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Acute Effects of the Chlorpyrifos Pesticide on Common Carp (*Cyprinus carpio* L., 1758)

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Abstract
In present study, an attempt has been made to investigate the effect of Chlorpyrifos insecticide on carp *Cyprinus carpio* L. through acute toxicity bioassay. The LC_{50} were estimated as 1897, 1266, 985 and 792 µg/L during 24 , 48, 72 and 96 hr. respectively by using the concentrations 500 , 1000, 1500 , 2000, 2500 and 3000 µg/L indicating high sensitivity of fish to chlorpyrifos. The behavioral response observed in the fish were erratic swimming, loss of reflex, hyperactivities, decrease in appetite secretion of mucus, increase movement of operculum and hyperventilation . These effects increased with increasing concentration of the chlorpyrifos and duration of exposure.

Keywords: *Cyprinus carpio*, LC_{50}, Chlorpyrifos, Bioindicator

التأثيرات الحادة لمبيد الكلوربايروفوس على اسماك الكارب الاعتيادي (*Cyprinus carpio* L., 1758)

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الخلاصه
في لدراسة الحاليه، تم إجراء محاولة لدراسة تأثير المبيد الحشري الكلوربايروفوس على اسماك الكارب الاعتيادي *Cyprinus carpio* من خلال دراسة التاثيرات السميه الحاده لهذا المبيد. قدرت التراكيز النصف القاتله لمبيد الكلوروبايروفوس بقيم بلغت 1897و 1266و 985 و792 مايكروغرام / لتر خلال 24 و 48 و 72 و 96 ساعة على التوالي باستخدام التراكيز 500 و 1000 و 1500و 2000و 2500 و 3000 مايكروغرام / لتر وتكشف هذه النتائج الحساسيه العاليه لاسماك الكارب اتجاه مبيد الكلوروبايروفوس. لوحظت في الأسماك المختبره بعض الاستجابات السلوكية مثل السباحة غير المنتظمة وفقدان رد الفعل، مع فقدان الشهيه للغذاء وفرط التنفس وزياده حركه الغطاء الغلصمي مع زياده في افراز الماده المخاطيه وقد زادت هذه التأثيرات مع زيادة تركيز المبيد ومدة التعرض.

Introduction

The widespread use of pesticides in modern agriculture is of increasing concern due to environmental pollution and subsequent biodiversity loss [1]. Pesticide pollution resulting from agricultural practices, and the consequential negative effects on non target species, is a problem characteristic of various types of farming activities in most parts of the world [2, 3]. The indiscriminate use of synthetic pesticides by the human activities is one of the main obstacles and cause high risk to non-target organisms [4]. Due to the high sensitivity of the aquatic life towards dissolving toxicant, the fish has been utilized as the biological measurement (Biomarker) to indicate the existence of toxicant exposure and/or the impact towards the evaluation of molecular, cellular to

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physiological level [5]. Fish are often used as sentinels because they play a number of roles in the trophic web, bioaccumulate toxic substances, and respond to low concentrations of mutagens [6, 7]. Variable concentrations of pesticides in aquatic environments cause structural and functional changes in aquatic organisms and this is more common than mortality, especially when the doses of pesticides that are not high enough to kill fish [8]. The aim of this study to determine (LC_{50}) and behavioral effects of chlorpyrifos on common carp (*Cyprinus carpio* L.) under constant exposure conditions.

Materials and methods

Physical and Chemical Characteristics

Hydrogen Ion Concentration (pH), Electrical Conductivity (EC) and Total Dissolved Solid (TDS) data were obtained at the time and location where fish were sampled, and in aquaria using a portable pH/ EC/ TDS meters. The results were expressed as microsemins/cm (µS/cm) for Electrical Conductivity (EC) and mg/L for TDS. While temperature was measured by using thermometer with a scale of (0-100) ^{0}C. The dissolved Oxygen is estimated through Lovibond meter.

Acclimatization and experimental site of *Cyprinus carpio* L.

The experiments was conducted at the College of Science, University of Baghdad, Environmental and Pollution Laboratory, Fish used in the present study were common carp *C. carpio* (range of length 14 -16 cm, range of weight 60 -80 gm) . During that, Fish were fed once daily with commercial fish food. For Acclimatization, 12 days in the glass aquaria before the commencement of the experiment, measuring (60X40X50) cm. During the acclimatization period, also observations of the aquaria were conducted daily to get rid of wounded, diseased and mortality

Acute Toxicity

Acute toxicity test of *C. carpio* was performed according to the [9] and carried out for a period of 96 hours. Jawadekar [10] defined the median lethal concentration (LC_{50}) by the toxicant that will kill 50 percent of test organism within a designated period of time. Acute toxicity test of *C. carpio* was performed and carried out for a period of 96 hours. Fishes were divided into seven groups of eight fishes in each group. They were kept deprived of feed and without aeration during the acute toxicity study period of 96-hr, in 40L-water containing glass flasks. The exposed fish samples were divided into two subgroups, where the first group was acting as a control and the other was subjected to toxicity test with three replicates each glass aquaria. The determined probit analysis methods [11]. The concentration that used in acute toxicity test of common carp are 500,1000,1500,2000,2500 and 3000 µg/L.

Behavioral changes

Change in behavior is considered to be a sensitive indicator to detect pollution due to pesticides. The effects of chlorpyrifos on aquatic systems are well documented. So any behavioral response must considered [12]. Fish behavior represents the fish physiological response towards the environmental factor. Moreover, the interaction of fish behavior related to the ecology can be easily observed even if it can quantify [13]. Observation of abnormal behavioral alternations of treated fish symptoms such as stress, movement, respiration, swimming, loss of appetite responses to the outer effects by comparison with normal control group (untreated fish) by many aspects of activity of fish and balancing of whole body from first exposure to chlorpyrifos.

Results and discussion

Physical and chemical characteristics of water in aquaria

Some physical and chemical properties were measured in the laboratory which gives a confirmation of the suitability of using of water in rearing the animals such as Temperature, pH, DO and EC. Table-1 shows the values of these parameters.

Table 1- Some physical and chemical properties of water for the experiments

Parameters	Range	Mean ± SD	Optimum values for aquatic life
Temperature (°C)	20-27	22.7± 3.4	10 -32
Dissolved oxygen (mg/l)	6.8-7.7	7.2 ± 0.30	> 5
pH	6.8-7.6	7.2±0.26	6.5-9
Electrical conductivity (µS/cm)	900-1210	1096±170	< 1800

Water temperature in the laboratory for glass aquaria varied between 20 – 27°C. Shamaaon and Alhabeb [14] noted that optimal temperature for carp from 23-28°C. Martinez-Jeronimo [15] emphasized the importance of temperature control and its modifying role to the toxicity studies results. The metabolic rate of fish is closely correlated to the water temperature: the higher the water temperature (i.e. the closer to the optimum values within the normal range), the greater the metabolism [16]. On the other hand, pH (hydrogen ion) is generally regarded as major factors controlling toxicity test to aquatic organisms [17]. The experimental pH range was 6.8 – 7.7; compared with the ideal values ranging between 6.5-8.5 [18]. There are variations in tolerance of fish, alkaline pH values above 9.2 and acidity below 4.8 can damage and kill salmonids (e.g. brown and rainbow trout); and pH values above 10.8 and below 5.0 may be rapidly fatal to cyprinids (especially carp). Thus salmonids, in comparison with cyprinids, are more vulnerable to high pH and more resistant to low pH [16]. Regarding dissolved oxygen, in the present study different fish species has different requirements for the concentration of oxygen dissolved in water. The values varied between 6.8-7.7 mg/l which close to the optimal values for dissolved oxygen is 7.5 mg/L [18]. Cyprinids are less demanding; they can thrive in water containing 6–8 mg/L and show signs of suffocation only, when the oxygen concentration falls to 1.5–2.0 mg/L [16]. About electrical conductivity: Consideration must be given to potential changes in bioavailability and toxicity of contaminated materials due to changes in ions strength of exposure water [19]. Overall, the range of the water temperature and other environmental parameters which recorded during this study was within the tolerable limits of living carp.

Acute tests

Median Lethal Concentration (LC_{50})

In the acute toxicity test in which six concentrations were used to obtain LC_{50} and use the log of concentration, the Probit unit in special tables through 24, 48, 72 and 96 hr. The obtained results of the present study indicate that the chlorpyifos varied in their acute toxicity to *C.carpio* with recorded values for median lethal concentrations 1897, 1266, 985 and 792 µg/L respectively with increased of exposure 24 ,48,72 and 96 hr. respectively by using the concentrations 500 ,1000,1500 , 2000, 2500 and 3000 µg/L and the results presented in Tables-2,-3,-4and -5 respectively.

Halappa [20] indicated that the LC_{50} of chlorpyrifos on commen carp founded to be 0.160 mg/L with impaired behavioural responses and morphological deformities were observed even under recovery periods. But Ramesh and Saravanan [21] found the acute toxicity (LC_{50}) of chlorpyrifos by static renewal (semi-static) bioassay test, and they found that, the LC50 was 5.28 ppm. Also, Ahmad [22] found that the 96h LC_{50} value of malathion concluded was 8.22 mg/l. In other studies the 96 h. LC50 values of chlorpyrifos in juvenile and adult of *Oreochromis niloticus* were determined to be 98.67 and 154.01 µg / l, respectively, and in mosquito fish was 297 µg /l. [23,24] Mahboob *et al.*[25] found that the organophosphates (OCP'S) and carbamates can cause pollution in freshwater ecosystem, as well as having a significant effect on the health of fish.

Table 2- LC_{50} (24 hr.) of chlorpyrifos for *C. carpio*

Concentrations µg/l	Log Concentration	Mortality %	Probits	LC_{50} (µg/l)
500	2.60	0	0	1897
1000	3.00	14	3.92	
1500	3.17	28	4.42	
2000	3.30	42	4.8	
2500	3.39	28	4.42	
3000	3.47	99	7.33	

Table 3- LC_{50} (48 hr.) of chlorpyrifos for *C. carpio*

Concentrations µg/l	Log Concentration	Mortality %	Probits	LC_{50} (µg/l)
500	2.60	14	3.92	1266
1000	3.00	42	4.8	
1500	3.17	57	5.18	
2000	3.30	42	5.18	
2500	3.39	71	5.5	
3000	3.47	99	7.33	

Table 4- LC50 (72 hr.) of chlorpyrifos for *C. carpio*

Concentrations µg/l	Log Concentration	Mortality %	Probits	LC_{50} (µg/l)
500	2.60	14	3.92	985
1000	3.00	42	4.8	
1500	3.17	57	5.18	
2000	3.30	57	5.18	
2500	3.39	99	7.33	
3000	3.47	99	7.33	

Table 5- LC_{50} (96 hr.) of chlorpyrifos for *C. carpio*

Concentrations µg/l	Log Concentration	Mortality %	Probits	LC_{50} (µg/l)
500	2.60	28	4.2	792
1000	3.00	57	5.18	
1500	3.17	71	5.55	
2000	3.30	85	6.04	
2500	3.39	99	7.33	
3000	3.47	99	7.33	

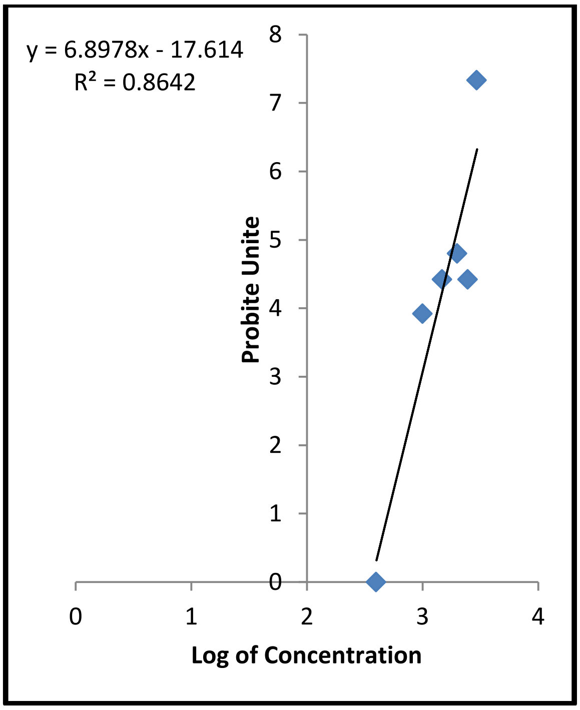

Figure 1- Toxicity curve of the chlorpyrifos after 24hr. of exposure for *C. carpio*

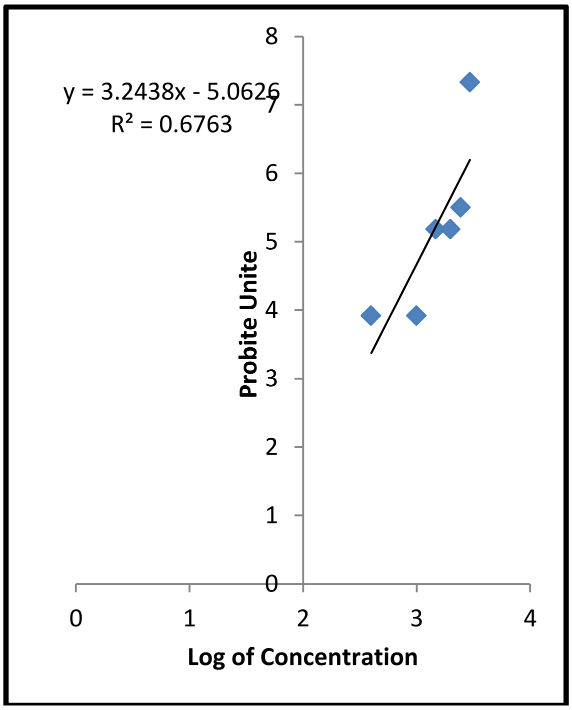

Figure 2- Toxicity curve of the chlorpyrifos after 48hr. of exposure for *C. carpio*

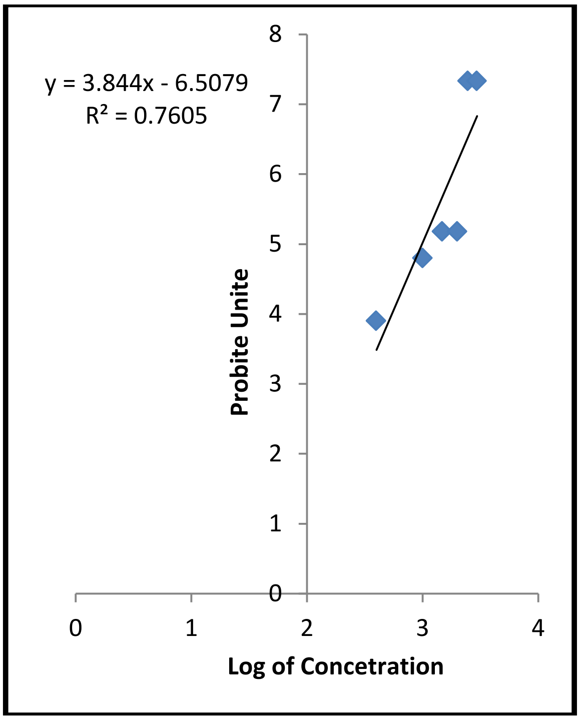

Figure 3- Toxicity curve of the chlorpyrifos after 72hr. of exposure for *C. carpio*

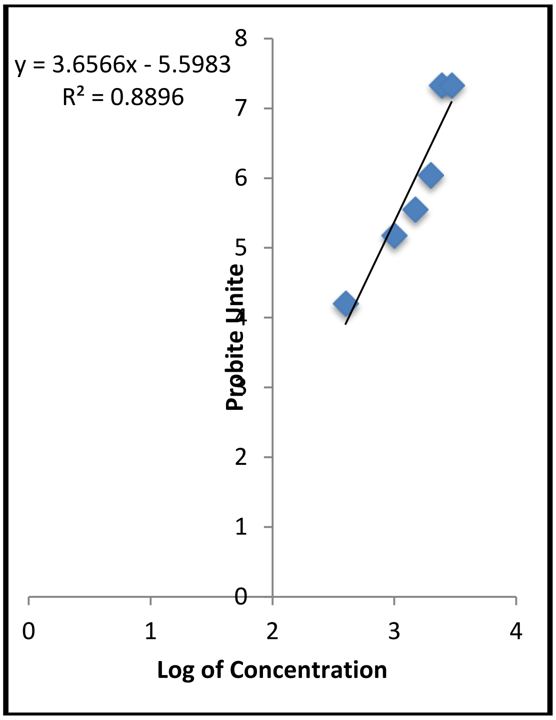

Figure 4- Toxicity curve of the chlorpyrifos after 96hr. of exposure for *C. carpio*

In present study all behavioral responses presented in Table-6. In experiment control show no change in the behavior and the swimming patterns and no mortality for this group and there was activity with their well-synchronized movements through the whole experimental period.

In 500µg/l, they were found in well coordinated manner and were alert to the slightest disturbances.

In 1000 µg/l there was jumping and sudden startling with one fish mortality when the herbicide was first added. The fish exhibited respiratory distress (such as gasping in air), loss of balance and erratic swimming prior to death.

In 1500µg/l showed abnormal swimming behavior increased and the fish were observed to hyperventilation. Fishes were initially surfaced, followed by vigorous and erratic swimming showing agitation. Quick opercular and fin movements were observed initially and gradually became feeble and often showed gulping of air.

In 2000µg/l jumping behavior increased and ascending of fish to the surface was increased, opercular opening became wider and exhibited respiratory distress. As the period of exposure increased, fishes were found to settle down to bottom and towards the final phase of exposure, fishes showed barrel-rolling indicating loss of equilibrium. Swimming with belly upwards and gradually became lethargic. 2500µg/l fish tried to breathe air from the surface more frequently begin to secrete mucus suggested to avoid contact to toxicant, and finally sluggishness was observed.

In the last concentration (3000µg/l) sudden jumping, loss of balance and swimming disorders, the fish were exhibited peculiar behavior that is the fish were trying to leap out from the test chamber which can be viewed as escape phenomenon.

These symptoms may be due to inhibition of acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine (ACh) in cholinergic synapses ensuing hyperstimulation. Since, inhibition of AChE activity is a typical characteristic of organophosphate compounds [26] . The behavioral and the swimming patterns of the fish exposed to different insecticides include changes in swimming behavior, feeding activities, predation, competition, reproduction and species social interactions such as aggression [27,28]. Bananee *et al.* [29,30] ,reported similar behavioral responses in common carp and rainbow trout exposed to sub- lethal levels of diazinon. In fact, most insecticides influence the behavior patterns of fish by interfering with the nervous systems and sensory receptors [31,32] and this incident may impair the identification of situations and development of appropriate response by the fish exposed to insecticides. The effect of certain insecticides on the activity of acetylcholinestrase may lead to a decreased mobility in fish [33].

Most insecticides affect the behavioral patterns of fish by interfering with the nervous systems and sensory receptors and consequently it can lead to disorders in the fish response to environmental stimuli [34]. The inhibitory effects of organophosphate insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation in relation to the behaviour and age [35,36] and is widely used for rapid detection to predict early warning of pesticide toxicity [37].

Table 6- The behavioral changes in common carp *C. carpio* for chlorpyrifos

Behavior	Concentrations µg/l						
	Control	500	1000	1500	2000	2500	3000
Sluggishness	-	-	-	-	+	+	++
Hyperventilation	-	-	-	+	+	+	++
Aquiline and darting swimming	-	-	-	+	+	+	++
Loss of reflex	-	-	-	+	+	+	++
Sudden jumping	-	+	-	-	+	++	++
Emotion nervous	-	-	+	+	+	+	++
Increase of operculum	-	-	+	+	+	++	++
loss of co-ordination	-	-	-	-	-	+	+
hyper excited fish	-	-	+	+	+	++	++
Secretion of mucus	-	-	-	+	+	++	++

(-) = none, (+) =mild response, (++) = severe response

References

1. Booth, L.H., Heppeithwaite,V. and Eason, C.T.**1998**. Cholinesterase and glutathione Stransferase in the earthworm *Aporrectodea caliginosa* as biomarkers of organophosphate exposure. Proceedings of the 51st New Zealand Plant Protection Conference, pp:138-142.
2. Sancho, E., Ceron, J.J. and Ferrando, M.D. **2000**. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla anguilla*. *Ecotoxicol Environ Saf.*, 46, pp: 81-86.
3. Rendon–Von Osten, J., Ortiz-Arana, A., Guilhermino, L.and Soares A. **2005**. In vivo evaluation of three biomarkers in the mosquitofish (*Gambusia yucatana*) exposed to pesticides. *Chemosphere*, 58, pp: 627-636.
4. Kumar, A., Prasad, M.R., Srivastava, K., Tripathi, S. and Srivastava. A.K. **2010**. Branchial Histopathological Study of catfish *Heteropneustes fossilis* following exposure to purified Neem Extract, Azadirachtin. *World Journal of Zoology*, 5(4), pp: 239-243.
5. Sabullah, M. K., Ahmad, S. A.,Shukor, M. Y., Gansau, A. J., Syed, M. A., Sulaiman, M. R. and Shamaan.N.A.**2015**. Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. *International Food Research Journal*, 22(2), pp: 435-454
6. Al-Sabti, K. and Metcalfe, C.D. **1995**. Fish micronuclei for assessing genotoxicity in water. *Mutat. Res*., 343, pp: 121- 135.
7. Stegeman, J.J. **2000**. Cytochrome P450 gene diversity and function in marine animals: past, present, and future. *Mar. Environ. Res.*, 50, pp:61–62.
8. Kegley, S., Neumeister, L. and Martin, T. **1999**. Ecological Impacts of Pesticides in California. Pesticide Action Network, California, USA. p:99.
9. Organization for Economic Co-Operation and Development (OECD). **1993**. *OECD Guidelines for Testing of Chemicals, Test No. 203: Fish Acute Toxicity Test*. OECD, Paris, France
10. Jawadekar ,N. **2009**. *Dictionary of Environment Science* .SBS Publishers and Distributors Pvt .Ltd. New DelhiFinney, D. J. **1971**. *Probit Analysis.* Third Edition, Cambridge University, Press, London. p:333.
11. Finney, D. J. **1971**. *Probit Analysis*. 3rd Edition, Cambridge University, Press, London.
12. Fountain, M. T., Brown, V. K., Gange, A. C., Symondson, W. O. C., and Murray, P. J. **2007**. The effects of the insecticide chlorpyrifos on spider and Collembola communities. *Pedobiologia*, 51(2), pp:147–158.

13. Scott, G. R. and Sloman, K. A. **2004**. The effects of environmental pollutants on complexfish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology*, 68, pp: 369-392.

14. Shamaaon, A. R. and Alhabeb, F. M. K. **1987**. Applications of quality control in fish Agriculturist. Offset press, Al-Basrah: p:47.

15. Martinez-Jeronimo, F. **2006**. Effect of culture conditions and mother's age on sensitivity of Daphnia *magna Straus* 1820 (Cladocera) neonates to hexavalent chromium. *Ecotoxicol*., 15, pp: 259-266.

16. Svobodová, Z.., Lloyd, A., Machova, J. and Vykusova, B. **1993**. Water quality and fish health. Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy.

17. Heijerick , D. G., De Shamphelaere , K.A.C. and Janssen , C.R. **2002**. Predicting acute zinc toxicity for *Daphnia magna* as a function of key water chemistry characteristics: development and validation of a Biotic Ligand Model . *Environ. Toxicol.Chem*., 21, pp: 1309-1315.

18. American Public Health Association (APHA). **2005**. Standard method for examination water and wastewater. American Public Health Association, Washington D.C.

19. Nebeker, A.V. and Schuytema , G.S.**1998**.Chronic effects of herbicide diuron on fresh Water cladocerans , Amphipods ,Midges , Minnows , and Snails . *Arch. Environ.Toxico*l.,35, pp:441-446.

20. Halappa, R., and David, M. (**2009**). Behavioural responses of the freshwater Fish , *Cyprinus carpio* (Linnaeus) following sublethal exposure to Chlorpyrifos, *Turk. J. Fish. Aquat. Sci*, 9, pp:233–238.

21. Ramesh, M. and Saravanan, M.**2008**. Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *Int J Integr Biol*, 3(1), pp: 80–83.

22. Ahmad Z. **2012**. Toxicity Bioassay and Effects of Sub-Lethal Exposure of Malathion on Biochemical Composition and Haematological Parameters of *Clarias gariepinus*. *African Journal of Biotechnology*, 11(34), pp: 8578-8585.

23. Kavitha, P.and Venkateswara Rao, J. **2008**. Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis* . *Environmental Toxicology Pharmacology*, 26, pp: 192-198.

24. Oruç, E.Ö. **2010**. Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. *Pestic. Biochem. Physiol.*, 96, pp: 160–166.

25. Mahboob,G.S., Al-Ghanim, K. A. , Sultana,S., Alkahem Al- Balawi, H. F. Sultana,T. and Al-Misned F. (**2014**). Acute toxicity II : effect of organophosphates and carbmates to *Catla catla* fingerlings. *The Journal of Animal & Plant Sciences*, 24(6), pp: 1795–1801.

26. Timchalk, C., Nolan, R.J., Mendrala, A.L., Dittenber, D.A., Brzak, K.A. and Mattsson, J.L. **2002**. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. *Toxicological Sciences*, 66, pp: 34-53.

27. Cong, N. V, Phuong, N. T, and Bayley, M. **2008**. Brain cholinesterase response in the snakehead fish (Channa striata) after field exposure to diazinon. *Ecotoxicology and Environmental Safety* , 71, pp: 314-318.

28. Cong, N. V, Phuong, N. T. and Bayley, M. **2009**. Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (Channa striata). *Ecotoxicology and Environmental Safety* , 72, pp:699-703.

29. Bananee M., Mirvaghefi A.R., Rafei G.R. and Majazi Amiri B. **2008**. Effects of sub-lethal diazinon concentrations on blood plasma biochemistry of common carp. *Int. J. Environ. Res.*, 2, pp: 189-198.

30. Banaee M., Sureda A., Mirvaghefi A.R. and Ahmadi K. **2011**. Effects of diazinon on biochemical parameters of blood in Rainbow trout (*Oncorhynchus mykiss*). *Pesticide Biochemistry and Physiology*, 99(1), pp: 1–6.

31. Keizer, G., D’agostino, R., Nagel, T., Volpe, P. and Gnemi, L. Vittozzi. **1995**.Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species. *Sci. Total Environ.* ,171, pp:213–220
32. Pan, G. and Dutta, H.M.(**1998**). The inhibition of brain acetylcholinesterase activity of juvenile largemouth bass Micropterus salmoides by sublethal concentrations of diazinon. *Environ. Res.* 79, pp:133–137.
33. Bretaud, S., Touttnt, J.P. and Saglio, P. **2000**. Effects of carbofuran, diuron and nicrosulfon on acetylcholinesterase activity in goldfish *(Carrasius auratus*). *Ecotoxicol Environ Saf.*, 47, pp: 117-124.
34. Banaee, M. **2012**. Adverse Effect of insecticides on various aspects of fish’s biology and physiology. Insecticides - basic and other applications. *Intechopen*, pp: 102-126.
35. Richmonds, C.R. and Dutta, H.M.**1992**. Effect of malathion on the brain acetyl- cholinesterase activity of bluegill sunfish *Lepomis macrochirus*. *Bull. Environ. Contam. Toxicol*., 49, pp: 431–435.
36. Dutta, H.M.,Munshi,J.S.D., Dutta, G.R.,,Singh, N.K.,Adhikari and S., Richmonds,C.R.**1995**. Age related differences in the inhibition of brain acetylcholinesterase activity of *Heteropneustes fossillis* (Bloch) by malathion. *Comp. Biochem. Physiol.,* 111, pp: 331–334.
37. Dutta, H.M. and Arends, D.A. **2003**. Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish. *Environ. Res.*, 91, pp: 157–162.