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Organophosphate poisoning to Some Fresh Water Teleosts—Acetylcholinesterase Inhibition

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The organophosphate (OP) insecticides are widely used in Northern India to control insect pests and disease vectors. Many of the treated areas contain fresh water resources like streams, lakes and ponds which harbour diverse aquatic fauna and flora. When released in them with the run-off rain water, the insecticides cause several adverse effects to the organisms living therein, including fishes. These insecticides act as nerve poisons by blocking synaptic transmission in cholinergic parts of the nervous system (KARCZMAR et al. 1970, METCALF 1971). The compounds bind to the active site of the enzyme acetylcholinesterase (AChE), prevent breakdown of acetylcholine (ACh) and cause its excessive accumulation (MIYAMOTO et al. 1963, ALDRIDGE 1971, METCALF 1971, FUKUTO 1971, KOELLE 1963).

The aquatic organisms show a broad range of response to OP insecticides, depending on the compound, exposure time, environmental conditions and species (EISLER 1970). As such the possible hazards of these compounds to the aquatic life should not be ignored. AChE measurements, probably are the best specific index of OP poisoning of fish in the environment (COPPAGE 1972, MACEK et al. 1972, COPPAGE and DUKE 1972, COPPAGE and MATTHEW 1974). Little information is available to indicate the sublethal effects of these toxicants on the activity of this enzyme in fresh water teleosts. The present communication deals with the quantitative reduction of AChE in few fresh water teleosts by sublethal exposure to some OP insecticides.

MATERIALS AND METHODS

Mature, well-fed, active and healthy fishes collected from the local fish market, were kept in rectangular glass aquaria at 24 ± 5°C. The size of Channa gachua varies from 120-167 mm and weight from 75-102 g while in Cirrhina mrigala, the size varies from 145-195 mm and weight from 88-109 g. The insecticides, Zolone (0,0-diethyl dithio-phosphorylmethyl-6-chlorbenzoxazolone), available in 30% emulsifiable concentrate (EC), manufactured by Mysore Insecticides Co. (P) Ltd. Madras, Rogor [0,0-dimethyl-S (N-methyl-carbamoyl-methyl) phosphorodithioate], available in 35% EC, manufactured by

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Tata Fison Ind. Ltd., Bombay and Malathion [0,0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate], available in 50% EC, manufactured by National Pesticides, Vidisha (M.P.) were used.

After the usual process of acclimatization and washing with 1% $KMnO_4$ solution to avoid the possibility of any infection, the fishes were transferred to glass aquaria. TL(50) values of different insecticides were determined by adopting the dilution techniques (STANDARD METHODS, 13th edition, 1971). The sublethal concentrations, 1/2, 1/3 and 1/5th of the TL(50) values for both the fishes were calculated and then the fishes were exposed for 3 and 7 day periods. During experimentation, fishes were fed with an artificial diet to avoid any possible effect of starvation on enzyme activity. The experimental solutions were renewed regularly at every 48 h interval. Controls were set side by side for comparison.

Control as well as treated fishes were sacrificed and the brain, liver and muscle were taken out. The activity of enzyme in different tissues was determined by measuring the amount of acetylcholine hydrolysed. The method given by METCALF (1951) was adopted for the purpose.

RESULTS AND DISCUSSION

The activity of AChE in the brain, liver and muscles of Channa gachua and Cirrhina mrigala was determined after exposure of fishes to sublethal concentrations of Zolone, Rogor and Malathion and the results so obtained are tabulated in Tables 1 and 2. The results are expressed as average $\pm$ S.E. of three observations. A significant fall in the AChE activity has been observed after treatment in both the fishes. A progressive significant fall in the enzyme activity has been observed in all the tissues, with the increased pesticide concentrations. The highest fall in the activity of this enzyme has been observed in brain of C. mrigala after three days exposure to Malathion, while least in liver of C. gachua exposed for seven days in Rogor.

The cholinesterases of vertebrates remain inhibited for several weeks after exposure because of the irreversible inhibition by extremely small quantities of dealkylated oxygen analog metabolites of thiophosphates (COPPAGE and DUKE 1972, MACEK et al. 1972). POST and LEASURE (1974) are of the opinion that a long time is required to recover brain AChE levels to near pre-exposure level and the recovery time varies with the fish species. AChE level in C. gachua and C. mrigala also recovered, not fully, but to some extent after seven days exposure. It indicates that the disposing of the organophosphate compounds even in lesser quantity into water resources, may not produce immediate loss of fishes living therein but it may result in an inability on the part of fishes to sustain their physical activity in the search of food, maintaining their body balance in flowing water and in escaping from their enemies. The fishes may die under these stresses.

TABLE 1

Effect on AChE in the brain, liver and muscle of *Channa gachua* exposed to sublethal concentrations of organophosphate insecticides.

Insecticides	Conc. in mg/L	Enzyme activity in u moles of ACh hydrolysed per mg of tissue protein per hr.					
		3 days exposure			7 days exposure		
		Brain	Liver	Muscle	Brain	Liver	Muscle
None	None	98.4±3.1	26.3±1.3	57.3±2.4	97.8±3.7	26.1±1.3	58.9±3.0
Zolone	0.0405	20.5±1.1**	10.5±0.4**	21.3±1.0**	30.5±1.8**	12.5±0.5**	26.0±1.6**
	0.0270	30.8±1.3**	14.8±0.4**	25.1±1.2**	41.8±2.1**	17.6±0.7*	32.8±2.1*
	0.0162	48.2±2.0**	19.3±0.6*	33.0±2.0*	57.9±2.9**	20.9±1.0*	41.1±2.4*
Rogor	2.2375	30.6±1.3**	15.9±0.9*	23.5±1.6**	39.7±2.1**	18.7±1.0*	31.0±2.2*
	1.4920	36.2±1.4**	18.2±1.0*	29.8±1.7**	49.0±2.4**	20.5±0.9*	35.8±2.0*
	0.8970	52.1±2.3**	19.5±0.9*	35.4±1.9*	63.6±3.1*	21.6±1.0*	43.9±2.3*
Malathion	3.8000	16.5±0.9**	10.1±0.4**	21.0±0.9**	29.3±1.4**	11.1±0.5**	24.5±1.8**
	2.5300	22.2±0.9**	12.5±0.4**	23.3±1.1**	43.8±2.1**	15.7±0.7*	30.8±2.1*
	1.5200	41.5±1.9**	17.1±0.6*	29.5±1.6**	57.1±2.9**	18.8±0.9*	40.7±2.4*

Each value represents the Mean ± Standard Error (S.E.) of 3 observations.
Values are statistically significant when determined by students t test: *P<0.05; **P<0.01; ***P<0.001.

TABLE 2

Effect on AChE in the brain, liver and muscle of Cirrhina mrigala exposed to sublethal concentrations of organophosphate insecticides.

Insecticides	Conc. in mg/L	Enzyme activity in u moles of ACh hydrolysed per mg of tissue protein per hr.					
		3 days exposure			7 days exposure		
		Brain	Liver	Muscle	Brain	Liver	Muscle
None	None	94.8±2.0	31.3±1.0	54.9±2.3	94.3±3.1	31.4±1.4	54.6±2.8
Zolone	0.0346	17.5±0.8***	12.1±0.5**	19.6±1.2**	25.6±1.1**	14.5±0.4**	22.0±1.1**
	0.0230	20.6±1.0***	16.7±0.5**	22.3±1.3**	29.8±1.3**	19.1±0.9*	27.6±1.2**
	0.0138	45.1±1.9**	21.4±1.0*	29.9±1.3**	53.3±2.1**	22.8±1.0*	36.6±1.2*
Rogor	1.5690	27.1±1.2***	18.2±1.0**	20.7±1.3**	35.1±1.6**	20.4±1.0*	24.7±1.3**
	1.0460	30.5±1.3***	20.2±1.1*	26.7±1.4**	44.9±1.9**	22.1±1.3*	30.5±1.7*
	0.6270	48.8±1.7**	22.0±1.2*	32.3±1.6**	59.7±2.1**	24.2±1.4*	38.8±2.0*
Malathion	2.7075	10.0±0.5***	11.5±0.3**	17.3±0.9**	23.7±1.0**	13.3±0.7**	21.1±1.1**
	1.8050	18.8±0.9***	13.1±0.4**	21.9±1.2**	28.4±1.5**	16.8±0.9**	25.8±1.5**
	1.0830	38.1±1.2**	18.2±0.6**	26.5±1.1**	52.4±2.4**	21.4±0.9*	35.0±1.8*

Each value represents the Mean ± Standard Error (S.E.) of 3 observations.
Values are statistically significant when determined by students t test: *P<0.05; **P<0.01; ***P<0.001.

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