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Research Article

IMPACT OF SYNTHETIC PYRETHROIDS CYFLUTHRIN AND FENVALERATE ON ACETYLCHOLINESTERASE ACTIVITY(ACHE) OF FRESH WATER FISH *GAMBUSIA AFFINIS*

S. Mohammed Ghouse*

Department of Zoology, Osmania College, Kurnool- 51800, Andhra Pradesh, India.

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ABSTRACT

Pesticides are used in agriculture for centuries for eradication of unwanted insects and controlling disease vectors. The term pesticide encompasses a group of chemical compounds used to eliminate or control of pests. These pesticides through the process of biomagnification increase their concentration at the top trophic level of the food chain, including the in humans. *Gambusia affinis* was subjected to sub lethal concentrations of cyfluthrin and fenvalerate from 24 to 96 hours Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

Keywords: Pesticides, Acetylcholinesterase enzyme, Decrease, tissues, *Gambusia affinis*.

INTRODUCTION

Pesticides are used in agriculture for centuries for eradication of unwanted insects and controlling disease vectors. The term pesticide encompasses a group of chemical compounds used to eliminate or control of pests. Under the U.S. Federal Environmental Pesticide Control Act, the term Pesticide has been defined to include any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (Barse *et al*., 2006). These pesticides by different routes finally reach the aquatic systems affecting the aquatic organisms (Santhakumar *et al.*, 1999). Pesticides are hazardous to the biodiversity including the man. Pesticides have detrimental effects on behaviour, reproduction, growth, hormonal regulation etc of the organisms .These pesticides through the process of biomagnifications increase their concentration at the top trophic level of the food chain, including the in humans. Fishes are usually exposed to highly contaminated water, which are in areas where waste water dilution rate is low (Bernet *et al*., 1999). Fish play a vital role in the toxicity testing for ecological effects as the most employed organisms for bio monitoring. In fish, the principle mode of uptake of pesticides is through the gills (Johnson, 1968), body surface and gastro-intestinal system (Costa & Murphy, 1983), through integument (Bowes, 1972) and by the ingestion of contaminated food (Macek *et al*., 1969). Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

*Corresponding Author: Dr. S. Mohammed Ghouse, Reader, Department of Zoology, Osmania College, Kurnool- 51800, Andhra Pradesh, India. Email: syed0002001@gmail.com, Mobile: +91 9501603265

MATERIALS AND METHODS

Fish collection and laboratory conditions

Adult fresh water teleost fishes *Gambusia affinis* with weight (0.7-1.5 gm), length (2.3 - 3.2 cm) from both sexes were selected randomly for the experimental studies were collected from water bodies and fisheries department of Kurnool district, Andhra Pradesh, India. The collected fish were transported in polythene bags filled with half water with least disturbance. They are kept in aquarium tanks containing well aerated de-chlorinated water to acclimatize to the laboratory conditions, with physic - chemical characteristics: temperature 24 ± 2°C, pH 7.1 ± 0.2 at 24°C, dissolved oxygen 9.6 ± 0.8 mg/L, carbon dioxide 6.3 ± 0.4 mg/L, total hardness 23.4 ± 3.4 mg as $CaCO_3$/L, phosphate 0.39 ± 0.002 µg/L, salinity in different cement tanks for one week. To prevent fungal infection the fish tanks were washed with potassium permanganate solution. Dead fish were removed as soon as possible to avoid water fouling. Fishes were fed daily on commercial fish food. The unused food is removed daily and water was renewed daily .Test fishes were screened critically for any signs of stress, disease, physical damage and mortality. Injured, severely, diseased fishes were discarded. The fishes were exposed to sublethal concentrations for treated and control period of 96 hours. A control group was maintained with identical environment. The toxicant water as well as normal water is renewed every day. The fish were sacrificed from both experimental and control groups on 96 hours.

Selection of pesticides

In the present investigation two synthetic pyrethroids were selected namely Cyfluthrin and Fenvalerate.

Characteristics of Cyfluthrin

Cyfluthrin, cyano (4-fluoro-3- phenoxyphenyl) methyl-3-(2,2dichloroethenyl)-2,2-imethylcyclopropanecarboxylate) is a broad spectrum synthetic type II pyrethroid insecticide, Cyfluthrin stable at room temperature, but sensitive to sunlight, CAS Registry Number: 68359-37-5, Chemical Class: Synthetic pyrethroid, Use: Broad spectrum insecticide for a variety of agricultural crops, Toxicity rating: Moderately toxic, Signal Words: Caution. Warning - Danger, Health Effects: it acts as a neurotoxic poison, causes organ inflammation and induces skin paresthesia as per the animal studies, Environmental Effects: Cyfluthrin is extremely toxic to bees, fish and other aquatic organisms. (Source: Cyfluthrin chemical watch Factsheet, August 2007).

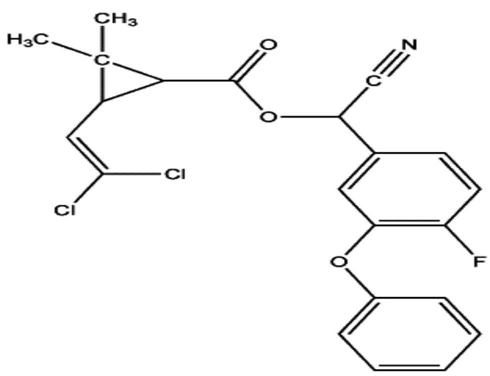


Figure 1

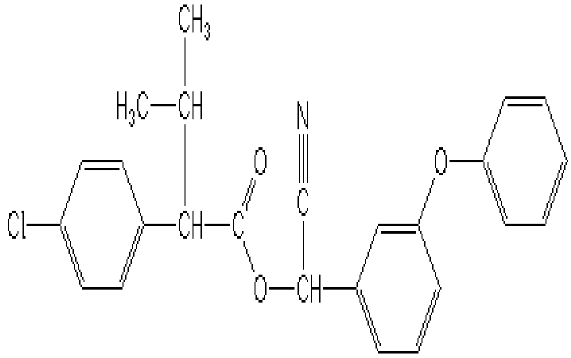


Figure 2

Figure 1. Cyfluthrin cyano (4-fluoro-3-phenoxyphenyl) methyl-3-(2,2-dichloroethenyl)-2, 2-dimethyl-cyclopropanecarboxylate. **Figure 2**. IUPAC name: (RS)-a-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate Chemical Abstracts name: cyano (3-phenoxyphenyl) methyl 4-chloro (1-methylethyl) benzene acetate.

Fenvalerate

Fenvalerate, is a clear viscious yellow liquid with a mild odor, a synthetic pyrethroid. Fenvalerate a synthetic pyrethroid is an effective against the bollworms, aphids, weevils, leaf hoppers, stem pod, fruit borers etc which are common pests of agriculture. The technical grade containing 92% fenvalerate and 8% related compounds were obtained from Searle India ltd, Mumbai. Generic name (IUPAC name): (RS)-alpha-Cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate, Common name: Fenvalerate, Trade names: Pydrin, Sumicidin, Belmark, CAS number: 51630-58-1, EPA Code: 109301, Year of initial registration: 1978.

Experimental design

Group 1: Fish exposed to tap water observed for 24 to 96 Hours (Untreated control). Group 2: Fish exposed to sublethal concentration of cyfluthrin of 24 to 96 hours. Group 3**:** Fish exposed to sublethal concentration of fenvalerate of 24 to 96 hours. The toxicity studies will conducted by using the technical grade formulation available formulation of Fenvalerate and Cyfluthrin. Solutions of desired concentrations were prepared in 95% acetone to get the stock solution as well as working solution as 100mg/100ml and 1mg/1ml of toxicant chemical Fenvalarate and Cyfluthrin in both technical formulation use of acetone in control as recommended by

EPA will be followed**.** The acclimatized fishes were kept in cement tanks and ten fishes in two groups control fishes will be kept in dechlorinated water only. Each set of experiment was replicated six times. Mortality was recorded every 24 h during the observation period of 96h.

Statistical analysis

The data obtained from the quantitative study were expressed as the mean ± S.E. The mean values were calculated from 6 individual observations; P-values were calculated by the students‘t’ test. The two mean values obtained were considered significant from each other with standard error.

Estimation of Acetylcholine esterase activity (AChE) activity

The method of AChE activity estimation is popularly known as Ellman's method (Ellman *et al*., 1961) named after George Ellman who developed this method in 1961 The esterase activity is measured by providing an artificial substrate, acetylthiocholinelodide (ACI). Thiocholine released because of the cleavage of ACI by AChE is allowed to react with the -SH reagent 5, 5’- dithiobis- (2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412nm. Assay was performed by adding 2.9ml of O.lmM sodium phosphate buffer (p.H-8) to 50ml of supernatant of different region of brain and then incubated at 37 for 5 minutes. After incubation 40ul of Acetylthiocholine iodide (154.38mM), 10ul of DTNB (10mM) was added, mixed by inverting cuvette and absorbance was taken at 412nm for 150 sec at interval of 30sec using UV spectrophotometer. The extinction coefficient of the thionitrobenzoic acid is 1.36 x 104/ molar/ centimeter. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is than taken as direct estimate of AChE activity.

RESULTS AND DISCUSSION

In the present observation when Gambusia *affinis* exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed significant decrease in the levels of AChE activity content in the gill when compared with the control group. The percent changes over the control with cyfluthrin (group-2) are - 10.21, - 18.97, - 28.62, - 36.08 for 24, 48, 72 and 96 hours respectively. Whereas in group-3 percent decrease in AChE activity over the control are - 12.41, - 20.67, - 31.43, - 40.96 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3). In the present study the observed values of AChE activity in the liver tissues of *Gambusia affinis* fish exposed to cyfluthrin (group-2) and fenvalerate (group-3) showed a significant decline ,when compare with the control group. The percent decline with cyfluthrin (group-2) are -8.89, - 16.23, - 26.72, - 34.67 for 24, 48, 72 and 96 hours respectively. The percent decrease with fenvalerate (group-3) are - 9.71, - 17.67, - 29.60, - 34.11 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3).

Pesticides deliberately added to the environment for eradication of pests but they are entering in to the environment affecting the non target organism like fishes. So fishes are used widely for the bioassay of these pesticides .Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

This enzyme is found in cholinergic synapses in the brain as well as in autonomic ganglia, the neuromuscular junction and the target tissues of the parasympathetic system (Silman & Sussman, 2005; Soreq & Seidman, 2001). AChE catalyses the hydrolysis of Ach and plays a role in cholinergic transmission by reducing the concentration of acetylcholine (ACh) in the junction to regulate nervous transmission (Kopecka *et al*., 2004). AChE inhibition of results in an accumulation of acetylcholine, which leads to tetany, paralysis, and eventual death due to the continuous and excessive stimulation of the nerve/muscle fibers. AChE inhibition of results to a variety of neurotoxic effects and decreased cholinergic transmission due to the buildup of acetylcholine within the nerve synapses (Mileson *et a*l., 1998). AChE inhibition results in functionally vital organs like gill, muscle and liver lead to impaired critical neurophysiological activity and block sodium channels of nerve filaments, thereby lengthening the depolarization phase. Pyrethroids delay the closing of the gate that allows sodium flow (Vijverberg & vanden Bercken, 1990) and thus, multiple nerve impulses rather than the usual single one occur. In turn, these impulses release the neurotransmitter ACh, which stimulates other nerves (Eells *et al*., 1992) ultimately resulting in buildup of ACh within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (Mushigeri & David, 2005). It is also known pyrethroid compounds which inhibit AChE activity were known to disrupt the normal behavioral patterns in the effected animals (De la Torre *et al*., 2002). In *Cyprinus carpio* and *Cnesterodon decemm-aculatus* showed reduce level of acetylcholinesterase (Roos *et al*., 1980).

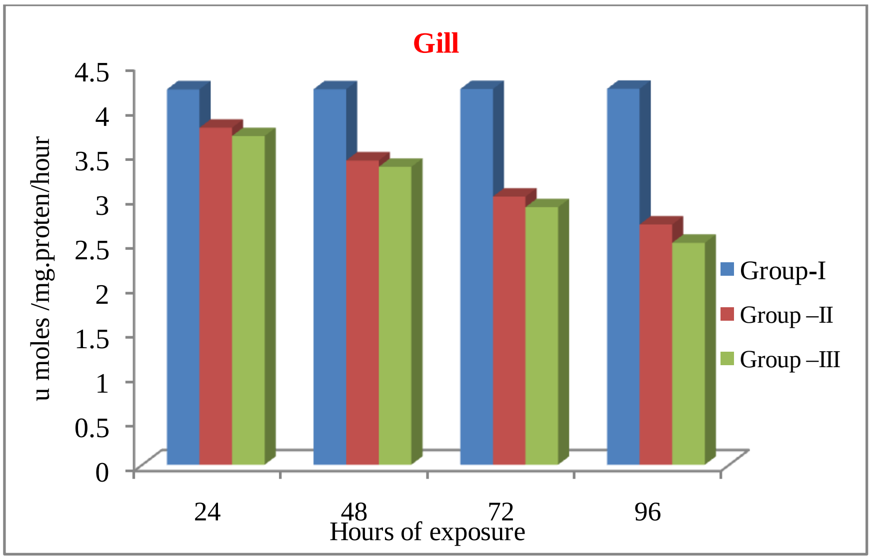
The AChE activity in the tissues of kidney in *Gambusia affinis* fish *exposed* to cyfluthrin (group-2) and fenvalerate (group-3) decreased in comparison with control group. The percentage of alterations are - 12.65, - 21.37, - 28.99, - 43.33 for 24, 48, 72 and 96 hours respectively in fish exposed to cyfluthrin (group-2) .The percentage of alterations in fish exposed to fenvalerate (group-3) are - 13.88, - 23.85, - 31.44, - 37 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3).

The levels of AChE activity in the muscle shown a declining trend in *Gambusia affinis* fish *exposed* to cyfluthrin (group-2) and fenvalerate (group-3) in comparison with control group. The percentage of decline in fish exposed to cyfluthrin (group-2) are - 8.46, - 15.86, - 24.59, - 33.11 for 24, 48, 72 and 96 hours respectively. The decline percentage in fish exposed to fenvalerate (group-3) are - 9.60, - 16.56, - 23.41, - 34.62 for 24, 48, 72 and 96 hours respectively. The levels of AChE content for 3 groups are recorded are statistically significant at 1% and 5% levels (Table 1 and Figure 3).

Table 1: Variations of Acetylcholine esterase (AChE) (umole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours

Tissues	Groups	24	48	72	96
		Hours of exposure			
Gill	Group-I Control	4.220 ± 0.056	4.222 ± 0.057	4.224 ± 0.058	4.226 ± 0.059
	Group –II- Cyfluthrin	3.789** ± 0.034	3.421** ± 0.033	3.015** ± 0.036	2.701** ± 0.034
	% COC	% - 10.21	% - 18.97	% - 28.62	% - 36.08
	Group –III- Fenvalerate	3.696** ± 0.044	3.349** ± 0.046	2.896** ± 0.046	2.495** ± 0.047
	% COC	% - 12.41	% - 20.67	% - 31.43	% - 40.96
Liver	Group-I Control	3.540 ± 0.071	3.542 ± 0.073	3.543 ± 0.076	3.544 ± 0.079
	Group –II- Cyfluthrin	3.225** ± 0.048	2.967** ± 0.051	2.596** ± 0.053	2.315** ± 0.061
	% COC	% -8.89	% - 16.23	% - 26.72	% - 34.67
	Group –III- Fenvalerate	3.196** ± 0.027	2.916** ± 0.028	2.494** ± 0.031	2.335** ± 0.034
	% COC	% - 9.71	% - 17.67	% - 29.60	% - 34.11
Kidney	Group-I Control	3.960 ± 0.029	3.962 ± 0.029	3.963 ± 0.034	3.962 ± 0.037
	Group –II- Cyfluthrin	3.459** ± 0.066	3.115** ± 0.068	2.814** ± 0.072	2.245** ± 0.076
	% COC	% - 12.65	% - 21.37	% - 28.99	% - 43.33
	Group –III- Fenvalerate	3.410** ± 0.091	3.017** ± 0.092	2.717** ± 0.092	2.496** ± 0.096
	% COC	% - 13.88	% - 23.85	% - 31.44	% - 37
Brain	Group-I Control	4.581 ± 0.081	4.583 ± 0.082	4.583 ± 0.085	4.584 ± 0.086
	Group –II- Cyfluthrin	4.193** ± 0.062	3.856** ± 0.065	3.456** ± 0.072	3.066** ± 0.076
	% COC	% - 8.46	% - 15.86	% - 24.59	% - 33.11
	Group –III- Fenvalerate	4.141** ± 0.074	3.824** ± 0.077	3.510** ± 0.079	2.997** ± 0.080
	% COC	% - 9.60	% - 16.56	% - 23.41	% - 34.62

Values are mean ± S.E Mean of six individual observations and student t-test. Significant at * P<0.05; Significant at * P<0.01 levels (+, -) denotes decreased and increased. %COC (change over control).



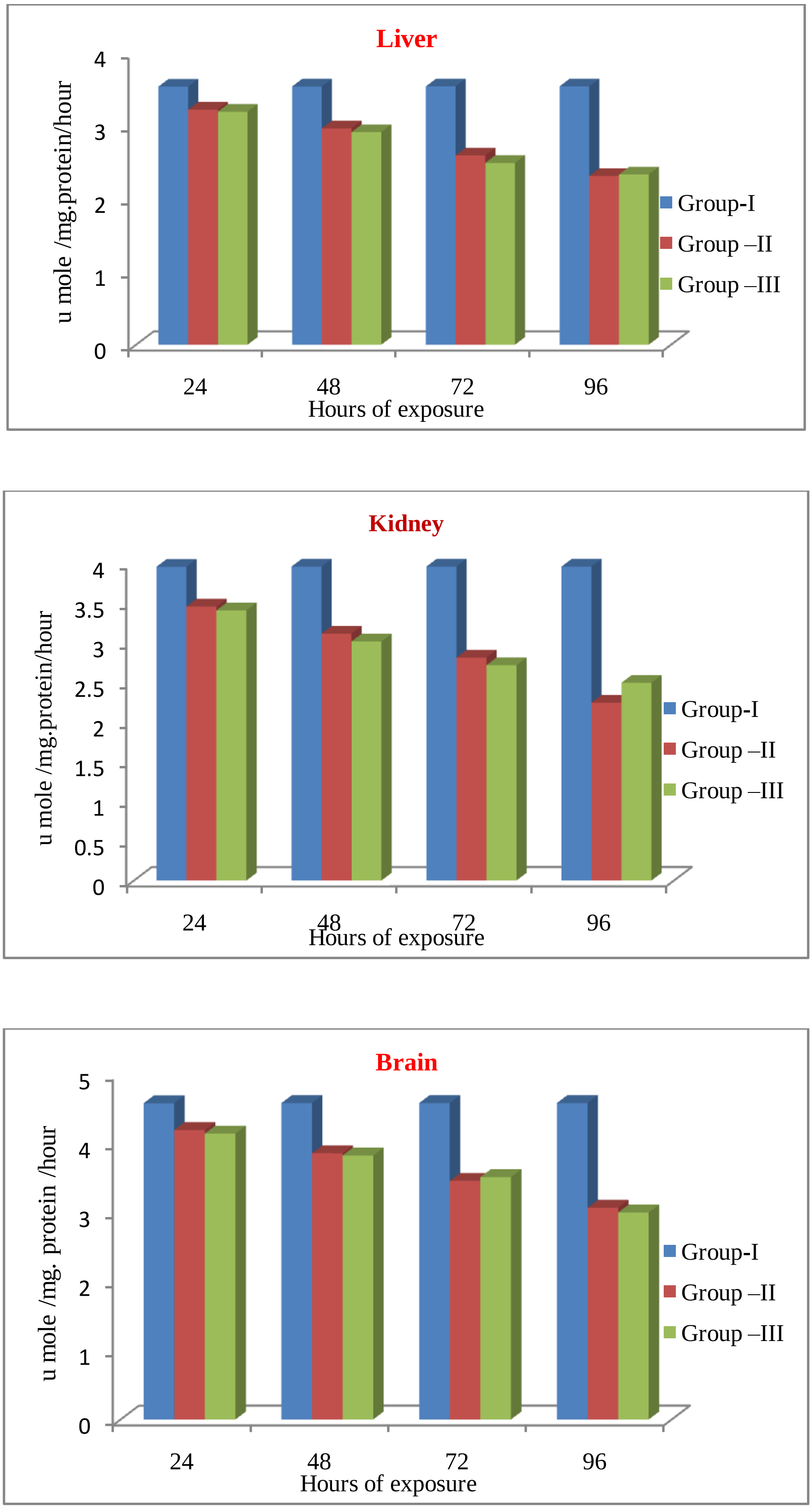


Figure 3. Changes in the level of Acetylcholine esterase (AChE) (u mole /mg.protein/hour) activity fresh water fish *Gambusia affinis exposed to* Cyfluthrin and Fenvalerate for 96 hours.

It has been established that the AChE enzyme unit consists of a negative subsite, which attracts the quaternary group of choline through both columbic and hydrophobic forces and an esterase subsite, where nucleophilic attack occurs on the acetyl carbon of the substrate (Key *et al*., 1998). OPs inhibitory action resulted in decrease in AChE level was probably due to the impairment of neural transmission. The decrease in AChE activity when grass shrimp exposed to sub lethal concentration of malathion, (Modesto & Martinez, 2010) decreased AChE activity in *Prochilodus lineatus* in the brain and the muscle of when exposed to glyphosate (Reddy *et al*., 1992).

Similar reports which collaborates our findings in the inhibition of AChE activity due to pyrethroids exposure is reported in *Cyprinus carpio* (Somnuek *et al*., 2007; Szegletes *et al*., 1995).Similar observation are made in the alterations of levels of the AChE activity in the brain, liver, muscle and gill tissues of hybrid catfish, *Clarias macrocephalus* and *Clarias gariepinus* exposed to a sublethal concentration of chlorpyrifos and a carbamate, carbaryl for 4 days and reported that AChE inhibition increased rapidly with insecticide concentration (Rodrıguez fuentes & Gold bouchot, 2000). AChE inhibition is observed under cypermethrin exposure in different fishes (Ahmad *et al*., 2000; Bretaud *et al*., 2000; Cattaneo *et al*., 2008; Ferrari *et al*., 2007; Lionetto *et al*., 2003; Van Cong *et al*., 2009; Wang *et al*., 2009).

CONCLUSION

It can be concluded that there is correlation between fish cyfluthrin and fenvalerate exposure and decline in the Acetylcholinesterase enzyme contents in selected tissues. Acetylcholinesterase enzyme involved in the maintenance of the structural and functional integrity of cellular membranes. For normal behaviour and muscular function AChE activity is vital. In the present investigation there is marked decrease in AChE activity in the gill, liver, kidney and brain tissues is observed, when the fish *Gambusia affinis* is exposed to the cyflthrin and fenvalerate. The intensity of inhibition of AChE activity might also correlated with the degree of accumulation of pesticide and their metabolite in different organs.

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