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IMPACT OF OXYDEMETONMETHYLE ON THE ACTIVITY OF ACETYLECHOLINE ESTERAGE ENGYME IN EDIBLE FISH *LABEO ROHITA*

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ABSTRACT

The consumption of pesticides is spectacularly increasing in our country both in agriculture and public health departments. The extensive and indiscriminate use of Organophosphorus insecticides has become an essential part for the better farming. The benefits due to use of OP insecticides are numerous, but at the same time they show considerable harm to biosphere. On exposure of *Labeo rohita* to sublethal concentration of oxydemetonmethyle organophosphorus insecticide were studied. Most of the OP compounds are similar with the ester part of acetylcholine (Ach) and they react with the esterase part of the Acetylcholinesterase. (AChE). After entering in to the organism. The conversion of ACh in to acetic acid and choline catalyzed by AChE is considered to be the key reaction in catabolism. A significant elevation in ACh content and inhibition in the activity of the AChE was observed in fishes with oxydemetomethyle insecticide for 48, and 72 hours. The interaction of OP insecticides with AChE indicate that their inhibitory action is reversible. The OP compound inhibits the activity of enzyme AChE and by doing this they disrupt the transmission of nerve impulses due to the enormous accumulation of Acetylecholine (ACh) at the synaptic cleft.

Key words: *insecticide, acetylcholine, esterase, organophosphorus.*

INTRODUCTION AND REVIEW OF LITERATURE

During the last three decades, the consumption of pesticides is spectacularly increasing in our country both in agriculture and public health departments. The great increase in the use of

pesticides which are accidentally or deliberately released in the environment has imbalanced the biology of ecosystem. The extensive and indiscriminate use of Organophosphorus insecticides has become an essential part for the better farming.

The benefits due to use of OP insecticides are numerous, but at the same time they show considerable harm to biosphere, pesticides through agricultural run off to the streams, lakes and ponds during rainy season adversely affect the non-target aquatic fauna. Zooplankton, insects, worms, mollusks and fishes suffer from various ill effects, like mass mortality, chronic changes in behavior, low survival rates and morphological changes in different organ system, by altering biochemical constituents of fish and other animals (Singh *et al:* 2004; Setha and Saxena, 2003, Mahboob and Siddique, 2002, Barcavolli and Martinez, 2004, Thangavel *et al:* 2004). Among the aquatic fauna fish are most sensitive to pesticides especially organophosphorus insecticides.

The wide range of application of organophosphorus (OP) insecticide in agriculture has resulted due to the ban on organochlorine (OC) compounds. . The use of OP insecticides is now increasing at a fast rate because of their high efficiency and biodegradability. Pollution of biosphere by insecticides is characterized by the presence of OP residues in food products and their circulation along the food chains. As the fish constitute an important link in the food chain its pollution causes threat to the entire aquatic environment.

In the present investigation an attempt has been made to study the toxic effects of technical grade OP insecticides i.e**.,** Oxydemetonmethyl on target enzyme Acetylcholinesterase and neurotransmitter acetylcholine (ACh) were studied in different tissues of normal and exposed fish for 48 hours and 72 hours of time period. The action of OP insecticides is generally neurotoxic and the inhibitors of acetylcholinesterase (AChE) enzyme.

OXYDEMETONMETHYL ;

It is a OP Insecticide used mainly as a foliage spray and has a relatively long residual life within plants for sucking insects.

Alternative Name: Metasystox-R

Chemical Name: S-2(Ethylsulfinyl)Ethyl O,O-Dimethyl Phosphorothioate.

Physical nature : Yellowish liquid .

Boiling point : 134^{o} C.

Toxicity : Acute oral LD_{50} for Rats 75-80 mg/kg

Solubility : Highly soluble in water and most organic solvents.

METERIAL AND METHODS

Healthy fresh water fish *Labeo rohita* weighing around 30-40 gm and 18-20 cm in length were collected from the local market and were acclimatized to laboratory conditions for 15 days in a cement tank under running tap water. The fish were fed with groundnut cake and white of the egg, but starved one day before the day of experimentation. The LC_{50} was determined by the method of Finney (1964) and Bayne et al: (1977). The fish were exposed to a sublethal concentration of Oxydemetonmethyl organophosphorus (OP) insecticide for 48 and 72 hours. Liver, brain ,muscle and gill tissues were isolated from the normal and exposed fish.

ACETYLCHOLINE (Ach) ESTIMATION;

Method.: Hestrin (1949) modified by Augustinson (1957)

Tissues weighing 50-100 mg were taken into test tubes containing 2.0ml of double distilled water and boiled for 5 minutes to release the bound acetylcholine (Ach) by inactivating the AChE. The tubes were cooled and the homogenates were prepared in 2.0 ml of double distilled water. The contents were centrifuged at 3000 rpm for 15 minutes and to 1.0 ml of the supernatant, 2.0 ml of 2M alkaline hydroxylamine hydrochloride reagent was added followed by 1.0 ml HCl (HCl : H_2O). The contents were centrifuged at 3000 rpm for 10 minutes. 2.0 ml of the clear filtrate 1.0 ml of 0.37 N Ferric Chloride was added and the color developed was react at 540 nm against the reagent blank. Acetylcholine chloride was used as standard.

Units: µ moles/g wet weight of the tissue.

ACETYLECHOLINESTERASE ENZYME (AChE).

Method**.** Metcalf (1951) described by Augustinson (1957)

The percent (w/v) tissue homogenates were prepared in 0.25 m sucrose solution which form the enzyme source. The reaction mixture in a total volume of 2 ml contained 100 micro moles of phosphate buffer (pH 7). 8 micromoles of acetylcholine chloride and 0.3 ml of enzyme source. The reaction mixture was incubated for 30 minutes at 29^0C and the reaction was stopped by adding 2 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 6N HCL. The content were shaken well and centrifuged. 2.5 ml of supernatant. 5 ml of 10% ferric chloride was added and the color developed was read at 540 nm against the reagent blank in a colorimeter.

Units; µ moles of acetylcholine hydrolyzed/mg protein/h.

RESULTS AND DISCUSSION

During the exposure of fish to different concentrations of of organophosphorus insecticides.Few changes were observed in the behavior of fish. there were no casualties in the controls. The test fish lies irregularly often comong to the surface followed by loss of equilibrium. The color of the fish skin gradually became very dark during the time of death.

The levels of ACh in tissues of control and insecticide exposed fish are presented in table-1. and Fig -1 The ACh content in control tissues viz., brain, muscle, gill and liver is 47.43, 33.89, 25.73 and 21.79 micro moles/gm wet weight of the tissues respectively. An increase in ACh content was observed in tissues of fish following treatment with Oxydemetonmethyl OP Insecticide

The percent changes in treated fish by exposed with Oxydemetonmethyl

at 48 and 72 hours, are +36.16 and +22.85 in brain, +27.50 and + 22.51 in muscle, +59.61 and +36.49 in gill finally in liver +57.09 and +45.06 respectively.

A significant elevation in Ach content and inhibition in the activity of the neuro transmitter enzyme AChE was observed in Fish on treatment with OP insecticide. The serine hydroxyl group is blocked by the OP compound leading to the inhibition of AChE which result in the excessive accumulation of ACH at the synaptic cleft disrupting the transfer of nerve impulses leading to paralysis and death of the fish. (O. Brien,1967).

TABLE NO.1: Changes in Acetylcholine content (µ moles/gm wet weight of the tissue) in tissues of control and insecticide exposed fish *Labeo rohita*.

Name of the Tissue	Control	48 hrs.	72 hrs.
BRAIN	47.43 ± 3.92	62.21 ± 1.21	58.27 ± 1.26
		PC = + 31.16	PC = + 22.85
MUSCLE	33.89 ± 2.73	43.21 ± 2.50	41.52 ± 2.49
		PC = + 27.50	PC = + 22.51
GILL	25.73 ± 1.62	40.27 ± 1.97	35.12 ± 2.21
		PC = + 59.61	PC = + 36.49
LIVER	21.79 ± 3.26	34.23 ± 2.11	31.61 ± 2.21
		PC = + 57.09	PC = + 45.06

Each value is mean ± S.D. of 6 individual observations.

Values are significant at 1% level (P<0.01).

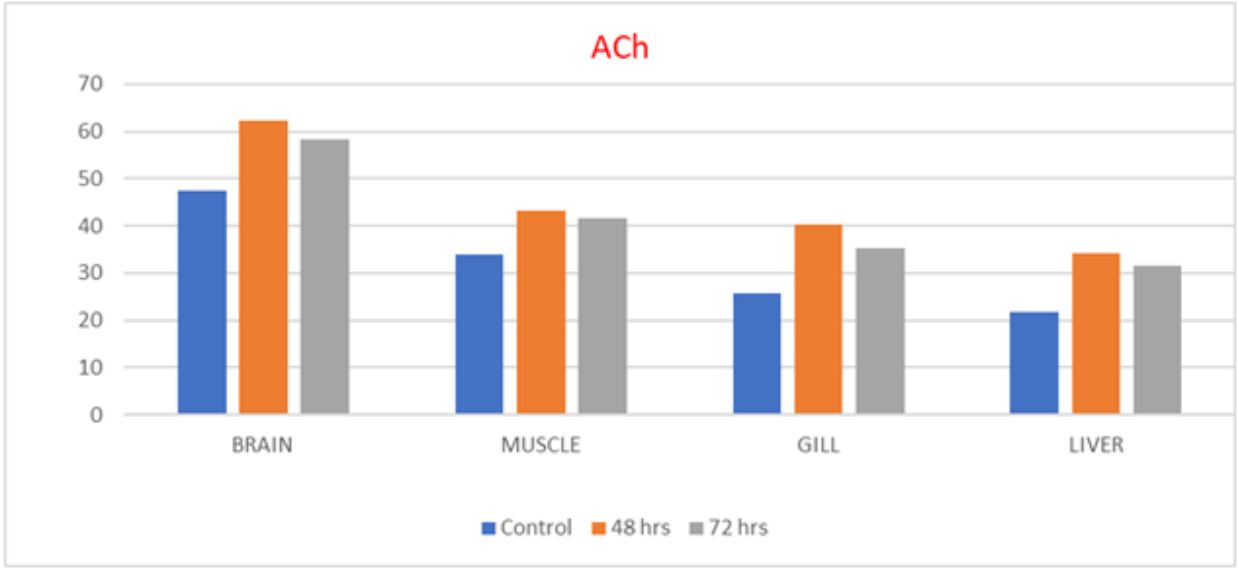

Fig.1.Levels of Acetylcholine in control and insecticide treated freshwater fish *Labeo rohita* at 48 hrs. and 72 hrs. of time period.

The activity of AChE in tissue of control and exposed fish are presented in table-2. and Fig-2. The AChE activity was inhibited in OP compound i.e. Oxydemetonmethyl OP Compound exposed fishes.

The percent changes in Oxydemetonmethyl insecticide . treated fish at 48 and 72 hours, are +31.16 and +22.85 in brain, +27.50 and + 22.51 in muscle, +59.61 and +36.49 in gill finally in liver +57.09 and +45.06 respectively.

The levels of AChE in tissues of control and insecticide exposed fish are presented in table-2. And Fig -2 The AChE content in control tissues viz., brain, muscle, gill and liver is 7.21, 4.72, 3.27 and 2.12, respectively.

The interaction of OP insecticides with AChE indicate that their inhibitory action is reversible. The OP compound inhibits the activity of enzyme AChE and by doing this they disrupt the transmission of nerve impulses due to the enormous accumulation of ACh at the synaptic cleft. The neurotransmitter ACh significantly increased whereas the enzyme AChE involved in terminating the action of ACh was inhibited in brain, muscle, liver and gill of fish (O. Brien, 1967, Shobha Rani 1990, Venkateshwarlu *et al:* 1992, Chitra et al: 1993, Sivakumari *et al*: 1997, Sridevi *et a*l: 1998, Ganesh *et al*: 2000, Janaiah *et al:* 2006).

TABLE NO.2: Changes in Acetylcholinesterase activity (µ moles of Acetylcholine Hydrolyzed/mg protein/h) in tissues of control and insecticide exposed fish *Labeo rohita*.

Name of the Tissue	Control	48 hrs.	72 hrs.
BRAIN	7.21 ± 0.39	3.98 ± 0.25 PC = - 44.79	4.72 ± 0.39 PC = - 34.53
MUSCLE	4.72 ± 0.16	2.96 ± 0.17 PC = - 37.29	3.42 ± 0.28 PC = - 27.54
GILL	3.27 ± 0.17	2.31 ± 0.30 PC = - 29.35	2.46 ± 0.28 PC = -24.77
LIVER	2.12 ± 0.15	1.42 ± 0.21 PC = - 33.02	1.64 ± 0.21 PC = - 25.00

Each value is mean ± S.D. of 6 individual observations.

Values are significant at 1% level (P<0.01).

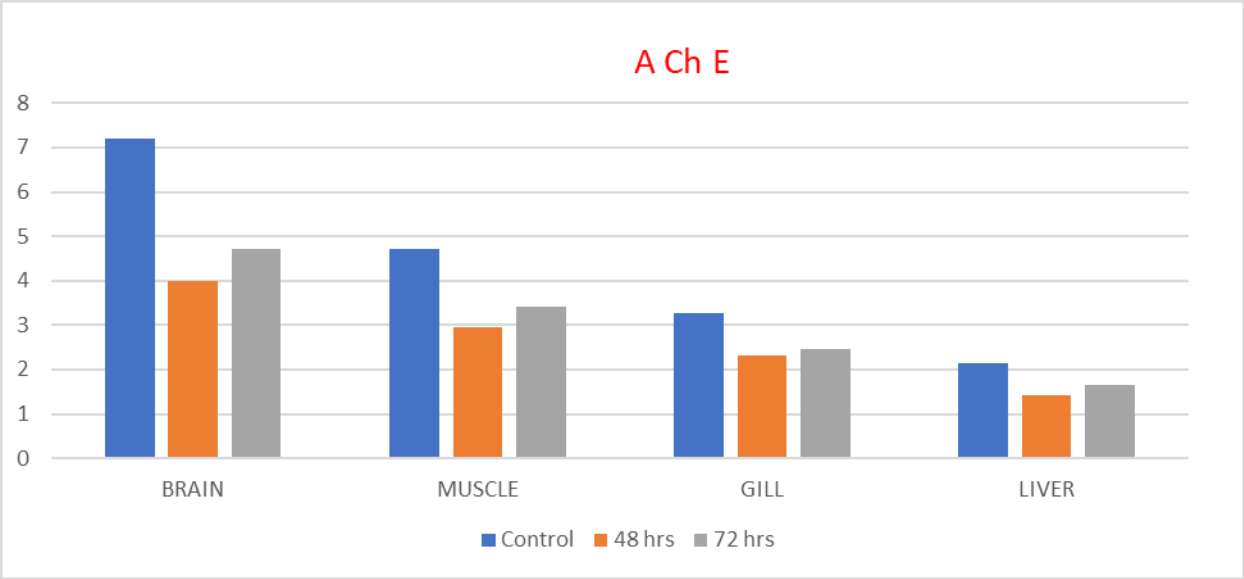

Fig.2.Specific activity levels of Acetylcholinesterase in control and insecticide treated freshwater fish *Labeo rohita* at 48 hrs and 72 hrs. of time period.

Oxydemetonmethyle treated fish the percent changes at 48 and 72 hrs. of time period are -44.79 and -34.53, in brain. -37.29 and -27.54. in muscle, in gill -29.35 and -24.77, and -33.02, -25.00 in liver respectively.

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