International Journal of Fisheries and Aquatic Studies 2016; 4(6): 388-393

ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(6): 388-393

www.fisheriesjournal.com
Received: 21-09-2016
Accepted: 22-10-2016

Abha Misha
Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, India

SnehaVerma
Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, India

Correspondence
Abha Misha
Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow-226025, India

Acute toxicity bioassay of organophosphorus pesticide, chlorpyrifos on freshwater catfish, *Heteropneustes fossilis* (Bloch, 1794)

Abha Misha, SnehaVerma

Abstract
Chlorpyrifos (CPF) is common pesticide. It reaches to water bodies though surface runoff and affecting the aquatic biota. The objective of the present study was to observe seasonal variation in acute toxicity of CPF with behavioural and morphological changes in freshwater catfish, *Heteropneustes fossilis*. This study was conducted by exposing the adult fish to different concentrations of chlorpyrifos (0.002-2.28 m.M.l^{-1}) for 96 h along with control groups in resting (December), preparatory (February), and prespawning (April) phase. The data were subjected to get LC_{50} and safe concentration in respective season. Susceptibility of catfish, *H. fossilis* to lethal effects of CPF was found to be both duration and concentration dependent. The LC_{50} values for CPF were found to be minimum during pre-spawning phase and maximum in resting season. Their tolerance to CPF was increasing with inactiveness of gonadal activity (resting season). At higher concentration of CPF, fish showed alterations in morphological and behavioural responses, especially erratic and jerky swimming, frequent surfacing and in gulping, mucus secretion, an increase in opercular movement and copious secretion of mucus all over the body. As per results, different reproductive phase fish responded different to toxicant concentrations.

Keywords: Acute toxicity, chlorpyrifos, LC_{50}, mortality, *Heteropneustes fossilis*, CPF

1. Introduction
The organophosphorus pesticide, chlorpyrifos (CPF) is a widely used and most preferred broad spectrum chlorinated organophosphate pesticide. It is frequently used on crops and in the warehouses, for the eradication of a wide range of insect-pests. This reaches to the aquatic ecosystem though rain and affecting aquatic organism [1-3]. The pesticides enter the food chain and their subsequent bioaccumulation and biotransformation at different trophic levels have disastrous effect to the ecosystem [4].
Aquatic contamination of pesticides causes acute and chonic poisoning in fish and other organisms directly or indirectly via food chain [5-7]. The responses of fish towards the toxic chemicals are broad-ranged depending on the toxicant, exposure duration, water quality and the species [8-10]. Toxicity tests are an important component in assessing the impact of chemicals on aquatic ecosystems because they indicate toxic effects of chemicals in organism by manipulating their morphology and behaviour at first and survival rate at last [11,12]. It gives first-hand information about the effects of such pesticides on organisms and the ecosystem as a whole. These tests are valuable in creating awareness regarding potential harmful effects of pesticide in the environment [13, 14]. In addition to mortality test, study of behavioral markers to assess the toxicant affects are the most promising and sensitive indicator of ecotoxicology [15-17]. The behaviour study is becoming prominent in toxicity assessment in a unicellular organism [18], insects [19, 20], fish [21, 22], and rodents [23]. Different studies reported that fish exposed to a wide range of pesticides exhibited abnormal behavioural and morphological alterations [24]. CPF directly inhibits acetyl cholinesterase enzyme activity in fishes and invertebrates [22, 25, 26] which may lead to decreased mobility of fish [24] therefore, it works primarily as nerve poison which is reflected in uncoordinated abnormal behaviour of fish after the toxicant application [25].
Various studies were reported on the toxicity of different organophosphate pesticides on fish [28-32]. In the present study, we have made an attempt to determine level of acute toxicity of chlorpyrifos with special emphasis on behavioural and morphological responses on a

freshwater catfish, *Heteropneustes fossilis* in resting, preparatory and pre-spawning phases of annual reproductive cycle.

2. Materials and Methods

2.1 Chemical

Hilban® (20% EC CPF; chlorpyrifos, Hindustan insecticide limited) were purchased from local suppliers. All other chemicals and reagents used were of analytical grade and were purchased locally.

2.2 Animal collection and their acclimatization

An experiment was performed in accordance with local/national guidelines of ethical committee for experimentation in animals.

The fresh water *H. fossilis* of relatively same size and weight were collected fromcommercial fisherman of Lucknow, Uttar Pradesh, India in resting (December), preparatory (February), and pre-spawning (April) phase of annual reproductive cycle. After formal quarantine treatment, fish were acclimatized in laboratory conditions under normal photoperiod and temperature for two weeks. Water was changes daily to remove faecal matter and waste metabolite of fish during acclimatization. During this period, fish were fed regularly with commercial fish food pellets and goat liver.

2.3 Seasonal acute toxicity bioassay

For toxicity bioassay, the concentrations of the test compound used were selected on the basis of our trial experiments that caused 0-100% mortality in respective reproductive phase. In each season, fish were exposed in a batch of ten to concentrations (0.002 to 2.28 m.M.l^{-l}) of Hilban® (20% EC CPF) with five replicates for each test concentrations along with a control group of same number of fish and replicates. Mortality data was recorded after 24, 48, 72 and 96 h to get LC_{50} of respective intervals. All solutions (control and test) were renewed daily and dead fish were immediately removed. Fish were not given food during the experiment.

The CPF effects on morphological, behavioural parameters were recorded during pre-spawning phase only to avoid repetition of observations in different reproductive phases. Control and CPF treated group fish were consistently monitored in intervals to get frequency. Their data were recorded as suggested by Gupta and Dua [33].

2.4 Data analysis

The mortality of the fish was recorded every 24 to 96 h study period. Significance level was checked by one way ANOVA among different annual reproductive phases. The $LC_{50}$with 95% confidence limits of 24, 48, 72 and 96 h was estimated by probit analysis [34] with the IBM SPSS (version 20) software. The safe concentration of CPF was also calculated [35]. The frequency of occurrence of different behaviour and morphological parameters during first hour of treatment in pre-spawning phase of annual reproductive cycle was recorded for group of exposed fish.

3. Results

3.1 Lethal toxicity test

Exposure to *H. fossilis* with different concentration of CPF showed varied degree of mortality in different reproductive season with a wide range of concentration. The percentage of mortality was significantly increased with the increase in concentration of the toxicant as well as duration of the experiment (F=52.27 in pre-spawning phase, F=134.24 in the preparatory phase, F=211.35 in resting phase; $p<0.05$; data not shown). On the basis of mortality, LC_{50} value was found higher in resting phase (1.547, 0.678, 0.299 and 0.174 m.M.l^{-l}), moderate in preparatory phase (0.332, 0.193, 0.152 and 0.123m.M.l^{-l}), whereas minimum in pre-spawning phase (0.296, 0.107, 0.044 and 0.026m.M.l^{-l}) for 24, 48, 72, and 96 h, respectively (Table 1). With the increase in duration of exposure the difference between lower and upper confidence limits of LC_{50} showed not much difference as in short duration (24 h). Results registered a higher safe concentration in resting phase as compared to preparatory and pre-spawning phase of reproductive cycle. During acute toxicity test (24, 48h) safe concentrations were higher as compared to longer duration (72, 48h) toxicity groups during all the thee reproductive phases. In general safe concentrations show only change during 24, 48 and 72 h but in later duration (96 h) the concentration was not different from the 72 h group.

Table 1: The LC_{50} values of chlorpyrifos (with 95% Confidence limit) in fresh water fish *Heteropneustes fossilis*. Lethal concentration shown in different reproductive (resting, preparatory and pre-spawning) phase. The LC_{50} values estimated by Finney Probit analysis. The LC_{50} and safe values calculated for each reproductive phase. Experiment was performed with five replicates of ten fish in each concentration.

Reproductive phase	Test duration (h)	Safe Concentration (m.M.l-1)	LC_{50} (m.M.l-1)	95% Upper confidence limits	95% Lower confidence limits
Resting phase	24	1.11	1.547	2.564	1.103
	48	0.037	0.678	0.969	0.514
	72	0.017	0.299	0.347	0.261
	96	0.017	0.174	0.191	0.159
Preparatory phase	24	0.011	0.332	0.41	0.283
	48	0.019	0.193	0.21	0.179
	72	0.02	0.152	0.166	0.14
	96	0.02	0.123	0.135	0.113
Pre-spawning phase	24	0.007	0.296	0.356	0.243
	48	0.004	0.107	0.143	0.074
	72	0.002	0.044	0.066	0.023
	96	0.002	0.026	0.051	0.01

3.2 Behavioural and morphological alterations

The stress behavioural alterations were positively correlated with the concentration of toxicant (Table 2). The behavioural changes were very clear in all the reproductive phases. But the occurrence of alterations in treatment groups were higher even in low concentrations during pre-spawning phase than preparatory and resting phase of reproductive cycle as compared to their control groups. The control group showed a normal behaviour and tend to move together. They came to the surface at intervals to gulp air. In low concentrations of

CPF (0.002 and 0.005 m.M.l^{-l}) fish behave similar to control group. They showed a number of abnormalities in their behaviour with increase in CPF concentration (>0.005 m.M.l^{-l}). The swimming speed of fish was higher and jerky in higher concentration and this behaviour was shown with immediate exposure of toxicant that slow down with the passage of treatment duration. The fish were calm down in lower concentration after some time, but in higher concentration, fish showed restlessness though out the experimental period. The schooling behaviour of fish was also disturbed in comparison to control group. Other recorded behaviour as hyperactivity, pectoral fin forwarded, frequent surfacing and in gulping, avoidance behaviour, escaping tendency, drooping of fins and loss of buoyancy by vertically hanging in the aquaria increased after CPF treatment. But fish became sluggish, lethargic and finally motionless with the increase in concentration and duration of CPF as compared to control group. Convulsion were most evident, the severity of which paralleled the concentration of CPF. The opercular movements were fast and of concentration dependent behaviour.

With the increase of CPF incubation period and concentration (>0.005 m.M.l^{-l}) fish registered significant, sharp abnormal morphological features (Table 3). It include pale yellow body color, discoloration of skin and barbels, lesion of skin, eye deformities, fin deformities and high mucus secretion and its coagulation all over the body as compared to control.

Table 2: The behavioral alterations of freshwater catfish, *Heteropneustes fossilis,* after exposure of organ phosphorus pesticide, chlorpyrifos. Data was recorded during first hour of treatment in pre-spawning phase of annual reproductive cycle. The frequency of occurrence of different behavior was counted for group of exposed fish

Behavioral and alterations	**Control**	**Concentration of CPF (m.M.l-1)**						
		0.002	**0.005**	**0.028**	**0.057**	**0.085**	**0.114**	**0.142**
Opercular movement	-	-	-	+	++	++	+++	+++
In gulping	-	-	-	+	+	++	+++	+++
Surfacing	-	-	-	+	+	++	+++	+++
Frequent swimming	-	-	-	-	++	++	+++	+++
Avoidance behavior	-	-	-	+	+	++	+++	+++
Hyperactivity	-	-	-	+	+	++	+++	+++
Pectoral fin forward	-	-	-	+	+	++	+++	+++
Convulsions	-	-	-	+	+	++	+++	+++
Abrupt swimming	-	-	-	+	+	++	+++	+++
Escaping tendency	-	-	-	+	++	++	+++	+++
Loss of buoyancy	-	-	-	+	++	++	+++	+++

Normal (-), Mild (+), Moderate (++), Maximum behavior (+++)

Table 3: The morphological alterations of freshwater cat fish, *Heteropneustes fossilis,* after exposure of organ phosphorus pesticide, chlorpyrifos (CPF). Data was recorded during 96 h of CPF treatment in pre-spawning phase of annual reproductive cycle. The frequency of occurrence of different morphological alterations was recorded for group of exposed fish.

Morphological observations	**Control**	**Concentration of CPF (m.M.l^{-1})**						
		0.002	**0.005**	**0.028**	**0.057**	**0.085**	**0.114**	**0.142**
Mucus secretion	-	-	-	+	++	++	+++	+++
Discoloration of skin	-	-	-	+	++	++	+++	+++
Fins drooping/ necrosis	-	-	-	+	+	++	+++	+++
Lesions of skin	-	-	-	+	++	+++	+++	+++

Normal (-), Mild (+), Moderate (++), Maximum morphological alterations (+++)

4. Discussion

The present investigation assured that CPF is lethal to fresh water fish, *H. fossilis*. Though the lethal concentration (LC_{50}) values of CPF varies with different reproductive phases in an annual cycle. The *H. fossilis* has higher tolerance limit (safe value) for CPF during resting phase but very minute concentration of CPF can cause mortality during pre-spawning phase. This study suggested that during gonadal maturity fishes are more vulnerable to aquatic toxicants. The level of aquatic toxification can be assessed by behavioural changes at very first and by morphological changes in a while.

The highest LC_{50} value was found in resting phase. Variation in the LC_{50} depends on the number of biological and phsico-chemical factors which have been reported by many earlier workers [36-40]. The same species may respond different to same toxicant depending on size, age, sex and condition of test species along with experimental factors [41].With the increase of duration of CPF exposure the difference between lower and upper limits of confidence for LC_{50} showed not much difference, this suggested possible bioaccumulation within animal tissue. The pattern of safe concentration suggest that in resting phase fish enjoy high concentration tolerance limit as compare to preparatory and pre-spawning phase. During pre-spawning phase even small concentration, i.e. safe in resting phase, may be lethal to fish. This suggested as reproductive stage and hormone may play important role in susceptibility to aqua-chemicals. A wide range of toxicant was found to increase the toxicity at higher temperature [42, 43]. It was related to the higher metabolic and respiration rate, which may largely be involved in toxicant response in an annual cycle [42, 44, 45]. Fish in general have low metabolic rate in resting phase [46]. This low metabolic activity results in slowdown toxicant absorption and its bioaccumulation [47]. The toxicant absorption and its bioaccumulation will increase comparatively in preparatory and pre-spawning phase with increase in metabolic activity [46]. That will increase comparatively in preparatory and pre-spawning phase with increase in metabolic activity [46]. Insecticides alter the immune system that may influence host defence system and its survival [48]. It is reported that steroid hormones may modulate immunity.

The behaviour changes are the most sensitive indicator of aqua-toxicity. These are considered as first signal of any kind of stress. The observation of behavioural changes was started immediately after CPF treatment as compare to control group. The pesticides influence the behaviour pattern of fish by interfering with the nervous systems and sensory receptors [49-51]. The degree of distress was increased as the toxicant concentration increased. The observed behavioural changes showed by the exposed fish to the chlorpyrifos are similar to those observed in other fish exposed to organophosphate pesticides [52-54]. By frequent surfacing behaviour toxicant exposed fish avoid contact with toxicant also satisfies its demand for oxygen in stressed condition [55, 56]. The opercular movement in fish was first increased after the exposure of CPF as sudden response to shock [32, 60]. *H. fossilis* showed adaptive feature to avoid toxicant intake by decreasing the opercular movement after the few hour of exposure. CPF cause severe damage to the gill membranes [7, 57]. The fade body color can be considered as symptoms of stress after intoxication [6, 58, 59]. Toxicant may manipulate number and size of chromatophores [32].

5. Conclusion
CPF toxicity shows seasonal variation within one species with difference in safe concentration. The observations indicated that CPF caused many behavioural and morphological alterations, which may result in severe physiological problems, ultimately leading to the death of fish. Therefore this investigation presents a relation among pesticide stress, behavioural disorders and mortality rates in light of reproductive stage of fish. This study suggest role of gonadal status and hormone to response with toxicant.

6. Acknowledgement
Sneha Verma is grateful to Rajiv Gandhi National Fellowship, UGC, New Delhi (F1-17.1/2011-12/RGNF-SC-UTT-7275) for financial assistance.

7. References

1. Chernyak SM, Rice CP, McConnell LL. Evidence of currently-used pesticides in air, ice, fog, seawater and surface microlayer in the Bering and Chukchi seas. Marine Pollution Bulletin 1996; 32(5):410-41.
2. Livingstone DR. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms Bulletin of Marine Pollutants 2001; 42:656-666.
3. Matsumoto ST, Mantovani MS, Malagutti MIA, Dias A, Fonseca IC, Morales Marin-MA. Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish, *Oreochromis niloticus* and chromosome aberrations in onion root-tips Genetics and Molecular Biology 2006; 29:148-158.
4. Grande M, Anderson S, Berge S. Effects of pesticides on fish. Norwegian Journal of Agricultural Sciences Supplement 1994; 13, 195-209.
5. Heger W, Jung ST, Martin S, Peter H. Acute and prolonged toxicity to aquatic organism of new and existing chemicals and pesticides Chemosphere 1995; 31:2702-2726.
6. Omitoyin BO, Ajani EK, Adesina BT, Okuagu CNF. Toxicity of lindane (Gamma Hexachloro-Cyclo Hexane) to *Clarias gariepinus* (Burchell 1822), World Journal of Zoology. 2006; 1:57-63.
7. Velmurugan B, M Selvanayagam, EI Cengizand E Unlu. The effects of monocrotophos to different tissues of freshwater fish *Cirrhinus mrigala*. Bulletin of Environmental Contamination and Toxicology 2007; 78(6):450-454.
8. Fisher SW. Changes in the toxicity of the pesticides as a function of environmental pH and temperature. Bulletin of Environmental Contamination and Toxicology 1991; 46:197-202.
9. Richmonds CR, Dutta HM. Effect of malathion on the brain acetylcholinesterase activity of bluegill sunfish *Lepomis macrochirus* Bulletin of Environmental Contamination and Toxicology 1992; 49:431-435.
10. Venkateswara RJ. Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis-mossambicus*. Ecotoxicology and Environmental Safety 2004; 59:217-222.
11. Misha A, Kaushal BT, Singh K. A toxicity study of heavy metal toxicant cadmium sulphate on behavioral and morphological changes of the fresh water fish *Channa punctatus* (Bloch), International Journal of Pharmaceutical Science Invention. 2012; 2(8):8-19.
12. Kaushal BT, Misha A. Investigation of acute toxicity of cadmium on snakehead fish *Channa punctatus*- A comparative toxicity analysis on median lethal concentration, International Journal of Advanced Biological Research. 2013; 3(2):289-294.
13. Adedeji OB, Adedeji AO, Adeyemoand OK, Agbede SA. Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*), African Journal of Biotechnology. 2008; 7:651-654.
14. Onyedineke NE, Odukoyaand AO, Ofoegbu PU. Acute toxicity tests of cassava and rubber effluents on the Ostracoda Strandesiaprava Klie, 1935 (Crustacea, Ostracoda), Research Journal of Environmental Sciences. 2010; 4:166-172.
15. Drummond RA, Russom CL. Behavioral toxicity syndromes, a promising tool for assessing toxicity mechanisms in juvenile fat-head minnows Environmental Toxicology and Chemistry 1990; 9:37-46.
16. Scherrer E. Behavioral responses as indicator of environmental alterations: approaches, results, developments, Journal of Applied Ichthyology. 1992; 8:122-131.
17. Cohn J, MacPhail RC. Ethological and experimental approaches to behaviour analysis: implications for ecotoxicology, Environmental Health Perspectives 1996; 104:299–304.
18. Tadehl H, Häder DP. Automated biomonitoring using real time movement analysis of *Euglena gracilis* Ecotoxicology and Environmental Safety 2001; 48:161-169.
19. Martin J. A portrait of loco motor behaviour in Drosophila determined by a video-tracking paradigm Behaviour Proceeding 2003; 67(2):207-219.
20. Venkateswara RJ, Parvathi K, Kavitha P, NM Jakkaand R Pallela. Effect of chlorpyrifos and monocrotophos on locomotor behaviour and acetylcholinesterase activity of subterranean termites, *Odontoter mesobesus* Pest Management Science 2004; 61(4):417-421.
21. Hansen JA, Marr JCA, Lipton J, Cacela D, Bergman HL. Difference in neurobehavioural response of Chinook

salmon, *Oncorhynchus tshawytscha*, and rainbow trout, *Oncorhynchus Mykiss* exposed to copper and cobalt, behavioural avoidance. Environmental Toxicology and Chemistry 1999; 18:1972-1978.
22. Rao JV, Begum G, Pallela R, Usman PK, Rao RN. Changes in behavior and brain acetylcholinesterase activity in mosquito fish *Gambusia affinis* in reference to the sublethal exposure of chlorpyrifos, International Journal of Environmental Research and Public Health. 2005; 2(3):478-483.
23. Dell' Omo G, Bryentonand R, Shore RF. Effects of exposure to an organophosphate pesticide on behavior and acetylcholinesterase activity in the common shew, *Sorex araneus* Environmental Toxicology and Chemistry 1997; 16:272-276.
24. Devi Y, Misha A. Study of behavioural and morphological anomalies of fry fish of fresh water teleost, *Channa punctatus* under chlorpyrifos intoxication, International Journal of Pharmacology and Biosciences. 2013; 4(1B):865-874.
25. Fulton MH, Key PB. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects Environmental Toxicology and Chemistry 2001; 20:37-45.
26. Agrahari S, Gopaland K, Pandey KC. Biomarkers of monocrotophos in a fresh water fish *Channa punctatus* (Bloch), Journal of Environmental Biology. 2006; 27:453-457.
27. Bretaud S, Toutant JP, Saglio P. Effects of carbofuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassius auratus*), Ecotoxicology and Environment Safety 2000; 47:117-124.
28. Dikshit TSS, Raizada RB. Toxicity evaluation of dimethoate technical in fish, Report to Shaw Wallace and Co India Ltd, 1981.
29. Verma SR, Bansal SK, Gupta AK, N Pa, Tyagi AK, Bhatnagar MC *et al*. Bioassay trails with twenty thee pesticides toa freshwater teleost *Saccobranchus fossilis* Water Research. 1982; 16:525-529.
30. Srivastava VK. A Singh. Studies of seasonal variation in toxicity of frequently used commercial organophosphate, carbamate and synthetic pyrethroid pesticides against freshwater fish *Channa punctatus* and behavioral responses to treated fish Malaysian Applied Biology 2001; 30:17-23.
31. Gul A. Investigation of acute toxicity of chlorpyrifos-methyl on Nile tilapia (*Oreochromis niloticus* L.) larvae Chemosphere 2005; 59:163-166.
32. Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Verma MS. Acute toxicity bioassay of mercuric chloride and malathion on air breathing fish *Channa punctatus* (Bloch), Ecotoxicology and Environmental Safety 2005; 61:114-120.
33. Gupta N, Dua A. Mercury induced behavioral alterations in *Channa punctatus* Pollution Research 2010; 29(4):721-723.
34. Finney DJ. Probit Analysis 2nd edition Cambridge University Press, Cambridge, UK 1971; 333:1953.
35. Hart WB, Doudoroff P, Greenbank J. The evaluation of the toxicity of industrial wastes, chemicals and other substances of freshwater fish. Atlant Refining Co, Phil, 1945, 317.
36. Srivastav AK, Srivastav SK, Misha D, Srivastav S, Srivastav SK. Ultimobranchial gland of freshwater catfish, *Heteropneustes fossilis* in response to deltamethrin treatment. Bulletin of Environmental Contamination and Toxicology 2002; 68:584-591.
37. Jaroli DP, Sharma BL. Effect of organophosphate insecticide on the organic constituents in liver of *Channa punctatus*, Asian Journal of Experimental Sciences. 2005; 19(1):121-129.
38. Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B. Genotoxicity assessment of acute exposure of chlorpyrifos to freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis Chemosphere 2008; 71:1823-1831.
39. Banaee M, Haghi BN, Ibrahim ATA. Sub-lethal toxicity of chlorpyrifos on common carp, *Cyprinus carpio* (Linnaeus, 1758): biochemical response, International Journal of Aquatic Biology. 2013; 1(6):281-288.
40. Padmanabha A, Reddy HV, Khavi M, Prabhudeva KN, Rajanna KB, Chethan N. Acute effects of chlorpyrifos on oxygen consumption and food consumption of freshwater fish, *Oreochromis mossambicus* (Peters), International Journal of Recent Scientific Research. 2015; 6(4):3380-3384.
41. Sprague J.B. Measurement of pollutant toxicity to fish: Bioassay methods for acute toxicity Water Research 1969; 3:793-821.
42. Macek KJ, Hutchinson C, Cope OB. The effects of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides, Bulletin of Environmental Contamination and Toxicology 1969; 4:174-183.
43. Muirhead-Thomson RC. Pesticide and freshwater fauna. Academic Press Inc, London, 1971, 248.
44. Wedemeyer GA, Mayer FP, Smith L. Environmental stress and fish diseases. Diseases of Fishes, Book 5 (eds. Srieszko S.F. and H.R Axelrod) T.H.F Publication, Neptune, 1976, 192.
45. Gordon MR, McLeay DJ. Sealed-jar bioassays for pulpmill effluent toxicity: Effects of fish species and temperature, Journal of Fish Research Board Canada 1977; 34:1389-1396.
46. Pickford GE, Atz JW. The physiology of the pituitary gland of fishes. New York Zoological Society, New York, 1957.
47. Hussein MA, Obuid-Allah AH, Mohammad AH, Scott-Fordsmand JJ, Abd El-Wakeil KF. Seasonal variation in heavy metal accumulation in subtropical population of the terrestrial isopod, *Porcellio laevis*. Ecotoxicology and Environmental Safety 2006; 63(1):168-74.
48. 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.
49. Keizer J, D' Agostino G, Nagel R, Volpe T, Gnemid and P, Vittozzi L. Enzymological differences of ACHE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species, The Science of the Total Environment 1995; 171:213-220.
50. Pan G, Dutta HM. The inhibition of brain acetylcholinesterase activity of juvenile largemouth bass, *Micropterus salmoides* by sub-lethal concentrations of diazinon Environmental Research, Section 1998; A(79):133-137.
51. Cong NV, Phuong NT, Bayley M. Effects of repeated exposure of diazinon on cholinesterase activity and

growth in snakehead fish (*Channa striata*) Ecotoxicology and Environmental Safety 2009; 72:699-703.
52. Ahmed AM. Acute toxicity of four organophosphorus insecticide to a freshwater teleost, *Channa punctatus* (Bloch), Pakistan Journal of Zoology. 1975; 7:135-140.
53. Ghose TK. Nuvan induced physiological, biochemical and behavioural changes in *Barbus stigma* Pollution Research 1986; 5:3.
54. Shukla M. Toxicological assessment of some common pollutants on a freshwater fish. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, India, 1995.
55. Halappa R, David M. Behavioural responses of the fresh water fish, *Cyprinus carpio* (Limmaeus) following sublethal exposure to chlorpyrifos, Turkish Journal of Fisheries and Aquatic Sciences. 2009; 9:233-238.
56. Katja S, Georg BOS, Stephan P, Chistian EWS. Impact of PCB mixture (Aroclor 1254) and TBT and amixture of both on swimming behavior, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*) Aquatic Toxicology 2005; 71:49-59.
57. Johal MS, Sharma ML, Ravneet. Impact of low dose of organophosphate monocrotophos on the epithelial cells of gills of *Cyprinus carpio communis* Linn- SEM study, Journal of Environmental Biology. 2007; 28:663-667.
58. Ree GE, Paney JF. Effect of toxaphene on reproduction of fish Chemosphere 1997; 34:855-867.
59. Wasu YH, Gadhikar YA, Ade PP. Sublethal and chonic effect of carbaryl and malathion on *Clarias batrachus* (Linn), Journal of Applied Sciences and Environmental Management 2009; 13(2):23-26.