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Acetylcholinesterase and acid phosphatase activity in Freshwater fish *Ctenopharyngodon idella* exposed to Profex

Haribabu Gogula, Lalitha V and Venkata Rathnamma V

Abstract
Insecticides are used extensively in agriculture, but their levels in superficial waters generally range far below lethal concentrations for aquatic organisms. However, sub lethal adverse effects might result from exposure of aquatic organisms to insecticides at environmentally relevant concentrations. Acetyl cholinesterase activity is routinely used as a biomarker of the exposure to certain groups of pesticides, such as organophosphates. The fish *Ctenopharyngodon idella* was exposed to lethal ($1/5^{th\ of}$ LC_{50} of 96hrs = $24\mu gL^{-1}$) and sub lethal (($1/10^{th\ of}$ LC_{50} of 96hrs = $12\mu gL^{-1}$) concentrations of Profex (profenofos 50%EC) for 4 days. The gill, liver, kidney, brain and muscle tissues were analysed for the activities of Acetylcholinesterase and Acid Phosphatase and all the experiments were compared with controls.

Keywords: Profex (50%EC), *Ctenopharyngodon idella*, biochemical changes

1. Introduction
Pesticides are one of the most harmful chemicals which have posed serious threat to animal's health worldwide (El-Shenawy *et al.*, 2009; Magar and Bais 2013) [1, 2] and plays an important role in modern agriculture. However, pesticides may have a beneficial effect on agricultural productivity, their indiscriminate use causes many serious problems to the environment and human health, since these compounds are toxic to non-target species (Diez, 2010; Coutinho *et al.*, 2005) [3, 4]. Acetylcholinesterase enzyme is widely used for rapid detection to predict early warning of pesticide toxicity (Dutta and Arends, 2003) [5]. The role of AChE in cholinergic transmission is to regulate nervous transmission by reducing the concentration of acetylcholine (ACh) in the junction through AChE-catalyzed hydrolysis of ACh (Kopecka *et al.*, 2004) [6]. AChE was identified as the enzyme responsible for termination of cholinergic transmission by cleavage of AChE to acetate and choline; AChE 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 and Sussman, 2005) [7].
The AChE activity is necessary to maintain normal behaviour and muscular function and represents a prime target called esteric site on which some toxicants can exert a detrimental effect by binding to it. Inhibition of the AChE activity results in a build-up process of acetylcholine causing prolonged excitatory postsynaptic potential. This results in repeated, uncontrolled firing of neurons leading to hyper stimulation of the nerve/muscle fibres, which leads paralysis, and eventual death. In the event of decreased ATPase system, phosphorylation may be preceded by activated phosphates to catalyse the liberation of inorganic phosphates from phosphate esters. Acetyl cholinesterase (AChE) activity is a frequently used in environmental monitoring, usually in areas contaminated with pesticides, heavy metals and effluents. It is an enzyme that catalyses three hydrolysis of acetylcholine to choline and acetate in synaptic cleft. The aim of this study was to verify the relationship between the lethal and sub lethal concentration (LC_{50}) of Profex (profenofos 50%EC) and the activity levels of Acetylcholinesterase (AChE) and ACP in gill, liver, kidney, brain and muscle tissues of the fish as the possible early biomarkers of exposure to this organophosphate insecticide.

2. Materials and Methods
The fish *Ctenopharyngodon idella* with an average size of 6 to7 cm total length (TL) and 6.5to 7.5 g body weight were brought from a local fish farm Kuchipudi village, Guntur District of Andhra Pradesh, India, and acclimatized at 28 ± 2^0 C in the laboratory for 15 days. Such acclimatized fish were exposed to sub lethal ($1/10^{th}$ of LC_{50}) and lethal ($1/5^{th}$ of LC_{50}) concentrations of profenofos 50% EC commercial grade for 4days.

The vital tissues like gill, liver, kidney, brain and muscle tissues of the fish exposed were taken for the estimation of activities of Acetyl chlorine esterase (AChE) and ACP all the test fish were compared with controls. Profex (profenofos 50% EC) manufactured by Nagarjuna Agrichem Limited., Hyderabad, India was purchased from the local pesticide dealer in Guntur and used in the experiment to evaluate the effects in different tissues at lethal and sublethal concentrations on freshwater fish, *Ctenopharyngodon idella.*

2.1 Estimation of Acetyl Cholinesterase (AChE) Activity
Enzyme activity was determined by the method based on Ellman *et al.*, (1961) [8].

2.2 Estimation of Acid Phosphatases (ACP)
The activity of acid phosphates was estimated by the method of Bodansky (1932) [9].

3. Results and Discussion
The AChE activity (µ moles/min/mg protein) was estimated in different tissues like gill, liver, kidney, brain and muscle of the fish *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC for a period of 4days along with controls and the percent change over the control are shown in table below.

Table 1: Changes in the AChE activity (µ moles/min/mg protein) in different tissues of the fish, *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC.

Tissues	Control	Profenofos 50% EC			
		Sub lethal (4days)	% Change	Lethal (4days)	% Change
Gill	0.126±0.0012	0.056±0.0013	-55.71	0.046±0.0004	-62.96
Liver	0.280±0.0022	0.136±0.0007	-51.43	0.122±0.0018	-56.43
Kidney	0.121±0.0051	0.072±0.0013	- 40.66	0.063±0.0010	- 48.11
Brain	0.391±0.0043	0.138±0.0005	- 64.71	0.112±0.0003	- 71.35
Muscle	0.317±0.0042	0.127±0.0002	- 59.93	0.115±0.0001	-63.74

Values are the mean of five observations: Standard Deviation is indicated as (±), Value are significant at $p< 0.05$

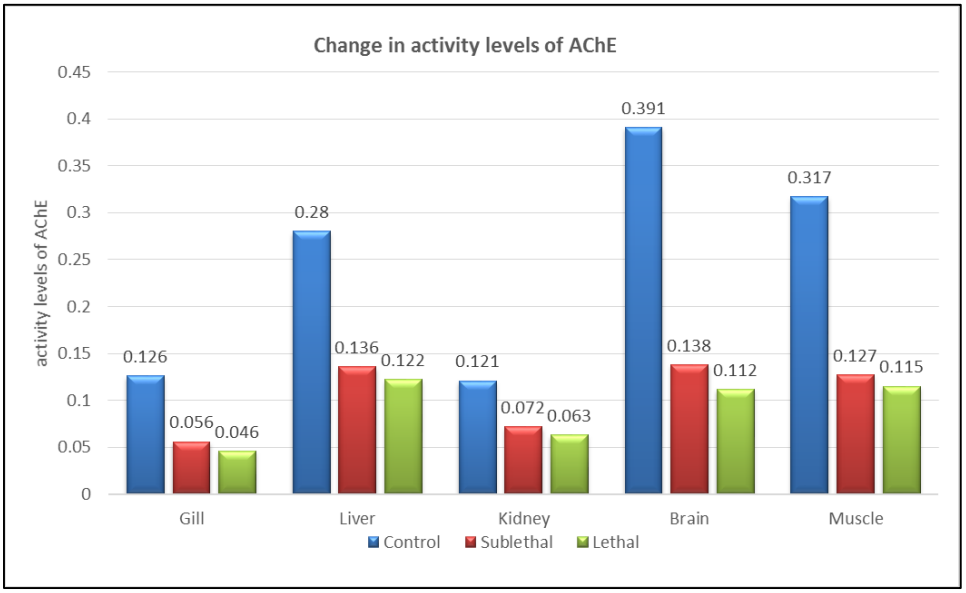

Fig 1: Changes in the AChE activity (µ moles/min/mg protein) in different tissues of the fish, *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC.

In profenofos 50% EC sub lethal exposure for 4days maximum percentage of depletion was (-64.71%) in brain followed by muscle (- 59.93), gill (-55.71), liver (-51.43) and minimum percentage was (-40.66%) in kidney. In lethal exposure maximum percentage of depletion was (-71.35%) in brain and followed by muscle (-63.74), gill (-62.96) liver (-56.43), and minimum percentage was (-48.11%) in kidney, all these were compared with controls.

Fish immune system, important for defence against a variety of harmful pathogens is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by biochemical profile of the nervous system. Insecticides can alter the immune functions of the body and result in immune-depression, uncontrolled cell proliferation, and alterations of the host defence mechanism against pathogens (Banaee M, 2012) [10]. Pesticides have been shown to cause alterations in the activities of many enzymes concerning to cellular metabolisms (Vani *et al.*, 2011) [11]. Organophosphate pesticides act by inhibiting the acetyl cholinesterase (AChE) at its esteratic site, resulting in an accumulation of the neurotransmitter acetylcholine in nerve tissue and at the effector organ (Ernest Hodgson, 2010; Mirjana B Colovic *et al.*, 2013) [12, 13].

According to Jaqueline Ineu Golombieski *et al.*, 2017) [14], accumulation of pesticides of organophosphate result in the continued stimulation of cholinergic synapses. Responses to organophosphate insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and the species (Fisher, 1991; Richmonds and Dutta, 1992) [15, 16]. Low concentrations of the compounds can inhibit AChE, which leads to an accumulation of acetylcholine at central cholinergic synapses and neuromuscular junctions (Sancho *et al.*, 1997; Varó *et al.*, 2003) [17, 18]. The inhibition of the Acetyl cholinesterase by pesticides can affect

locomotion and equilibrium of exposed organisms (Saglio & Trijasse, 1998; Bretaud *et al.,* 2000) [19, 20].

According to the study of Jaqueline Ineu Golombieski *et al.*, 2008) [21] after diafuran exposure, the AChE activity decreased ($p< 0.05$) for all concentrations in both tissues in relation to the control. Maximum inhibition of the AChE activity for all species was reached when exposed to 1 mg L-1diafuran. Maximum percentage AChE activity for 1 mg L-1 of diafuran in brain and muscle tissue compared to control was 28.92 and 28.89% for common carp, 30.17 and 55.45% for grass carp, and 55.22 and 64.54% for bighead carp AChE inhibition was higher in the brain of common and grass carps exposed to 1 and 2 mg L-1 of diafuran than in bighead carp. Changes in brain and muscle AChE activity observed in common, grass and bighead carps exposed to diafuran probably reflected in movement disturbances, with fish lethargic and immobile in the boxes, help to explain behaviour alterations induced by insecticides.

Erratic swimming, convulsions and lethargy were also observed in fathead minnows (*Pimephales promelas*) exposed to carbofuran (0.2 g L-1) (Health *et al.,* 1997) [22] and European eel (*Anguilla anguilla*) (Sancho *et al.*, 1997); Fernández-Vega *et al.,* 2002) [17, 23] after exposure to fenitrothion and thiobencarb, respectively, and silver catfish (*Rhamdia quelen*) fingerlings exposed to 10 mg L-1 clomazone for 96 h (Miron *et al.,* 2005) [24]. Cholinesterase inhibition in brain and muscle produce adverse effects in movement because the AChE participates in neuronal and neuromuscular transmissions (Fernández-Vega *et al.,* 2002) [23].

When inhibition occurs in AChE activity the neuro transmitter, acetylcholine is not hydrolysed in the nerve synapse and neuromuscular junction, causing an abnormal amount of Ach in these areas, which leads to an over activation of the brain and muscular tissues (Roex *et al.*, 2003; Candida Toni *et al*., 2011) [25, 26]. The mechanism of a toxic effect of diazinon, a thiophosphoric acid ester is the same as of other organophosphorus substances. There is an inhibition of a whole series of enzymes and mainly of Acetylcholinesterase (Mahboob *et al.,* 2011); Akhtar *et al.*, 2012) [27, 28]. Organophosphate compounds phosphorylate AChE and inhibit its activity causing accumulation of acetylcholine (Ach) at the nerve synapse, which leads to disruption of the central nervous system and eventually death of the animal. AChE activity decreased at both the concentrations in liver, kidney and gills of *Ctenopharyngodon idella* as compared to the control.

A significant inhibition in the activity of AChE was observed and it increased with the increase in the exposure to chlorpyrifos in the organs of the fish studied (R. Jindal and M. Kaur, 2014) [29]. Chronic exposure to low levels of pesticides might have more significant effects on fish populations than acute poisoning (Capkin and Altinok, 2013) [30]. Biochemical changes lead to metabolic disturbances, inhibition of enzymatic activities and retardation of growth of aquatic organisms (Dogan, 2006) [31]. However, disturbances in their catalytic properties as results of pesticide interactions can cause impairment in cellular homeostasis affecting other biochemical parameters and enzymatic activities, which can lead to adverse effects at higher levels of biological organizations such as tissues, organs or individuals (Orrego *et al.,* 2011) [32]. Incidentally, a great deal of attention has been paid to evaluate the hazardous effects of various pesticides on physiology of many non- target organisms.

Acid phosphatase activity (ACP)

The calculated values of acid phosphatase (ACP) activity along with controls and percent change over the control are given in table and graphically represented in figure. The acid phosphatase activity in the control fish is in the order of:

Controls: Kidney > Gill > Liver > Muscle > Brain

Under exposure to sub lethal and lethal concentration of profenofos 50%EC for 4days. The percent change in acid phosphatase activity is in the order of:

Sublethal 4days: Gill > Brain > Muscle > Liver > Kidney
Lethal 4days: Brain > Gill > Liver > Muscle > Kidney

In sub lethal exposure maximum percentage of elevation in ACP activity was (74.86%) in gill followed by brain (59.06), muscle (54.43), liver (44.07) and minimum elevation was (32.25%) in kidney. But in lethal exposure maximum percentage of elevation was (86.57%) in brain followed by gill (83.52), liver (69.62), muscle (68.98) and minimum percentage of elevation was (56.27%) in kidney.

The acid phosphatase (ACP) is a lysosomal enzyme and the raise in this activity probably released to the cellular damage. It is difficult, however, to relate the decrease in ACP activity with tissues damage. Increase in acid phosphatase activities can be interpreted as a shift of the tissues emphasis on energy breakdown pathway from normal specific pH and are usually termed phosphomonoesterases. Pesticide poisoning increases ACP activity in the fish (Abdul *et al.*, 2004); Carla *et al*., 2005) [33, 34]. Pesticides are reported to reduce glycogen levels and increase phosphorylase activities; the boost of phosphatases activity reveals the increase the transportation of metabolites through cellular membrane (Abdel *et al*., 2010) [35].

Table 2: Changes in the specific activity levels of Acid Phosphatase (ACP) (mg pi/gram protein/hr) in different tissues of the fish, *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC.

Tissues	Control	Profenofos 50% EC			
		Sublethal	% Change	Lethal	% Change
Gill	1.87± 0.0316	3.27± 0.044	74.86	5.38± 0.051	83.52
Liver	2.70± 0.058	3.89± 0.048	44.07	4.58± 0.054	69.62
Kidney	2.79± 0.048	3.69± 0.050	32.25	4.36± 0.032	56.27
Brain	1.49± 0.057	2.37± 0.048	59.06	2.78± 0.051	86.57
Muscle	1.58± 0.041	2.44± 0.047	54.43	2.67± 0.051	68.98

Values are the mean of five observations: Standard Deviation is indicated as (±). Values are significant at $p< 0.05$

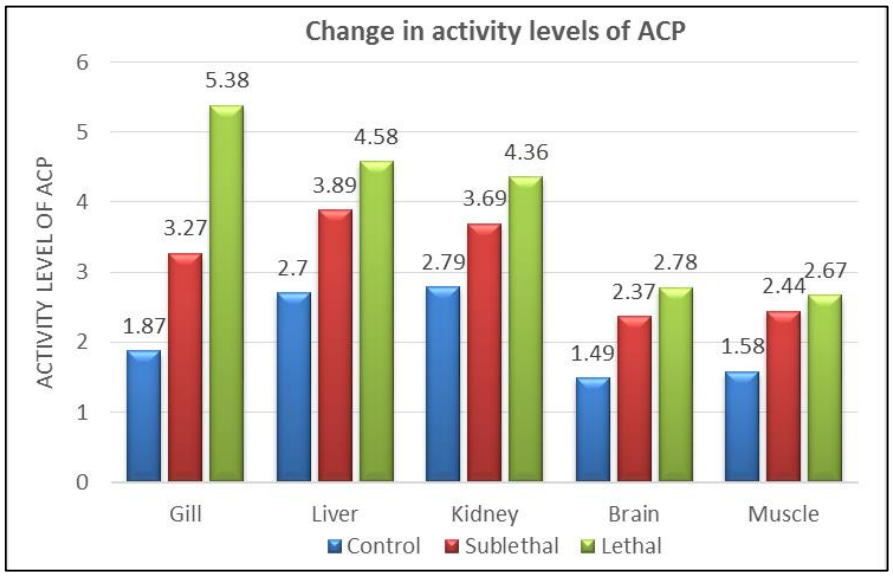

Fig 2: Changes in the specific activity levels of Acid Phosphatase (ACP) (mg pi/gram protein/hr) in different tissues of the fish, *Ctenopharyngodon idella* exposed to sub lethal and lethal concentrations of profenofos 50% EC.

Alterations in alkaline phosphatase and acid phosphatase (ACP) activities in tissues and serum have been reported in fish (B. Jyothi and G. Narayan, 2000) [36]. Borah *et al.*, 1996) [37] noticed significant decline in the activity of acid phosphatase of *Heteropneustes fossilis* on exposure to rogor. The decrease in acid phosphatase activity might be due to histopathological changes such as necrosis. It may be due to decrease in the rate of trans-phosphorylation. Dalela *et al.*, 1980) [38] are also on the opinion that uncoupling of oxidative phosphorylation has been the main reason for inhibition of acid phosphatase. The acid phosphatase activity in the liver and muscle tissues after exposure to the Malathion. They were showed significant decrease after exposing *Channa punctatus* to malathion for four days thus the activity of alkaline phosphatase was found to decrease in the experimental fish when compared with that of the control fish, Magar RS and Afsar Shaikh, 2013) [39].

4. Conclusion

In the present study, it is concluded that exposure to sub lethal and lethal concentrations of profenofos for a period of 4 days, adversely affected the AChE and ACP in different organs of freshwater fish, *Ctenopharyngodon idella* and these alterations might be attributed to increased autolysis in the tissues due to cytotoxicity.

In the present study, brain AChE is the most inhibited than all the tissues. This might be due to the pesticide activity on the brain. Since, the compound is neurotoxic hence the activity levels of AChE were inhibited. This study is in agreement with the earlier reports of Ray and Gosh, (2006) [40]; Wang *et al.*, 2009) [41]; R. Jindal and M. Kaur, 2014) [29]. The elevation in alkaline phosphatase suggests an increase in the lysosomal mobilization and cell necrosis due to pesticide toxicity. Elevation of ACP activity in brain was reported earlier in stress-exposed *Labeo rohita,* 2000b) [42]. Sub-acute studies with monocrotophos showed increased activities of ACP content in plasma, which are conventional indicators of liver injury (Jyothi and Narayan, 2000) [36].

But profenofos intoxication caused remarkable elevation in the activity levels of ACP in all the test tissues it is significant in contrast to the pyrethroid effect on acid phosphatase, the results were in agreement with the study of Patil V.N.*et al.*, 2014) [43]. The acid phosphatase (ACP) activity in the liver tissue of *Ctenopharyngodon idella* after profenofos exposure was found to be higher when compared to that in control fishes. Increase in acid phosphatase can be interpreted as a shift of the tissues emphasis on energy breakdown pathway from normal ATPase system to phosphatase system. In the present investigation it is found that, significant alterations in the enzyme contents in all the tissues as compared to control were observed (Santha Kumar *et al.*, 2000a; Patil V.N. *et al.*, 2014) [44, 43].

The parameter measured could be biomarker of toxicological effects of the pesticide on the fish and help in the diagnosis of the pollution and control on indiscriminate use of the pesticide is suggested. The results demonstrated that of organ phosphorus pesticides have the capacity to act based on the concentration of the toxicant. Thus, the effect of the increasing concentration of pesticide profenofos exhibited in increasing alterations in the biological, behavioural and biochemical aspects of the test species. This is consistent with a priori assumption of this concept, which is dose dependent to contribute to the overall effect on the test species *Ctenopharyngodon idella*.

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6. References

1. El-Shenawy NS, Al-Eisa RA, El- Salmy F, Salah O. Prophylactic effect of vitamin E against hepatotoxicity, nephrotoxicity, haematological induces and histopathology induced by diazinon insecticide in mice, Curr. Zool. 2009; 55(3):219-226.
2. Magar RS, Bias UE. Histopathological Impact of Malathion on the Ovary of the Fresh Water fish Channa punctatus. Int. Res. J Environment Sci. 2013; 2(3):59-61.
3. Diez MC. Biological aspects involved in the degradation of organic pollutants, journal of Plant Nutrition and Soil Science. 2010; 10:244-267.

4. Coutinho CFB, Tanimoto ST, Galli A, Garbellini GS, Takayama M, Amaral RB, *et al*. Pesticidas: mecanismo de ação, degradação e toxidez. Pesticidas: Revista de Ecotoxicologia e Meio Ambiente. 2005; 15:65-72.
5. Dutta HM, Arends DA. Effects of endosulfan on brain Acetylcholinesterase activity in juvenile bluegill sunfish. Environ. Res. 2003; 91:157-162.
6. Kopecka J, Rybakowas A, Pempkowiak J. A ChE levels in mussels and fish collected off Lithuania and Poland (southern Baltic). Ocenologia. 2004; 46(3):405-418.
7. Silman I, Sussman JL. Acetylcholinesterase: classical and non-classical functions and pharmacology. Curr. Opinion in Pharmacol. 2005; 5:293-302.
8. Ellman GL, Courtney FKD, Andres Jr. V, Featherstone RM. A new and rapid colorimetric determination of Acetylcholinesterase activity. Biochem. Pharmacol. 1961; 7:88-95.
9. Bodansky A. Determination of serum Phosphatase, Bur's Law and interfacing, 1932.
10. Banaee M. Adverse Effect of Insecticides on Various Aspects of Fish's Biology and Physiology, Insecticides - Basic and Other Applications, Dr. Sonia Soloneski (Ed.), In Tech, 2012, 101-126.
11. Vani T, Saharan N, Mukherjee SC, Rajastan Kumar R, Brahmchari RK. Deltramethrin induced alterations of Catla Catla (Ham.) and their amelioration by dietary supplyment of Vitamin C. pesticide Biochemistry and Physiology. 2011; 101(1):16-20.
12. Ernest Hodgson. A Textbook of Modern Toxicology, 4th Edition, A John Wiley &Sons, inc., Publication., USA, 2010. ISBN 978-0-470-46206-5,
13. Mirjana B Colovic, danijela Z Krstic, Tamara DLazarevic-Pasti, Aleksandra M Bondzic, Vesna M Varsic. Acetylcholinesterase inhibitors: Pharmacology and Toxicology, Curr Neuropharmacol. 2013; 11(3):315-335.
14. Jaqueline Ineu Golombieski, Enio Marchesan, Edinalvo Rabaioli Camargo, Joseânia Salbego, Joele Schmitt Baumart, Vania Lucia Loro, *et al*. Acetyl cholinesterase enzyme activity in carp brain and muscle afteracute exposure to diafura n, Animal Science and Pastures, Sci. agric. (Piracicaba, Braz.) vol.65 no.4, Print versio n ISSN 0103-9016 Online version ISSN 1678-992X, 2017.
15. Fisher SW. Changes in the toxicity of the three pesticides as a function of environmental pH and temperature. Bull. Environ. Contam. Toxicol. 1991; 46:197-202.
16. Richmonds RC, Dutta HM. Effect of Malathion on the brain Acetylcholinesterase activity of bluegill sunfish Lepomis Macrochirus. Bull. Environ. Contam. Toxicol. 1992; 49:431-435.
17. Sancho E, Ferrando MD, Andreu E. Response and recovery of brain acetylcholine sterase in the European eel, Anguilla Anguilla, exposed to feniltrothion. Ecotoxicology and Environmental Safety. 1997; 38:205-209.
18. Varó I, Navarro JC, Amat F, Guilhermino L. Effect of dicholorvos on cholinesterase activity of the European sea bass (*Dicentrarchus labrax*). Pesticide Biochemistry and Physiology. 2003; 75:61-72, 2003.
19. Saglio P, Trijasse S. Behavioural responses to atrazine and diuron in goldfish. Archives of Environmental Contamination and Toxicology. 1998; 35:484-491.
20. Bretaud S, Toutant JP, Saglio P. Effects of carbofuran, diuron, and nicosulfuron on Acetylcholinesterase activity in goldfish (Carassius auratus). Ecotoxicology and Environmental Safety. 2000; 47:117-124.
21. Jaqueline Ineu Golombieski, Enio Marchesan, Edinalvo Rabaioli Camargo, Joseânia Salbego, Joele Schmitt Baumart, Vania Lucia Loro, *et al*. Acetylcholinesterase enzyme activity in carp brain and muscle after acute exposure to diafuran, Scientia Agricola Print version ISSN 0103-9016On-line version Sci. agric. (Piracicaba, Braz.) Piracicaba. 2008; 65:4. ISSN 1678-992X,
22. Health AG, Cech JJ, Brink L, Moberg P, Zinkl JG. Physiological responses of fathead minnow larvae to rice pesticides. Ecotoxicology and Environmental Safety. 1997; 37:280-288.
23. Fernández-Vega C, Sancho E, Ferrando MD, Andreu E. Thiobencarb-induced changes in Acetylcholinesterase activity of the fish Anguilla anguilla. Pesticide Biochemistry and Physiology. 2002; 72:55-63.
24. Miron DS, Crestani M, Shettinger MR, Morsch VM, Baldisserotto B, Tierno MA. Effects of the herbicides Clomazone, Quinclorac, and Metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae). Ecotoxicology and Environmental Safety. 2005; 61:98-403.
25. Roex EWM, Keijzer R, Gestel CAM. Acetylcholinesterase inhibition and increased food consumption rate in the zebra fish, Danio rerio, after chronic exposure to parathion. Aquat. Toxicol. 2003; 64:451-460.
26. Candida Toni, Vania Lucia loro, Adriana santi, Charlene cavalheiro de Menezes, Roberta cattaneo, Barbara estevo clasen, *et al*. Exposure to tebuconazol in rice field and laboratory conditions induces oxidative stress in carp (*Cyprinus carpio*). Comparative Biochemistry and physiology, Part C. 2011; 153:128-132.
27. Mahboob S, Niazi F, Asi MR, Sultana S, AlGhanem KA. Det ermination of organochlorine and nitrogen containing pestcide residues in Labeo rohita. Toxicol. Environ. Chem. 2011; 93:1851-1855.
28. Akhtar M, Mahboob S, Sultana S, Tayyaba S. Assessment of Pesticide Residues in Sediments Collected from River Ravi and its Tributaries between its stretches from Shahdara to Balloki Headworks, Pakistan. J Biol. Agri. Heal. 2012; 2:127-133.
29. Jindal R, Kaur M. Acetylcholinesterase Inhibition and assessment of its recovery response in some organs of Ctenopharyngodon idellus induced by chlorpyrifos, International Journal of Science, Environment and Technology. 2014; 3(2):473-480.
30. Capkin E, Altinok I. Effects of chronic carbosulfan exposure on liver antioxidant enzymeactivities in rainbow trout. *Environ* Toxicol Pharmacol. 2013; 36(1):80-87.
31. Dogan S. The *in vitro* effects of some pesticides on carbonic anhydrase activity of *Oncorhychus mykiss and Cyprinus carpio* fish. J Hazard Matter A. 2006; 132:171-176.
32. Orrego Rodrigo, Zacharias Pandelides, John Guchardi, Douglas Holdways. Effects of pulp and paper mill effluent extracts on liver anaerobic and aerobic metabolic enzymes in rainbow throut. Ecotoxicology and environmental Safety. 2011; 74:761-768.
33. Abdul Naveed P, Venkateswarlu, Janaiah C. The action of sublethal concentration of endosulfan and kelthan on regulation of protein metabolism in the *Clarias*

*baturach*us. J. Nat. Environ. Pollut. Tech. 2004; 3(4):539-544.
34. Carla-Fenoglio, Eleonora Boncompagni, Mauro Fasola, Carlo Gandini Sergio, Comizzoli, Gloria, *et al*. Effect of environmental pollution on the liver parenchyma cells and kupffer melanomacrophagic cell of the frog *Rana esculenta*. J Econ. toxic environ. Saf. 2005; 60(3):259-268.
35. Abdel-Salam Mohamed Ibraik Ohaida, Nuha Mahmoud, Akrawee A. The effects of exposure to organophosphorus compounds on some aspects of carbohydrate metabolism in carbohydrate metabolism in Tilapia zillii. The Egyptia Journal of Hospital Medicine. 2010; 39:223-228.
36. Jyothi B, Narayan G. Pesticide induced alterations of non-protein nitrogenous constituents in the serum of a freshwater catfish, *Clarias batrachus* (Linn.). Indian J Exp. Biol. 2000; 38:1058-1061.
37. Borah Sabita, Yadav RNS. Effect of rogor on the activity of lactate dehydrogenase, acid and alkaline phosphatase in muscle and gill of a fresh water fish *Heteropneustes fossilis.* J Environ. Bipl. 1996; 17(4):279-283.
38. Dalela RC, Rani S, Verma SR. Physiological stress induced by sublethal concentrations of phenol and pentachlorophenol in Natopterus notopterus. Environ. Pollu, 1980, 213.
39. Magar RS, Afsar Shaikh. Effect of Malathion on acid phosphatase activity of fresh water fish *Channa punctatus*, International journal of pharmaceutical, chemical and biological sciences. 2013; 3(3):720-722.
40. Ray AK, Ghosh MC. Aquatic toxicity of carbamates and organophosphates. In: Gupta, R. (Ed.), Toxicology of organophosphate and carbamate compounds. Section VII. Aquatic life & wildlife Academic Press, USA. 2006; 45:657-672.
41. Wang C, Lu Gui J, Wang P. Sublethal effect of pesticide mixtures on selected biomarkers of Carassius suratus. Environ. toxicol. Pharmacol. 2009; 28(3):414-419.
42. Das BK, Mukherjee SC. Chronic toxic effects of quinolphos on some biochemical parameters in *Labeo rohita* (Hamilton), Toxicol. Lett. 2000b; 114(1-3):11-8.
43. Patil VN, Kadam GH, Raut PD. Enzymological alterations produced by chronic malathion exposure in freshwater crab, paratelphusa (barytelphusa) jacquemontii, International Journal of Science, Environment and Technology. 2000b; 3(1):303-313.
44. Santha kumar M. Balaji M, Ramudu K. Effect of monocrotophos on plasma phosphatase activity of a fresh water fish, Anabastestudineus (Bloch). Poll. Res. 2000a; 19(2):257-259.