreased at high doses.

Table 4: Acetylcholinesterase Activity in the Kidney after Treatment with Cypermethrin (Pyrethroid)

Conc. (µg/individual)	Time (Sec)	Mean Enzyme AA	% ACTIVITY
Control	0	5778.98	100
	30	5802.44	100
	60	5865	100
	90	5904.1	100
250	0	4160.24	72
	30	4144.6	71.5
	60	4183.7	71.4
	90	4222.8	71.6
500	0	6021.4	104.1
	30	6091.78	104.9
	60	6146.52	104.8
	90	6193.44	104.9
1000	0	3659.76	63.4
	30	3667.58	63.3
	60	3667.58	62.6
	90	3683.22	62.4

3.5. Acetylcholinesterase Activity in the Muscles after Treatment with Cypermethrin

Results reported in Table 5 present the muscle AChE activity of *Anas platyrhynchos* after being subjected to cypermethrin. The post-treatment cypermethrin dose of 250 µg/ind was 79.5%, 82.2%, and 81%, respectively. Whereas at 500 µg/ind, the doses were 62.3%, 63.1%, and 61.9%; at 103 µg/ind, the doses were 97.6%, 100.8%, and 98.7% after 30, 60, and 90 seconds, respectively (Table 5). The control group showed no change in enzyme activity over the observed period (2322.54-2416.38 AA/min), representing 100% of the activity. At a concentration of 250 µg/individual, middle-range inhibition resulted in moderate activity reduction (approximately 79-82% of control), indicating minor inhibition of AChE in muscle tissue. A stronger inhibitory effect was observed at 500 µg/individual, with enzyme activity reduced to

approximately 62-65 percent of the control, indicating a pronounced neuromuscular cholinesterase suppression by pesticide stress. But even at the highest concentration (1000 µg/individual), AChE activity approached baseline levels (97.7101% of control), suggesting a rebound or an adaptive response at high exposure. This implies that muscle tissue could be adaptively tolerant or enzymatically reactivated to high levels of toxic stress, possibly due to upregulation of enzyme synthesis or physiological compensation.The general trend of AChE activity in muscle showed a dose-dependent effect that was not linear, with peak inhibition at intermediate levels of exposure and near-normal levels at higher levels. This tendency supports the supposition that cypermethrin causes non-linear enzymatic modulation rather than linear inhibition at the expense of tissue specificity.

Table 5: Acetylcholinesterase Activity in Muscles after Treatment with Cypermethrin (Pyrethroid)

Concentration µg/individual	Time (Sec)	Mean Enzyme AA	% ACTIVITY
Control	0	2322.54	100
	30	2361.64	100
	60	2332.92	100
	90	2416.38	100
250	0	1837.7	79.2
	30	1876.8	79.5
	60	1915.9	82.2
	90	1955	81
500	0	1509.26	65
	30	1470.16	62.3
	60	1470.16	63.1
	90	1493.62	61.9
1000	0	2259.98	97.3
	30	2306.9	97.6
	60	2353.82	100.8
	90	2385.1	98.7

3.6 Comparative Analysis of AChE Activity in Liver, Kidney, and Muscle of *Anas platyrhynchos* after Cypermethrin Exposure

A comparative analysis of acetylcholinesterase (AChE) activity in liver, kidney, and muscle tissues of *Anas platyrhynchos* after exposure to cypermethrin showed dose- and tissue-dependent alterations in enzyme inhibition.A sharp inhibition of AChE was also observed at 250 µg/individual in the liver, with enzyme activity reduced to about 46% of the control. This marked reduction in this factor indicates that the liver, as the primary organ of detoxification, is highly sensitive to the oxidative and neurotoxic stresses induced by cypermethrin. Interestingly, AChE activity partially recovered (~70% of control) with 500 µg/individual and was almost normalized (99% of control) with 1000 µg, suggesting metabolic adaptation or enzyme reactivation at higher exposures. There was a somewhat inconsistent response in kidney enzyme activity. An intermediate level of inhibition was observed at 250 µg/individual (72 percent relative to the control), and an unanticipated slight positive response beyond control levels (105 percent) was observed at 500 µg/individual. This response was likely due to either increased compensatory metabolic processes or increased renal clearance. Nonetheless, AChE activity decreased once more to 1000 µg/individual (= 63% of control), indicating that the organ's detoxifying capacity was exceeded by extensive or excessive exposure.AChE activity in muscle tissue was steadily reduced as cypermethrin levels increased, with the highest levels being approximately 62-65 percent of the control at 500 µg/individual. As with liver trends, enzyme activity at 1000 µg/individual increased again to near baseline levels (around 98101), which could be explained by physiological adaptation of the enzyme or by the production of additional enzyme molecules.In general, the findings reveal that cypermethrin influences cholinesterase activity differently across tissues: the liver shows the most significant initial inhibition, followed by the muscle and kidney. The trend of enzyme recovery with increasing doses in the liver and muscle is indicative of a non-linear dose-response, perhaps due to detoxification or the induced provision of the enzyme to its own feedback. These data are consistent with previous research that has documented tissue-specific, reversible pyrethroid cholinesterase inhibition [12, 13, 21].

3.7. Acetylcholinesterase Activity in the Liver after Treatment with Methamidophos

Acetylcholinesterase activity in the liver after treatment with methamidophos at a dose of 250 µg/ind was 88.7%, 88.1%, and 88%, respectively. Whereas at the 500 µg/ind dose, the percentages were 47.1%, 46.8%, and 46.3%, respectively. And at 1000µg/ind 36.1%, 35.7% and 35.6% after 30, 60, and 90 seconds, respectively (Table 6). The findings in Table 6 show a significant dose-dependent inhibition of acetylcholinesterase (AChE) in the liver tissue of *Anas platyrhynchos* exposed to methamidophos, an organophosphate insecticide. At the lowest concentration (250 µg/individual), only a slight decrease in enzyme activity was observed, with mean values of 88-89% of the control at 30, 60, and 90 seconds. This weak inhibition indicates primary interference with normal cholinergic function, rather than total suppression of enzyme activity. There was a considerable loss of AChE activity at the 500 µg/individual dose, with the enzyme activity reduced to about 4647% of the control, indicating strong inhibition of cholinesterase activity. This type of inhibition is

indicative of the high affinity of organophosphates for the active site of AChE, resulting in phosphorylation and irreversible inhibition of the enzyme.

Inhibition of the enzyme at the maximum concentration (1000 µg/individual) was even more pronounced; activity was approximately 3536 times the control values, and a dose-response relationship was evident. This massive decrease demonstrates that Methamindophos has strong neurotoxic effects and perturbs the normal transmission of nerve impulses in hepatic tissue by preventing the hydrolysis of acetylcholine. Overall, the results clearly establish that methamidophos induces robust and progressive AChE inhibition in liver tissue, whereas Cypermethrin (Pyrethroid) induces less intense, occasionally reversible AChE inhibition. The toxicity of methamidophos can be attributed to its organophosphate structure, which binds more tightly and remains permanently attached to the enzyme's active site. These results are consistent with earlier reports highlighting the strong inhibitory effect of organophosphates on cholinesterase enzymes in both avian and mammalian species [13, 21, 25].

Table 6: Acetylcholinesterase Activity in Liver after Treatment with Methamidophos (Organophosphate)

Concentration µg/individual	Time (Sec)	Mean Enzyme AA	% ACTIVITY
Control	0	10938.52	100
	30	10932.36	100
	60	11026.2	100
	90	11096.58	100
250	0	9626.42	88.9
	30	9696.8	88.7
	60	9720.26	88.1
	90	9676.18	88
500	0	5114.28	47.2
	30	5145.56	47.1
	60	5153.38	46.8
	90	5129.92	46.3
1000	0	3917.82	36.2
	30	3941.28	36.1
	60	3925.64	35.7
	90	3941.28	35.6

3.8. Acetylcholinesterase Activity in the Kidney after Treatment with Methamidophos

Acetylcholinesterase activity in kidney after treatment with methamidophos at 250 µg/ind was 103.6%, 102.8%, and 102.5%, respectively. At a dose of 500 µg/ind, the percentages were 84.6%, 83.8%, and 83%, respectively. And at 1000µg/ind dose 47.9%, 47% and 46.7% after 30, 60, and 90 seconds, respectively (Table 7). The findings, as indicated in Table 6 suggest that Methamidophos exposure had a dose-dependent effect on acetylcholinesterase (AChE) activity in the kidney tissue of *Anas platyrhynchos*. When the lowest concentration (250 µg/individual) was entered, a small increase in AChE activity was observed, ranging from 103.7% to 102.5% of the control values. Nevertheless, at the 500 µg/individual dose, inhibition was significant, as enzyme activity decreased to approximately 83.85% of the control, demonstrating toxic interference with normal cholinergic enzyme activity. At the highest concentration (1000 µg/individual), AChE activity was strongly inhibited, with values falling to 4648% of the control at all time intervals.The regulated trend, characterized by a first mild activation followed by progressive inhibition, indicates that kidney tissues are initially resistant to the effects of organophosphate stress. Still, as the dose increases, the toxicity exceeds the detoxification threshold, leading to substantial enzyme inactivation. These results are consistent with those of other researchers who have found dose-dependent inhibition of AChE activity in renal tissues in the presence of organophosphates [15, 26, 13, 20], confirming that methamidophos is a strong neurotoxicant that not only impacts the nervous system but also critical detoxifying organs, such as the kidney.

Table 7.Acetylcholinesterase Activity in the Kidney after Treatment with Methamidophos (Organophosphate)

Concentration µg/individual	Time (Sec)	Mean Enzyme AA	% ACTIVITY
Control	0	5778.98	100
	30	5802.44	100
	60	5865	100
	90	5904.1	100
250	0	5997.94	103.7
	30	6013.58	103.6
	60	6029.22	102.8
	90	6052.67	102.5
500	0	4926.6	85.3
	30	4910.96	84.6
	60	4910.96	83.8

	90	4895.32	83
1000	0	2776.1	48.1
	30	2774.82	47.9
	60	2752.64	47
	90	2752.64	46.7

3.9. Acetylcholinesterase Activity in the Muscles after Treatment with Methamidophos

Acetylcholinesterase activity in muscle after treatment with methamidophos at a 250 µg/ind dose was 77.5%, 78.5%, and 76.7%, respectively. At the 500 µg/ind dose, the percentages were 102.4%, 103.6%, and 99.3%, respectively. And at 1000µg/ind dose 178.1%, 182% and 177% after 30, 60, and 90 seconds, respectively (Table 8). The data in Table 6 indicate that the exposure of the *Anas platyrhynchos* muscle tissue to methamidophos resulted in a significant and variable acetylcholinesterase (AChE) activity change, which reflects the complicated biochemical response of muscular tissue to organophosphate toxicity. The first level of concentration (250 µg/individual) showed a distinct decrease in AChE activity of 76-79% of control, indicating that the inhibitor methamidophos was in contact with the catalytic site of AChE.On the contrary, at the 500 µg/individual dose, there was a slight stimulation in AChE activity, with the control reaching 99-105%. Enzyme activity increased significantly (177–182% of control) at a dose of 1000 µg/individual. This hyperactivation may be explained by compensatory processes, such as upregulation of AChE synthesis, or by the presence of nonspecific esterases that reacted with the assay substrate.

In general, exposure to methamidophos generated a non-linear dose-response relationship in muscle tissue, where it initially inhibited and then activated at low concentrations, and hyperactivated at the highest concentration. These variations demonstrate the tissue-specific metabolic plasticity of muscle, which can differ in detoxification ability compared with the liver and kidney. The results are consistent with earlier research indicating that organophosphate insecticides can also disrupt cholinesterase dynamics in muscle tissues, depending on dose and duration of exposure [26, 13, 25].

Table 8: Acetylcholinesterase Activity in Muscles after Treatment with Methamidophos (Organophosphate)

Concentration µg/individual	Time (Sec)	Mean Enzyme AA	% ACTIVITY
Control	0	2322.54	100
	30	2361.64	100
	60	2332.92	100
	90	2416.38	100
250	0	1837.7	79.2
	30	1829.88	77.5
	60	1829.88	78.5
	90	1853.34	76.7
500	0	2447.66	105.3
	30	2418.74	102.4
	60	2418.74	103.6
	90	2400.74	99.3
1000	0	4175.88	179.7
	30	4207.16	178.1
	60	4246.26	182
	90	4277.54	177

Pesticide toxicity is not specific to a target 'pest', and lethal effects are observed in non-target organisms as well. Several non-target species can be affected when pesticides are used in the environment, as they reduce cholinesterase activity in these species. This enzyme is found in wild animals, and its primary function in the nervous system is to break down the neurotransmitter acetylcholine, which can result in sub-lethal toxicity and death [27]. The extent of cholinesterase inhibition and intoxication from insecticides depends on several factors, including the type of insecticide, the amount to which birds are exposed, the duration and frequency of exposure, the route of exposure, species variation, and the degree of environmental contamination. [28-32]. Results indicated that the low dose (250 µg/ind) produced greater inhibition of liver ChE activity than the other doses (500 µg/ind and 1000 µg/ind). In comparison, a 500 µg/ind dose produced a slight elevation (4.9%) in the kidney. The effects of acute exposure can be delayed, whereas chronic exposure may result in lethal outcomes. In some cases, sublethal concentrations can be more effective than high doses. Pesticides affect the nervous system by ChE inhibition. It leads to nerve exhaustion, and when the nervous system fails, muscles do not receive the Electrical input they require to move. When the respiratory muscles are affected, respiratory paralysis is often the cause of death. In the present study, Acetylcholinesterase activity after treatment with cypermethrin in the liver at a dose of 250 µg/ind was 46.6%, 46.7%, and 47.1%, respectively. At the 500 µg/ind dose, the percentages were 69.7%, 69.6%, and 69.3%, respectively. And at a 1000 µg/ind dose, 99.1%, 99%, and 99.3% after 30, 60, and 90 seconds, respectively. In the kidney, at 250 µg/ind, the percentages were 71.5%, 71.4%, and 71.6%, respectively, while at 500 µg/ind doses, the percentages were 104.9%, 104.8%, and 104.9%, respectively. And at a 1000 µg/ind dose, 63.3%, 62.6%, and 62.4% after 30, 60, and 90 seconds, respectively. In muscle, at a 250 µg/ind dose, the percentages were 79.5%, 82.2%,

and 81%, while at a 500 µg/ind dose, the percentages were 62.3%, 63.1%, and 61.9%, respectively. And at 1000µg/ind 97.6%, 100.8% and 98.7% after 30, 60 and 90 seconds, respectively.

After treatment with methamidophos, the acetyl cholinesterase activity in the liver at a 250 µg/ind dose was 88.7%, 88.1%, and 88%, respectively. Whereas at the 500 µg/ind dose, the percentages were 47.1%, 46.8%, and 46.3%, respectively. And at 1000µg/ind 36.1%, 35.7% and 35.6% after 30, 60, and 90 seconds, respectively. In the kidney, at the dose of 250 µg/ind, the values were 103.6%, 102.8%, and 102.5%, respectively. At a dose of 500 µg/ind, the percentages were 84.6%, 83.8%, and 83%, respectively. And at a 1000 µg/ind dose, 47.9%, 47%, and 46.7% after 30, 60, and 90 seconds, respectively. In muscle at a dose of 250 µg/ind, activity was 77.5%, 78.5%, and 76.7%, respectively. At a 500 µg/ind dose, the percentages were 102.4%, 103.6%, and 99.3%, respectively. And at 1000µg/ind dose 178.1%, 182% and 177% after 30, 60, and 90 seconds, respectively

Burnand and Leighton[6] observed inhibition of activity in Japanese quail under the effect of the Organophosphate (azinphos-methyl, 0.5, 5, and 50 mg/kg). In the Present investigation, Organophosphate (Methamidophos) at 250 µg/ind, 500 µg/ind, and 1000 µg/ind doses showed inhibition in the liver, kidney, and muscles of ducklings (*Anas platyrhynchos*). The present investigation supports the findings of previous research [33] that organophosphate pesticides affect the nervous system and other organs by inhibiting acetylcholinesterase (AChE) activity. In the present and previous research, organophosphates inhibited ChE activity in the test organisms (Liver, kidney, and muscle).

Martin and Forsyth [33] studied the effects of Organophosphate (Dimethoate, 48g on 1-day-old mallard (*Anas platyrhynchos*) ducklings and observed a 20% inhibition of cholinesterase activity. In the present study, methamidophos (an Organophosphate) produced inhibition in the liver and kidneys of *Anas platyrhynchos* at doses of 250 µg/ind, 500 µg/ind, and 1000 µg/ind. At 1000 µg/ind, it produces greater inhibition in liver and kidney than the 250 µg/ind and 500 µg/ind doses. The present results indicate that high levels of pesticide exposure lead to greater disruption of vital biological processes in target organisms. Their mode of action (ChE inhibition) led to the death of the organs. Khan *et al*. [34] studied the effects of two pyrethroids (Lambda cyhalothrin, 0.1% and Permethrin, 1%) on the amphibians *Rana cyanophlyctis* and *Rana tigrina*. Cholinesterase activity was observed in the livers and kidneys of these frog species. It decreased by up to 34.6% and 46.3% in the liver, and by up to 25.08% and 57.1% in the kidney under the effect of pyrethroid. In the case of Pennethrin treatment, cholinesterase activity decreased by up to 23% in the liver and by 6.76% and 35% in the kidney, respectively. In the present investigation, ChE activity decreases after the application of cypermethrin (Pyrethroid) at doses of 250 µg/individual, 500 µg/individual, and 1000 µg/individual.

3.10. Comparison of Acetylcholinesterase reaction in the treatment of Cypermethrin and Methamidophos.

Comparative analysis of cypermethrin and methamidophos treatments showed different trends of the inhibition of acetylcholinesterase (AChE) activity in the liver, kidney, and muscle tissues of *Anas platyrhynchos* (Fig. 1). Although both insecticides influenced the enzyme activity, the extent, direction, and persistence of the inhibition were quite different, which is attributed to their chemical structures, toxicokinetics, and mechanisms of action. Results showed that the Organophosphates irreversibly inhibit acetylcholinesterase (AChE) by phosphorylating the serine hydroxyl group at the enzyme's active site. This prevents the breakdown of acetylcholine, leading to nerve overstimulation.

Enzymatic Biochemical Reaction

$$AChE–OH + (RO)_2P(=O)X \rightarrow AChE–O–P(=O)(OR)_2 + HX$$

The above reaction showed the formation of a phosphorylated enzyme (AChE–O–P bond), and the –OR group is lost, making inhibition irreversible; consequently, accumulation of acetylcholine in the liver, kidney, and muscle led to overstimulation. While there is no substantial covalent chemical reaction between pyrethroids and AChE, the observed variable enzyme activity may be related to indirect effects on sodium channel kinetics rather than direct AChE inhibition. It may indirectly cause secondary inhibition of AChE through oxidative stress or membrane perturbation, which is associated with lower toxicity than that of organophosphates.

3.10.1 Liver Tissue

Methamidophos showed a strong dose-dependent effect on AChE activity in the liver. The enzyme activity decreased from approximately 89% at 250 µg/individual to 47% at 500 µg/individual, and then to 36% at 1000 µg/individual, indicating a progressive and irreversible inhibition of cholinesterase activity. Conversely, cypermethrin had less deleterious effects, with approximately 46% inhibition at 250 µg/individual, incomplete recovery at 500 µg/individual, and nearly normal at 1000 µg/individual.

This distinction underscores the fact that organophosphates (such as methamidophos) are irreversible AChE inhibitors because they phosphorylate the active site of the enzyme and pyrethroids (such as cypermethrin) are reversible or transient AChE inhibitors, primarily because they alter the functioning of ion channels, as opposed to binding covalently to the enzyme [12-13].

3.10.2 Kidney Tissue

The response pattern in kidney tissues was somewhat inconsistent. At 250 ug/individual (=72) and 1000 ug/individual (=63), treatment with cypermethrin led to moderate inhibition and slight activation, respectively. Similarly, methamidophos induced a slight activation at the lowest level (10^3), followed by progressive inhibition at higher concentrations: 85% at 500 µg/individual and 47% at 1000 µg/individual.

It shows that both compounds induce compensatory enzyme activation at initial sublethal doses, which may be due to stress-induced upregulation of AChE (or other esterases). Nonetheless, methamidophos showed stronger inhibition at higher doses, suggesting a greater potential for nephrotoxicity than cypermethrin.

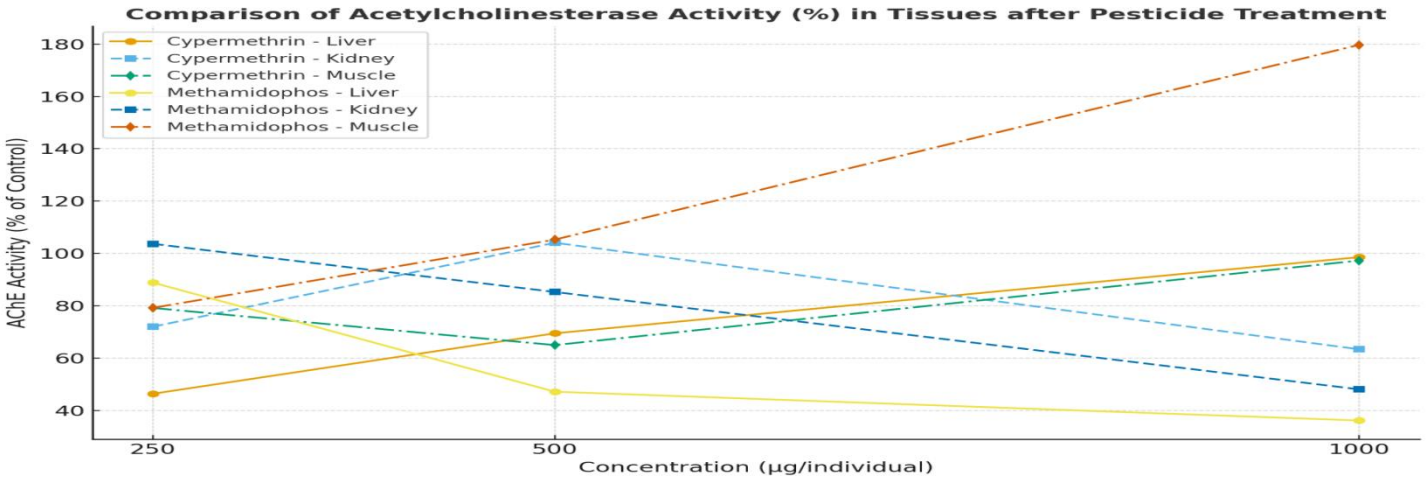

Figure1. Comparison of Acetylcholinesterase activity after treatment with Cypermethrin and Methamidophos pesticides.

3.10.3 Muscle Tissue

In muscle, cypermethrin inhibited AChE activity, with approximately 79-82% inhibition at 250 µg, 62-65% at 500 µg, and nearly normal levels at 1000 µg. Methamidophos, on the other hand, exhibited a biphasic effect: primary inhibition (approximately 7779% at 250 ug), slight activation (approximately 99105% at 500 ug), and spectacular hyperactivation (approximately 177182% at 1000 ug). The cause of this abnormal rise may be nonspecific esterase activity, enzyme induction, or a biochemical feedback response to address the high neurotoxic stress. The trend also suggests that muscle tissue has specific compensatory mechanisms in response to organophosphate exposure.

CONCLUSION

It was concluded that both Cypermethrin (pyrethroid) and Methamidophos (Organophosphate) significantly affected growth (weight) and acetylcholinesterase (AChE) activity in the liver, kidney, and muscle tissues of *Anas platyrhynchos* (ducklings), however, with divergent intensities and mechanisms. Methamidophos showed stronger, dose-dependent, and largely irreversible inhibition of AChE, predominantly in the liver and kidney, indicating enzyme phosphorylation and high neurotoxicity typical of organophosphate poisoning. In contrast, cypermethrin caused variable enzyme activity, likely due to indirect effects on sodium channel kinetics rather than direct AChE inhibition. The liver was the most sensitive tissue, highlighting its key role in detoxification. Overall, methamidophos was more neurotoxic and biochemically disruptive than cypermethrin, emphasizing the need for stricter regulation of organophosphate pesticides near avian habitats. AChE activity proved to be a valuable biomarker for assessing neurotoxicity and pesticide exposure in birds.

Acknowledgment

We acknowledge the PCSIR Laboratories Complex, Karachi, for providing the necessary facilities during the research work.

References

1. G.A. Page. Encycl. Occup. Health Saf., Int. Labour Office, Geneva:62.9–62.40, (1998).
2. R.A. Reylea, N.H. Schoeppner, J.T. Hoverman. Ecol. Appl. 15(4):1125–1134, (2005).
3. W. Stone, S.R. Overmann, J.C. Okoniewski. Condor 86:333–336, (1984).
4. M.F. Khan, M.I. Khan, M. Aslam, S.N.H. Naqvi. Proc. Int. Symp. Hazard. Eff. Pollut. Women Workers, (1996).
5. M.A. Cairns, C.C. Maguire, B.A. Williams. Environ. Toxicol. Chem. 10:657 664, (1991).
6. J.D. Burnand, F.A. Leighton. J. Wildl. Dis. 32:216–224, (1996).
7. B.W. Wilson. In: Wexler, P. (ed.), Clin. Chem. Lab. Anim., Taylor & Francis, Philadelphia, PA, USA:339–454, (1999).
8. W.M. Iko, A.S. Archuleta, F.L. Knop. Environ. Toxicol. Chem. 22:119–125, (2003).
9. R. Claudie, G. Grolleau, S. Chamoul, J. Riviere. J. Wildl. Dis. 41:184–208, (2005).
10. J.R. Osten, A.M. Soares, L. Guilhermino. Environ. Toxicol. Chem. 24:313–317, (2005).
11. C. Roy, G. Grolleau, S. Chamoulaud, J. Riviere. J. Wildl. Dis. 41:184–208, (2005).
12. E. Hodgson. Elsevier/Academic Press, (2012).

13. M. Jokanović. PubMed M., (2018).
14. R. Singh. Environ. Toxicol. Pharmacol. 77:103383, (2020).
15. M. Abdollahi, A. Ranjbar, S. Shadnia, S. Nikfar, A. Rezaiee. Med. Sci. Monit. 10(6): RA141–RA147, (2004).
16. P. Kumar. Ecotoxicol. Environ. Saf. 243:113969, (2022).
17. R, Kaur, H.S. Sandhu.Toxicology International, 15(2), 111–116. (2008).
18. S. Sharma, P. Chadha. SpringerPlus 5:1344, (2016).
19. K.J. Fildes, J.K. Szabo, L. Astheimer, M.J. Hooper, W.A. Buttemer. Environ. Toxicol. Chem. 43(2):321–332, (2024).
20. M. Umar, M. Hussain, S.K. Maloney. Braz. J. Biol. 83:1–9, (2021).
21. A. Kumar, D.K.Singh, V.Singh. Environmental Toxicology and Pharmacology, 94, 103899. (2022).
22. S. Singh, V. Kumar, P.Singh, S.Thakur, R.Kaur.. Environmental Science and Pollution Research, 27(3), 3017–3031. (2020).
23. M. Asif, T. Ahmad, M. Hussain. J. Environ. Toxicol. Public Health 6(3):145–155, (2021).
24. K.J. Fildes, J.K. Szabo, L. Astheimer, M. Hooper, W.A. Buttemer. Sci. Papers, Univ. Wollongong:41–41, (2009).
25. A. Kumar, D.K.Singh, V.Singh. Environmental Toxicology and Pharmacology, 94, 103899. (2022).
26. D. Sharma, A. Nagpal, Y.B. Pakade, J.K. Katnoria, B.Singh. Environmental Toxicology and Pharmacology, 63, 103–114. (2016).
27. K. Cooper. Handbook of Pesticides Toxicology, Vol. 1. Academic Press, New York, USA:463–496, (1991).
28. G.D. Osweiler. Williams and Wilkins, Philadelphia, PA, USA:231, (1996).
29. B.W. Wilson, S.A. McCurdy, J.D. Handerson, S.A. McCarty, J.E. Billiti. Humans 1:23–26, (1998).
30. R. Kanwal, P.A. Sidddui, T. Saqib, R. Tabassum, S.N.H. Naqvi. Int. J. Biol. Biotech. 1(4):723–724, (2004).
31. B.W. Wilson. In: Wexler, P. (ed.), Encycl. Toxicol., 2nd ed., Vol. 1, Elsevier, New York, NY, USA:588–599, (2005).
32. B.W. Wilson, D.E. Arrieta, J.D. Handerson. Chem. Biol. Interact. 157–158:253–256, (2005).
33. P.A. Martin, D.J. Forsyth. Ecotoxicol. 7:81–87, (1998).
34. M.Z. Khan, M. Nazia, F. Fatima, R. Tabassum, K. Gabol. Bull. Pure Appl. Sci. 22A(1):43–49, (2003).

Received: September29th 2025 **Accepted: October 20th 2025**