Asian J. Exp. Sci., Vol. 19, No. 2, 2005, 119-126

Enzyme Inhibition (AChE) in Muscles and Skin of *Oreochromis mossambicus* due to Pesticidal Pollution of Herbicide "Pursuit"

Farhina Pasha[1] and Romsha Singh[2]*

1. Department of Biotechnology, Unique College, Bhopal (M.P.)
2. Department of Zoology,
Govt. M. L. B. Girls P. G. (Autonomous) College, Bhopal (M.P.)

Abstract : In the present investigation the effect of three sub lethal concentrations of Pursuit *i.e.*, 63.7 ppm, 85 ppm and 127.5 ppm in *Oreochromis mossambicus* were studied. Pursuit inhibited acetylcholinesterase of muscle and skin of *Oreochromis mossambicus* by increasing the K_m and V_{max}, thereby acting as a mixed inhibitor. The assay of muscle and skin AChE is thus useful for monitoring pesticide toxicity of fish.

Key words : Acetylcholinesterase, *Oreochromis mossambicus*, pursuit and mixed inhibitor.

Introduction :

Accumulation of xenobiotic compounds as recalcitrant is a major problem faced by developing and developed countries, where approximately 92% is pesticide pollution. Amongst pesticides carbamates are potential inhibitors of acetylcholinesterase (Coppage *et al.*, 1975; Satyadevan, 1994). Acetylcholine is released from pre-ganglionic neurons of parasympathetic division of autonomic nervous system. It is a unanimously accepted fact that hydrolysis of acetylcholine (ACh) to choline and acetic acid is catalyzed by enzyme cholinesterase in animal system. The enzyme prevents accumulation of excessive acetylcholine at cholinergic synapse and at neuromuscular junction (Konar, 1979). Quantitative estimation of acetylcholinesterase (AChE) is taken as a good indicator of the extent of pesticide pollution in animals. Enhanced ACh accumulation results in affecting metabolism, muscle coordination, and irregular transmission of impulse and ultimate death of the animal. The test pesticide pursuit (10% st) is a herbicide (a carbamate compound) used extensively for effective control of annual grasses, sludge and broad leaf weeds in soybean and groundnut etc. Its main chemical imazethapyr ($C_{15}H_{22}N_4O_3$) blocks protein

* Corresponding Author.

synthesis. Therefore, the present study was undertaken to investigate long-term exposure of pesticides pursuit on muscle and skin AChE enzyme kinetics and activity of an exotic carp *Oreochromis mossambicus* which may be used as a diagnostic tool to assay toxicity of carbamate compounds to vertebrates and as a controlling measure to check the growth of *Oreochromis mossambicus* which is commonly known as a neuscence fish as it destroys the indigenous fauna.

Materials and Methods :

Healthy fingerlings of *Oreochromis mossambicus* of nearly uniform size (5 cm length) of both the sexes were collected from local fish farm, were kept in glass aquaria and acclimatized to laboratory conditions for two weeks. They were fed daily during the acclimatization period until two days prior to acute and chronic exposure of pursuit (American Cynamide Co., USA). Feeding of control as well as treated fishes was stopped during the experimental period. LC_{50} value of pursuit for 96 hrs was determined by standard method as reported by Doudoroff *et al.* (1951) and estimated to be 0.51 ml/l. Three sublethal concentrations were taken from 2/3 of LC_{50} value and were 63.7 ppm, 85 ppm and 127.5 ppm. Each group of 10 fingerlings was exposed to 3 sublethal concentrations for 96 hrs and control was also maintained for the same duration. At the end of the experiment, the control and experimental fishes were dissected and the muscles and skin were removed. 5% tissue homogenate was prepared in ice-cold 0.25 M sucrose solution and centrifuged at 12,000 rpm for 7 minutes. AChE activity was measured spectrophotometrically at 540 nm by the method of Metcalf (1951) using AChI as substrate. Protein estimation was done according to Lowry *et al.*, (1951) method using Bovine serum albumin as standard. Calculating K_m and V_{max} by applying Lineweaver Burk plot did enzyme kinetic study.

Results and Discussion :

In muscles of fingerlings of *Oreochromis mossambicus,* the K_m in control was 2.13×10^{-3} M which raised to 4.22×10^{-3} M with 63.5 ppm, 5.25×10^{-3} with 85 ppm and reached a maximum at 18.34×10^{-3} M with 127.5 ppm. The V_{max} of control with 1.99 A/mg protein/30 minutes

increased to 2.08 A/mg protein/30 minutes with 63.7 ppm, 2.30 A/mg protein/30 minutes with 85 ppm and 4.28 A/mg protein/30 minutes with 127.5 ppm. The increasing K_m and V_{max} in acute exposure exhibited the mixed competitive non-competitive inhibitory nature of AChE (Table 1). The slope obtained from uninhibited enzyme and for three different concentrations of pursuit intercepted at different ordinates showing the competitive non-competitive nature of inhibition (Fig. 1). The chronic effect of pursuit toxicity in muscles recorded K_m 2.10×10^{-3} M in control which increased to 5.47×10^{-3} M in fifteen days and 7.07×10^{-3} M in 30 days exposure with V_{max} as 1.94 A/mg protein/30 minutes in 15 days and remained constant on 30^{th} day (Table 2). The slope intersecting at different ordinates again confirm the mixed inhibitory nature of pursuit .

Table 1 : Acute effect of different concentrations of pursuit on kinetic parameters K_m and V_{max} of AChE of muscle of *O. mossambicus* (substrate used was AChI)

Pursuit Concentration (ppm) 96 hrs.	**KINETIC PARAMETERS**		
	$K_m \times 10^{-3}$M		**V_{max} Absorbance/mg protein/ 30 min.**
Control	2.13	± 0.78	1.99
63.7 ppm	4.22	± 0.69	2.08
85.0 ppm	5.25	± 0.48	2.30
127.5 ppm	18.34	± 0.52	4.28

In skin of fingerlings, the inhibitory effect of pursuit on enzyme kinetics of AChE was studied, in control it was 0.83×10^{-3} M , with 63.7 ppm it raised to 1.25×10^{-3} M ppm, with 85 ppm the K_m was 1.61×10^{-3} M and at the highest sublethal concentration of 127.5 ppm the K_m was 3.34×10^{-3} M . The V_{max} also showed a similar trend. It was observed as 5.0 A/mg protein /30 min. in control, which remained stable at 63.7 ppm, with 85 ppm raised to 5.5A/mg protein/30 min and with 127.5 ppm it was 7.5A/mg protein/30 min. (Table 3, Fig. 2). The skin of fingerlings treated with 63.7 ppm pursuit for 15 and 30 days was observed as 6.23×10^{-3} M and 7.85×10^{-3} M, respectively, showing an increasing trend with a control

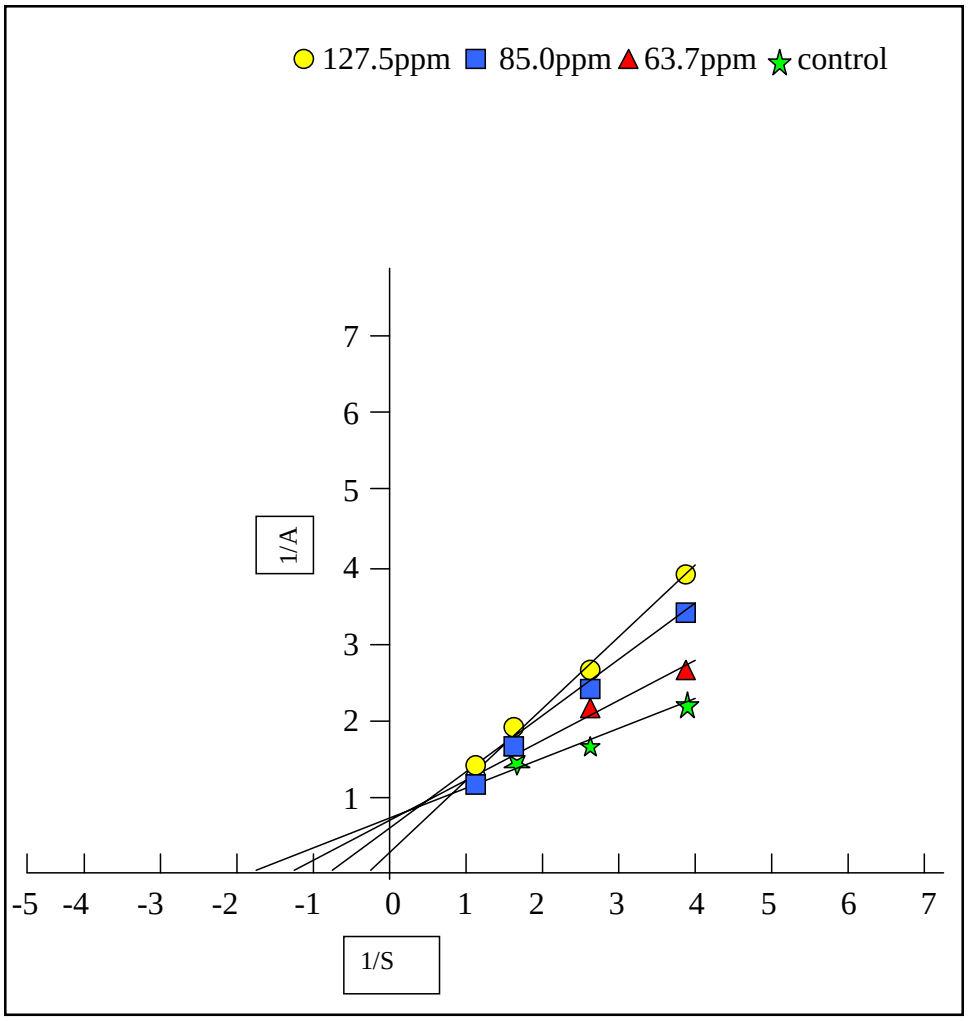

Fig. 1 : Line Weaver Burk Plot of inhibitory effect of 63.7; 85.0; 127.5 ppm PURSUIT on AChE of muscles of *O. mossambicus* treated for 96 hrs. (S is the concentration of AChI).

value of 0.8×10^{-3} M. The V_{max} for 15 days and 30 days at 63.7 ppm pursuit concentration was 4.6A/mg protein/30 min.with a control value of 4.29 A/mg protein/30min. (Table 4). The studies conducted by various earlier workers revealed a similar trend in AChE assay of muscles (Carter, 1971; Shivpratap Rao *et al.*, 1982; Satyadevan *et al.*, 1994.).

Table 2 : Chronic effect of different concentrations of pursuit on kinetic parameters K_m and V_{max} of AChE of muscles of *O. mossambicus* (substrate use was AChI)

Pursuit Concentration (ppm)	**KINETIC PARAMETERS**		
	$K_m \times 10^{-3}$M		**V_{max} Absorbance/mg Protein/30min**
15 Days			
Control	2.10	± 0.96	1.94
63.7 ppm	5.47	± 0.48	2.01
30 Days			
Control	2.10	± 0.68	1.94
63.7 ppm	7.07	±0.59	2.01

Table 3 : Acute effect of different concentrations of pursuit on kinetic parameters K_m and V_{max} of AchE of skin of *O. mossambic.* (substrate used was AChI)

Pursuit Concentration (ppm) 96 hrs.	**KINETIC PARAMETERS**		
	Km $\times 10^{-3}$M		**V_{max} Absorbance/mg protein/ 30 min.**
Control	0.83	± 0.9	5
63.7 ppm	1.25	± 0.75	5
85.0 ppm	1.67	± 0.86	5.5
127.5 ppm	3.34	±0.54	7.5

Table 4: Chronic effect of different concentrations of pursuit on kinetic parameters K_m and V_{max} of AchE of skin of *O. mossambic*. (substrate use was AChI)

Pursuit Concentration (ppm)	KINETIC PARAMETERS	
	Km × 10^{-3}M	V_{max} Absorbance/mg protein/ 30 min.
15 Days		
Control	0.8 ± 0.46	4.3
63.7 ppm	6.23 ± 0.52	4.6
30 Days		
Control	0.8 ± 0.59	4.3
63.7 ppm	7.85 ±0.62	4.6

Thus, the present study reveals that pursuit inhibited the muscles and skin AchE in *Oreochromis mossambicus* fingerlings in both acute and chronic exposures and showed increasing K_m and V_{max} showing a mixed competitive non-competitive nature of inhibition. The phenomenon is studied as the non-competitive inhibitor lowers the maximum velocity attainable within a given amount of enzyme with K_m dependent on concentration, since, I and S may combine at different sites, formation of Enz I and Enz S complex is possible. The Enz I-S may breakdown to form product at slower rate than does Enz S. Our results are in conformity with Bashamohideen and Sailbala (1989) and Coppage *et al.* (1974), although with different fish species and with Rao *et al.* (1984) and Tembhre and Kumar, (1995), who also reported similar trends of mixed inhibition. Moreover, *Oreochromis mossambicus* (Tilapia), which has created a serious problem for survival of indigenous fauna, can be controlled to certain extent by this carbamate compound pursuit as the fish is hardy and resistant to majority of toxicants pursuit being a carbamate and a mixed inhibitor is strong toxicant and its recovery is far more difficult.

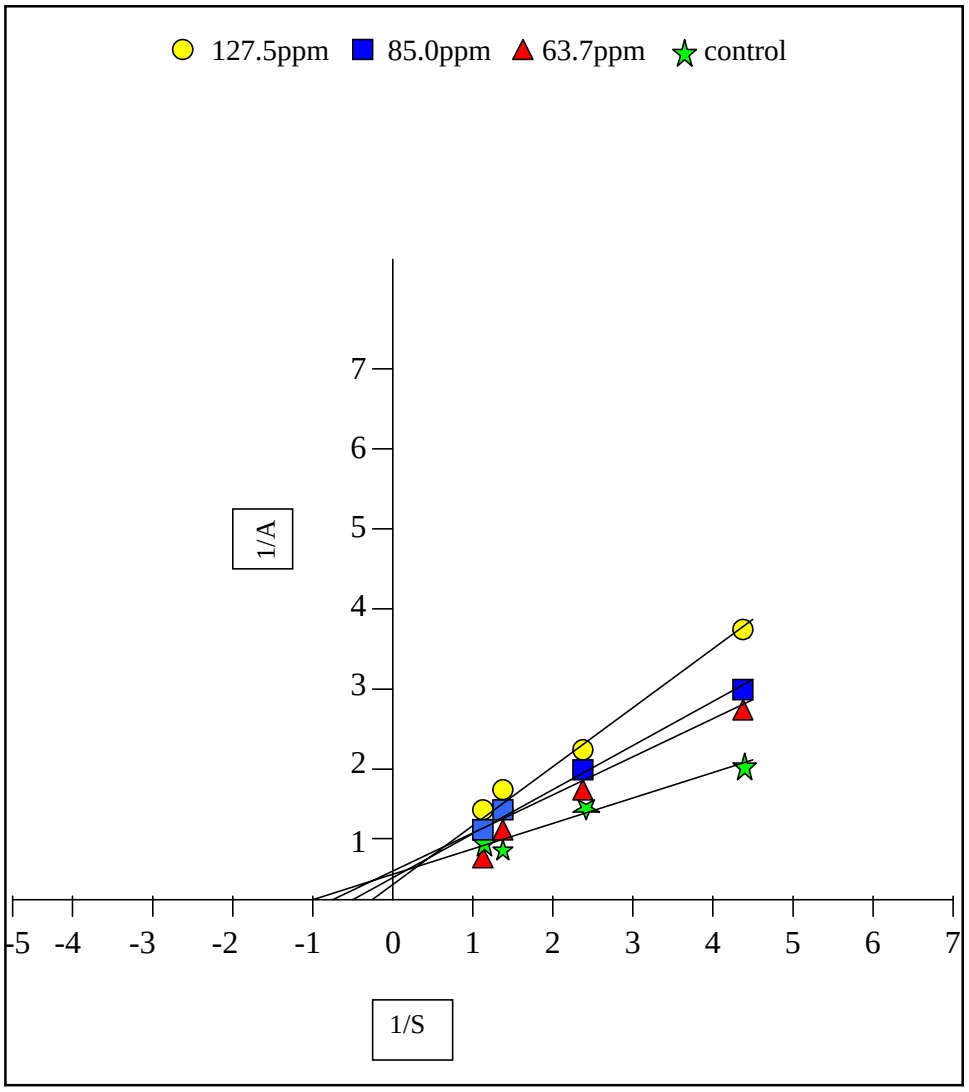

Fig. 2 : Line Weaver Burk Plot of inhibitory effect of 63.7; 85.0; 127.5 ppm PURSUIT on AChE of Skin of *O. mossambicus* treated for 96 hrs. (S is the concentration of AChI).

References :

Bashamohideen M. and Sailbala T. (1989) : AChE activity in the tissue of *Cyprinus carpio* subjected to the sublethal exposure of malathion., *J. Environ. Biol*., **10(1),** 51-57.

Carter F.L. (1971) : *In vivo* studies of brain acetylcholinesterase inhibition by organophosphate and carbamate insecticides in fish. *Ph.D. Dissertation, Louisiana State Univ., Baton*, Rouge, Louisiana.

Coppage D.L., Mathews E., Cook G. H. and Knight J. (1974) : Brain acetylcholinesterase inhibition in fish as a diagnosis of environmental poisoning by malathion pesticide., *Biochem. and physiol.,* **5,** 536-542.

Coppage D.L., Mathews E., Cook G. H. and Knight J. (1975) : Brain acetylcholinesterase inhibition in marine teleost during lethal and sublethal exposure to 2 –dibromo-2,2-dichloroethyl dimethyle phosphatein sea water., *Toxicol.Appl.Pharmacol*., **31,** 128-129.

Duodoroff P., Anderson B.G., Burdwick G.E., Galtsaff P.S., Hart W.B., Patrick R., Strong E.R., Surber E.W. and Vanhern, W.M. (1951) : Standard methods for determination of LC_{50} values., *Sew.Indust. Water*, **23,** 130.

Konar S.K. (1979) : Pesticides and aquatic ecosystem., *Indian J. Fish*, **22,** 80-85.

Lowry O.H., Rosenbrough N.J. AND Randall R.J. (1951) : Protein measurement with Folin phenol reagent., *J.Biol.Chem.,* **193,** 265-275.

Metcalf R.L. (1951) : Methods in biochemical Analysis. D.Click (Ed.), .New York; Intrinsic Publishers Inc. 1.

Rao K. J., Azhar Baig M.D., Rammurthy K., Madhu L.H. and Murthy V.S.R. (1984) : Chronic sublethal effects of phosphamidon on a fresh water teleost *Tilapia mossambica., Bull. Environment. Sci.,* **1(1),** 3-4.

Satyadevan S. (1994) : Cholinesterase system of brain of *Cyprinus carpio*; Biochemical kinetics and histopathological study, *Ph.D.Thesis*, Barkatullah University, Bhopal.

Shiva Pratap Rao K., Kabeer Ahmad I. and Rama Rao K.V. (1982) : Impact of methyl parathion on lactate dehydrogenase isozymes of a teleost *Tilapia mossambica* (Peters)., *Indian J.Fish,* **29,** 185.

Tembhre M. and Kumar S. (1995) : Effect of sublethal concentrations of methylamine on AChE activity and enzyme kinetics of alimentary canal of *Cyprinus carpio., Indian J. Spect.,* **6(2),** 39-42.