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Toxicity Effects of Malathion, Dichlorvos and Temephos on Acetylcholinesterase in Climbing Perch (*Anabas Testudineus*)

Asysyuura Adytia Patar, Wan Rozianoor Mohd Hassan, Nooraain Hashim , Farida Zuraina Mohd Yusof

University Teknologi MARA (UiTM), School of Biological Science, Faculty of Applied Sciences, 40450 Shah Alam Selangor, Malaysia.

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ABSTRACT

Malathion, dichlorvos and temephos pesticides are globally used for pest control in agricultural field. Widespread use of pesticides is a worldwide phenomenon. However, applications of pesticides are posing great danger to aquatic environment such as fish. The main objective of this study is to determine the acetylcholinesterase (AChE) activity in different tissues after exposure to malathion, dichlorvos and temephos. Total protein and enzyme activity was determined by Bradford, 1976 and Ellman et al, 1962 respectively. This study showed the optimum condition to be at pH 7 of phosphate buffer with 0.015 M of acetylthiocholine iodide (ACTHI) at 25 °C for 30 minutes. After exposure, the AChE activity decreased compared to the control in all fish organs exposed to high concentration of malathion (0.05 mg/L), dichlorvos (0.47 mg/L) and temephos (5.0 mg/L). However, the exposed fish recovered their AChE activity and the recovery was greater in liver, kidney and gill than in brain. It is concluded that exposure of pesticides in fish lead to inhibition of AChE activity which will cause physiological and biochemical disorders. Thus, the use of pesticides should be properly and strictly controlled and regulated to prevent indiscriminate use.

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INTRODUCTION

Pesticides are routinely employed in the integrated farming practice to protect crops and animals from weeds, insects and diseases [1]. Organophosphates (OPs) are more frequently used among other classes of pesticides because of its high insecticidal property, relatively low degrees of toxicity, less persistence and rapid biodegradability in the environment [2]. Increased use of pesticides at different stages of crop production, starting from seed processing to storage of agricultural produce, is posing great danger to aquatic environment [2]. These pesticides become readily available in the food chain and subsequent bioaccumulation in aquatic environment by directly applied, surface runoff from sites of application and watersheds with possible unquantifiable disastrous consequences on the ecosystem [3]. Thus, indiscriminate use of these pesticides causes the aquatic ecosystem being adversely affected as they serve as ultimate sink for these pesticides, at the same time faced with a threat of a shrinking genetic base and biodiversity [2, 4]. Due to the residual effects of pesticides, the physical and chemical changes in aqueous environment often cause some physiological changes in fish, therefore the water quality of an aquatic body is very crucial because it determines the productivity and other measures necessary for fish survival [5].

Pesticides exert their action by inhibiting AChE activity. Most pesticides attack the nervous system and will cause physiological and biochemical disorders [6]. The pesticides can cause damage to vital organs, skeletal system and biochemical alterations in the exposed fishes. Moreover, heavy contamination of aquatic environment by pesticides can lead to mass mortality of fish and other aquatic fauna [2].

Fish is a non target organism to the use of pesticides but it is affected through loss of habitat and food supply [7]. *Anabas testudineus*, the climbing perch is a fish species in the family of Anabantidae. It is a very hardy fish and important as a food fish because of its ability to survive out of water for a long period of time [8].

Corresponding Author: Wan Rozianoor Mohd Hassan, Universiti Teknologi MARA (UiTM), School of Biological Science, Faculty of Applied Sciences, 40450 Shah Alam Selangor, Malaysia.
Tel: 60193507981 E-mail: rozianoor@salam.uitm.edu.my

MATERIALS AND METHODS

Toxicity Test:

The LC_{50} value of malathion, dichlorvos and temephos was determined as 0.25 mg/L, 2.35 mg/L and 25 mg/L respectively, following the probit analysis method as described by Finney 1952 [9]. Based on the LC_{50} value, the three test concentration of malathion, dichlorvos and temephos, concentration I (1/5th of LC_{50}), concentration II (1/50th LC_{50}) and concentration III (1/500th of LC_{50}) were estimated.

The fish were divided into ten groups and exposed to the different test concentrations, three malathion treatments groups (0.05, 0.005 and 0.0005 mg/L), three dichlorvos treatment groups (0.47, 0.047, 0.0047 mg/L), three temephos treatment groups (5, 0.5 and 0.05 mg/L) and one water control. The fish were exposed for 40 days with water and pesticides replaced once every two days [10, 11].

At the end of the exposure, fishes were sacrificed by decapitation. Then, the liver, kidney, gill and brain were separated immediately and were placed on an ice-cold plate washed in physiological saline solution. Part of the fresh tissue was weighed for further protein and enzyme analysis.

Recovery Test:

Ten fishes from the exposed fish of each group were kept in pesticide free water for 20 days in a large aquarium with filters and continuous aeration. After 20 days, the fish treatment and tissue isolation methods were the same as in toxicity test.

Determination of protein and AChE activity:

Each organ was homogenized in 0.2 M phosphate buffer. Then the homogenates were centrifuged at 2500 rpm for 40 minutes at -4°C. The supernatants were used for total protein and AChE activity determination. The AChE activity was determined according to Ellman method [12] using ACTHI as the substrate. The absorbance was recorded at 412 nm. The enzymatic activity was expressed as µmol/mg/min.

Total protein content was determined by Bradford method [13] by using bovine serum albumin (BSA) as standard with absorbance reading of 595 nm.

Results:

The effects of malathion exposure on the AChE activity in different tissues of climbing perch are presented in Table 1 to Table 4. After exposure (40 days), the AChE activity in all organs decreased at all three concentrations. At the lowest concentration of malathion (0.0005 mg/L), AChE activity in brain, liver, kidney and gill decreased to 28%, 58%, 56% and 49% respectively, compared to control (100%). Maximum enzymatic inhibition after exposure by 0.05 mg/L malathion was 16% in brain, 32% in liver, 29% in kidney and 25% in brain respectively. After transferring the fish to fresh water for 20 days (recovery test), the AChE activity in all treatment groups and examined tissues were different compared during exposure, which restored up to 74% in brain, 89% in liver and kidney, and 86% in gill at the lowest concentration of malathion (0.0005 mg/L).

Table 1: Effects of malathion on AChE activity in brain of *A. testudineus*

Concentration of malathion (mg/L)	Control Treated (40 days) Recovery (20 days)		
	AChE activity (µmol/mg/min)		
0.0005	28.85 (100%)	8.08 (28%)	21.35 (74%)
0.005	28.85 (100%)	5.77 (20%)	16.73 (58%)
0.05	28.85 (100%)	4.62 (16%)	10.39 (36%)

Table 2: Effects of malathion on AChE activity in liver of *A. testudineus*

Concentration of malathion (mg/L)	Control Treated (40 days) Recovery (20 days)		
	AChE activity (µmol/mg/min)		
0.0005	18.65 (100%)	10.82 (58%)	16.60 (89%)
0.005	18.65 (100%)	7.65 (41%)	13.06 (70%)
0.05	18.65 (100%)	5.97 (32%)	11.38 (61%)

Effects of dichlorvos exposure on the AChE activity in different tissues of climbing perch are presented in Table 5 to Table 8. After 40 days of exposure, the AChE activity in brain, liver, kidney and gills were reduced at all concentrations compared to the control. The AChE activity reduced in all examined tissues with increasing dichlorvos concentration. At highest concentration 0.47 mg/L of dichlorvos, the AChE activity reduced to 18% in brain, 33% in liver, 49% in kidney and 37% in gill. After 20 days withdrawal to untreated water, the AChE

activity in brain, liver, kidney and gill restored up to 75%, 83%, 88% and 89% respectively at lowest concentration of dichlorvos (0.0047 mg/L).

Table 3: Effects of malathion on AChE activity in kidney of *A. testudineus*

Concentration of malathion (mg/L)	Control Treated (40 days) Recovery (20 days) AChE activity (µmol/mg/min)		
0.0005	12.35 (100%)	6.92 (56%)	10.99 (89%)
0.005	12.35 (100%)	5.93 (48%)	9.76 (79%)
0.05	12.35 (100%)	3.58 (29%)	7.29 (59%)

Table 4: Effects of malathion on AChE activity in gills of *A. testudineus*

Concentration of malathion (mg/L)	Control Treated (40 days) Recovery (20 days) AChE activity (µmol/mg/min)		
0.0005	10.57 (100%)	5.18 (49%)	9.09 (86%)
0.05	10.57 (100%)	3.28 (31%)	7.19 (68%)
0.05	10.57 (100%)	2.64 (25%)	6.02 (57%)

Table 5: Effects of dichlorvos on AChE activity in brain of *A. testudineus*

Concentration of dichlorvos (mg/L)	Control Treated (40 days) Recovery (20 days) AChE activity (µmol/mg/min)		
0.0047	20.48 (100%)	6.35 (31%)	15.36 (75%)
0.047	20.48 (100%)	4.71 (23%)	13.11 (64%)
0.47	20.48 (100%)	3.69 (18%)	10.04 (49%)

Table 6: Effects of dichlorvos on AChE activity in liver of *A. testudineus*

Concentration of dichlorvos (mg/L)	Control Treated (40 days) Recovery (20 days) AChE activity (µmol/mg/min)		
0.0047	19.76 (100%)	10.28 (52%)	16.40 (83%)
0.047	19.76 (100%)	8.69 (44%)	14.03 (71%)
0.47	19.76 (100%)	6.52 (33%)	11.66 (59%)

Table 7: Effects of dichlorvos on AChE activity in kidney of *A. testudineus*

Concentration of dichlorvos (mg/L)	Control Treated (40 days) Recovery (20 days) AChE activity (µmol/mg/min)		
0.0047	12.35 (100%)	7.78 (63%)	10.87 (88%)
0.047	12.35 (100%)	6.92 (56%)	9.02 (73%)
0.47	12.35 (100%)	6.05 (49%)	7.53 (61%)

Table 8: Effects of dichlorvos on AChE activity in gills of *A. testudineus*

Concentration of dichlorvos (mg/L)	Control Treated (40 days) Recovery (20 days) AChE activity (µmol/mg/min)		
0.0047	9.80 (100%)	5.00 (51%)	8.72 (89%)
0.047	9.80 (100%)	4.31 (44%)	6.96 (71%)
0.47	9.80 (100%)	3.63 (37%)	6.27 (64%)

Effects of temephos exposure on the AChE activity are presented in Table 9 to Table 12. After exposure for 40 days, the AChE activity in brain, liver, kidney and gills were decreased at all concentrations compared to the control. At the lowest temephos concentration (0.05 mg/L), brain, liver, kidney and gill decreased to 37%, 53%, 58% and 52% in the AChE activity respectively. However, after the recovery, the AChE activity increased to 73% in brain, 79% in liver, 83% in kidney and 87% in gill. While at the highest concentration of temephos (5 mg/L), the AChE activity decreased 19% in brain, 30% in liver, 33% in kidney and 31% in gill. Meanwhile after the 20 days recovery, the AChE activity increased to 47%, 54%, 62% and 60% in brain, liver, kidney and gill respectively. Overall, the recovery was greater at lower concentration of malathion, dichlorvos and temephos.

Table 9: Effects of temephos on AChE activity in brain of *A. testudineus*

Concentration of temephos (mg/L)	Control Treated (40 days) Recovery (20 days)		
	AChE activity (µmol/mg/min)		
0.05	26.45 (100%)	9.79 (37%)	19.31 (73%)
0.5	26.45 (100%)	7.41 (28%)	16.93 (64%)
5.0	26.45 (100%)	5.03 (19%)	12.43 (47%)

Table 10: Effects of temephos on AChE activity in liver of *A. testudineus*

Concentration of temephos (mg/L)	Control Treated (40 days) Recovery (20 days)		
	AChE activity (µmol/mg/min)		
0.05	21.88 (100%)	11.60 (53%)	17.29 (79%)
0.5	21.88 (100%)	10.06 (46%)	15.32 (70%)
5.0	21.88 (100%)	6.56 (30%)	11.82 (54%)

Table 11: Effects of temephos on AChE activity in kidney of *A. testudineus*

Concentration of temephos (mg/L)	Control Treated (40 days) Recovery (20 days)		
	AChE activity (µmol/mg/min)		
0.05	18.55 (100%)	10.76 (58%)	15.40 (83%)
0.5	18.55 (100%)	8.16 (44%)	12.99 (70%)
5.0	18.55 (100%)	6.12 (33%)	11.50 (62%)

Table 12: Effects of temephos on AChE activity in gills of *A. testudineus*

Concentration of temephos (mg/L)	Control Treated (40 days) Recovery (20 days)		
	AChE activity (µmol/mg/min)		
0.05	12.96 (100%)	6.74 (52%)	11.28 (87%)
0.5	12.96 (100%)	5.83 (45%)	9.46 (73%)
5.0	12.96 (100%)	4.02 (31%)	7.78 (60%)

Discussions:

The widespread use of pesticides in the environment is causing a great concern about the consequences on the health of humans, wildlife and ecosystems [14]. The degree of enzyme inhibition in fish of all treatment groups were based on pesticide concentration [15]. Referring to the results obtained, a similar trend of inhibition was marked in all four organs (brain, liver, kidney and gill) of climbing perch where the degree of AChE inhibition increased with the increased concentration of malathion, dichlorvos and temephos.

In this study, a reduction in the AChE activity by malathion, dichlorvos and temephos were observed in brain, liver, kidney and gill after 40 days of exposure compared to the control. Therefore, it showed that the pesticides inhibited the AChE. However, the inhibited AChE undergoes spontaneous recovery around 20 days following transfer of the fish to pesticide free water. It was noted that fast rates of AChE recovery were always associated with the high levels of accumulated ACh [16]. These findings of inhibition of AChE were supported by previous research of Kabeer *et al*. [17] and Rath and Misra [15] on *Tilapia mossambica*, as well as other study by Ansari and Kumar [18] on *Brachydanio Rerio*.

In addition, the inhibition of AChE activity was different in all the tissues due to the presence of isoenzymes with different affinities for the substrate and the inhibitor. Furthermore, the pesticides itself may be present in different amounts in the different tissues or the inhibitor may be metabolized at different rates [17]. Moreover, inhibition of AChE was accompanied by an increase in ACh levels and can lead to increase of catecholamines which can affect the activity of enzyme involved in glycogenolysis and glycogen synthesis [6]. Then, continuous stress may affect the synthesis site of AChE. The inhibitory effect on AChE activity indicates that pesticide might interfere in vital processes. All these pesticides not only inhibit AChE but also affect other metabolic activities and the combined effect leads to death [18]. This was supported by Nevin *et al*. [6] which also reported inhibition of AChE that is responsible for the degradation of ACh will result in excessive stimulation of cholinergic nerves. It can lead in tremors, convulsions and finally death of the fish [6].

Besides, behavioral changes also observed in the exposed fish such as increase in surfacing and gulping of surface waters which appears to be an attempt by the fish to avoid breathing in the poisoned water [2]. Then, hypoxic condition due to damage of gills which disrupt oxygen uptake may also contribute to increase surfacing [19]. Moreover, erratic movements and abnormal swimming are triggered by deficiency in muscular and

nervous coordination which due to accumulation of ACh in synaptic and neuromuscular junction. Meanwhile, gradual loss of equilibrium and drowning are caused by adverse effects of pesticides on central nervous system. While increased mucus secretion is probably an adaptive response to counter the irritating effect of the pesticide on body surface and mucus membrane. Other than that, discoloration, which is one of the indicative damage may be caused by impairment of pituitary function reflected by reduction in number and size of chromatophores and their pigment content [2]. Next, defecation also increased in the exposed fishes compare to control group, this is accordance to hyper stimulation of muscarinic receptors in the smooth muscles, gastrointestinal tract and secretory gland [20]. These observation was supported by Alkahem *et al*. [21] which reported on *Oreochromis niloticus* exposed to trichloroform and also Fafioye [22] which reported similar changes in freshwater fish exposed to plant extract.

Conclusion:

Therefore, based on this finding and supported by several previous studies, it was approved that malathion, dichlorvos and temephos were poisonous to *A. testudineus* and these pesticides exert their action by inhibiting AChE activity. Furthermore, this study can help to secure additional knowledge on the toxicology and the use of pesticides in agriculture. Besides, its public awareness should be enhanced in the country.

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