academicJournals

Vol. 15(9), pp. 303-314, 2 March, 2016
DOI: 10.5897/AJB2015.14801
Article Number: 0A6CB0457400
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Histopathological effects of Cyperdicot and vitamin E supplementation on selected organs of *Clarias gariepinus* (Burchell, 1822) reared in a tropical fish farm in Nigeria

Odo, G. E.[1]*, Agwu, J. E.[1], Madu, J.[1], Ezea, C. O.[2], Nnamonu, E. I.[1] and Eneje, V. O.[3]

[1]Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu, Nigeria.
[2]Federal University of Technology, Owerri, Imo State, Nigeria.
[3]Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria.

Received 15 June, 2015; Accepted 29 December, 2015

This study conducted in 2014 investigated the histopathological effects of Cyperdicot and vitamin E supplementation on some selected organs in juveniles of *Clarias gariepinus*. Fish were exposed to 0. 0.08 and 0.16 mg/L Cyperdicot and vitamin E. Fish were divided into six groups: control, 0.80 mg/L; Cyperdicot, 0.16 mg/L; Cyperdicot, vitamin E, vitamin E + 0.08 mg/L Cyperdicot, and vitamin E + 0.16 m/L Cyperdicot insecticide. There was significant relation between temperature, pH, and dissolved oxygen with Cyperdicot concentration. The LC_{50} value based on probit analysis was found to be 0.08 mg/L for 96 h. Samples were taken at fixed times for histopathological studies. The fish exhibited behavioural and dermatological changes. Vitamin E + 0.08 mg/L Cyperdicot and vitamin E + 0.16 mg/L Cyperdicot treated fish showed abnormalities in their behaviour. Gills, liver, and kidneys of the 0.08 mg/L Cyperdicot treated group also showed several histopathological changes during the experimental periods. The organs of the fish treated with vitamin E + 0.16 mg/L Cyperdicot induced histopathological changes. The toxic effect of Cyperdicot is clear on the behavioural and histopathological aspects of the fish gills, liver, and kidney tissues, while vitamin E had no amelioration effects on them.

Key words: Cyperdicot, vitamin E, *Clarias gariepinus*, pesticide, sub lethal toxicity.

INTRODUCTION

The application of environmental toxicological studies on non-mammalian vertebrates is rapidly expanding, and for aquatic system, fish have become the indicators for the evaluation of the effects of noxious compounds (Ervnest, 2007). Pesticides occupy a unique position among many chemicals which are encountered daily by man. Cyperdicot is an agrochemical pesticide formulated with dimethoate and cypermetrin. It is a broad-spectrum

*Corresponding author. E-mail: gregory.odo@unn.edu.ng.

pesticide widely used throughout the world for agriculture and domestic purposes (Ali et al., 2009). Pesticides at high concentrations are known to reduce the survival, growth, and reproduction of fish and produce many visible effects on fish (Rahman et al., 2002). Peebua et al. (2007) recorded that, the 96-h LC_{50} values of Cyperdicot freshwater fish rainbow trout and fat head minnows were 15 and 0.8 µg/L, respectively.

A great proportion of acute poisoning cases are caused by exposure to pesticides, especially organophosphate (OP) compounds. The primary mechanism of action of OP pesticides is based on inhibition of the acetylcholinesterase (AChE) enzyme (Eto, 1979). Once AChE has been inactivated, acetylcholine (Ach) accumulates throughout the nervous system, resulting in overstimulation of muscarinic and nicotinic receptors. Like all organophosphate insecticides, Cyperdicot acts on the nervous system as inhibitor of AchE, an enzyme that hydrolyzes acetylcholine (Ach). Ach is a molecule that is involved in the transmission of nervous signals from nerves to nerves and between neurons in the brain (Jungera, 2014). Due to Cyperdicot toxicity, the Environmental Protection Agency (EPA) has classified it as restricted use pesticide (RUP) that warrants special handling (ATSDR, 2005). Large amounts of pesticides find their ways into water bodies due to repeated application for the control of pests. The indiscriminate use of pesticides, careless handling, accidental spillage or discharge into natural water ways have harmful effects on fish population and other aquatic organisms and may contribute to long-term effects (Nwani et al., 2013b)

Histopathological studies help to establish casual relations between exposure to contaminants and various biological responses and have proven to be a sensitive tool used in detecting direct effects of chemical compounds within target organs of laboratory experiments (Altinok and Capkin, 2007; Boran et al., 2012). A study of histopathology provides very important and useful data concerning changes in cellular or sub-cellular structure of an organ much earlier than external notification. One of the advantages of using histopathological biomarkers in environmental studies is that it allows the examination of target organs and the alterations found in these organs are easier to identify than functional ones (Fanta et al., 2003). These alterations serve as warning signs of damage to the wellbeing of the organism.

Histopathological biomarkers in the gills may be valuable as indicators of the general health of the fish and mirror effects of exposure to a variety of anthropogenic pollutants (Wiieyaratne and Pathiratne, 2006). The histopathological effects on the gills of freshwater fish showed necrosis, abnormalities to gill lamellae, extensive fusions of secondary lamellae, and a thick coat of mucus on the gill filaments upon exposure to Cyperdicot (Rao et al., 2005). In addition, hyperplasia of the gill filaments, edema separation of primary gill lamellae, haemorrhage in the blood vessel, clubbing, fusion of adjacent filaments, and hyperplasia in the secondary lamellae in freshwater *Clarias gariepinus* exposed to Cyperdicot were also observed.

The kidney, for instance, is a target of toxic chemicals, which can disrupt its functions, and cause temporary or permanent derangement of homeostasis. Several authors recorded histopathological changes in the kidney and liver of freshwater fish, *Puntius conchoniu* and *Channa punctatus* exposed to organophosphate insecticides, such as diazinon, monocrotophos, dimethoate, and elsan, respectively (Miller, 2002). Todd and Van Leeuwen (2002) reported that pesticides present in aquatic environments can affect aquatic organisms in different ways which include toxic effects at both lethal and sub-lethal concentration which may change their growth rate, development, reproduction, histopathology, biochemistry, physiology, and behaviour. The most histopathological changes in the kidney and liver of freshwater catfish *(Heteropneutes fossilis)* and zebra fish *(Danio rerio)* exposed to insecticide Cyperdicot were shrunken glomeruli, dilated lumina of the renal tubules, vacuolated blood cells in the glomerular tuft, caryolysis, and widespread vacuolar degeneration of the hepatocytes (Scheil et al., 2009).

Clarias species are mostly freshwater fish which are distributed throughout African and Asian lakes, swamps, and rivers. *Clarias* can be obtained throughout the year in Nigerian rivers and are anadromous (Akinwole and Faturoti, 2007; Adewolu et al., 2008). The fish is in high demand because of its flesh and good taste. To meet the ever increasing demand, *C. gariepinus* is the fish of choice in Nigerian aquaculture. There has been paucity of information on the effect of Cyperdicot on freshwater cat fish in Nigeria aquatic ecosystem. Hence, this work determined the half-lethal concentration (96 h LC_{50}) of the insecticide to juveniles of *C. gariepinus* in order to evaluate the effect of its sub-lethal toxicity on some selected organs (gills, liver and kidneys) of the fish, and the effect of vitamin E supplementation against the different concentrations of Cyperdicot pesticide were also evaluated.

MATERIALS AND METHODS

Experimental fish

Three hundred African catfish, *C. gariepinus* juvenile (mean weight 150 ± 5.20 g, length = 35.00 ± 2.50 cm) were obtained from Sacen Fish Farm, Enugu, Nigeria and transported in a 300 L capacity plastic tank to the Fisheries Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria Nsukka. The fish specimens were treated with 0.05% potassium permanganate to avoid possible dermal infections. They were acclimatized for 20 days in a 1000 L plastic tank during which they were fed 3% body weight (BW) in divided rations twice daily (7.00 am and 7.00 pm) with locally laboratory prepared pelleted diet containing 35% crude protein (Eyo et al., 2013). The feeding was terminated 24 h prior to the range finding and toxicity test to avoid interference of faeces

(Reish and Oshida, 1987). The ethical guidelines of the Animal Care Committee (UNN-EGACC, protocol no. 0430/2013) of the University of Nigeria, Nsukka were strictly followed.

Pesticide

Cyperdicot is composed of cypermethrin and dimethoate. Cypermethrin is an insecticide in the synthetic pyrethroid family, first marketed in 1977. The primary manufacturers in the U.S. are Zeneca Inc., FMC Corp., and American Cyanamid Co. Common brand names are Demon, Cymbush, Ammo, and Cynoff. Dimethoate, first marketed in 2001 by FAO, is an organ phosphorus and systemic pesticide with stomach and cholinesterase inhibition actions. The trade names are Danadim, Rogo, and Roxion. The primary manufacturers in Denmark and Italy are Cheminouta.

Diets

The basal control diet (diet C) was formulated according to Kim et al. (2003) from practical ingredients to satisfy all known nutrient requirements of *C. gariepinus* with adequate levels of vitamin E, a-tochopheryl acetate in diet (a-TA).

Vitamin E

Vitamin E is composed of a compound that includes tocopherols and tocotrienols. The molecules that contribute a-tocopherols activity are four tocopherols and four tocotrienols, identified by alpha (a), beta (β), gama (y), and delta (ð). The brand names are Aqua-E, Aquasol E, Aqueous Vitamin E and E-400 clear (Packer et al., 2001).

Toxicity test

Determination of the LC_{50} concentration

A toxicity assay to determine the 96 h LC_{50} values of Cyperdicot was conducted with a definitive test in a semi-static system in the laboratory following standard methods (APHA, AWWA, WPCF, 2005). A range-finding tests (5, 4, 3.5, 3, and 2.5 mg^{-1}) was carried out to determine the concentrations of the test solution for the definitive test. The test solution was changed on every alternate day to counter-balance the decreasing pesticide concentrations. To prevent oxygen depletion, experimental tanks were continuously oxygenated using an air pump. Dead fish were immediately removed to avoid possible deterioration of the water quality. Behavioural changes in fin and opercular movements, equilibrium status, swimming rate, air gulping, and skin coloration during the test period were observed. In the definitive test, a set of 10 fish specimens was randomly exposed to Cyperdicot at 5, 4, 3.5, 3, and 2.5 mg^{-1} concentration. Another set of 10 fish specimens was simultaneously maintained in tap water, without test chemical, and considered as control. The experiment was set in triplicate to obtain LC_{50} values of the test chemical under a photoperiod of 12 h light and 12 h dark.

Determination of sub-lethal concentrations

The 96 h LC_{50} value of Cyperdicot on *C. gariepinus* was determined to be 0.80 mg/L. Based on this value, two sub-lethal concentrations of 0.08 and 0.16 mg/L corresponding to 1/20 and 1/10th of the 96 h LC_{50} of the pesticide, respectively, were prepared by serial dilution of the stock solution and were used for *in vivo* exposure. A total of 90 fish from the acclimatized batch were used during the *in vivo* experiment. The fish were randomly divided into three groups of 30 fish without regard to sex. Fish in the first treatment group were exposed to tap water and served as control, while those in the second and third groups were treated with 0.08 and 0.16 mg/L of Cyperdicot, respectively. The exposure lasted for a period of 4 weeks during which the fish were fed daily with small quantity of food approximately 1% of total body weight about an hour before the test solution was renewed to avoid catabolism and subsequent mortality.

Morphometric data

The condition factor (CF) and hepatosomatic index (HSI) of the fish were calculated after White and Fietcher (1985) as follows:

$$\text{Condition factor (CF)} = \text{Bodyweight (g)}/\text{Standard length (cm)}^{3} \times 100$$

$$\text{Hepatosomatic index (HIS)} = \text{Liver weight (g)}/\text{Bodyweight (g)} \times 100$$

Experimental design

The 96 h LC_{50} of Cyperdicot insecticide to juveniles of *C. gariepinus* was determined to be 0.80 mg/L. In the sub lethal toxicity test, fish were exposed to 0.08 mg/L (1/10 LC_{50}) and 0.16 mg/L (1/5 LC_{50}) Cyperdicot and/or vitamin E and as a result, six concentrations were established and used for the definitive test for four weeks: (i) Group 1: Control; (ii) Group 2: 0.08 mg/L Cyperdicot; (iii) Group 3: 0.16 mg/L Cyperdicot; (IV) Group 4: vitamin E; (v) Group 5: vitamin E + 0.08 mg/L Cyperdicot; (vi) Group 6: vitamin E + 0.16 mg/L Cyperdicot.

Fish were collected at the end of the 1st, 2nd, 3rd and 4th week for behavioural and histopathological studies. The cumulative mortality was recorded throughout the period of study and the fish were examined to determine the cause of their death. The immediate behavioural changes of the fish were recorded before death.

Histopathological examinations

On 1, 7, 14, 21 and 28 days, one fish from each replicate treatment group and control was sacrificed after anesthetizing with tricaine methanesulfonate (MS 222) to minimize stress. The fish were dissected and gill, kidney, and liver tissues were removed, preserved in 10% phosphate buffered formalin for 24 h, dehydrated by a series of upgraded ethanol solution, embedded in paraffin, and sectioned at 5 µm thick. A total of three tissue sections of gill, kidney, and liver each per fish for each replicate concentration were routinely processed and stained with Hematoxylin and Eosin (H&E) and examined by light microscopy according to Bancroft and Gamble (2002). Photomicrographs were then taken and the fish of the control groups were compared with that of exposed groups under the guidance of a pathologist.

Statistical analysis

Mean values were analyzed for significant differences ($p \leq 0.5$) using the analysis of variance (ANOVA). Differences between means were partitioned using the Duncan new multiple range test. The mean physicochemical parameters of the test concentrations of Cyperdicot on *C. gariepinus* were observed. Condition factor and hepatosomatic indices were calculated. The Statistical Package for Social Sciences (SPSS), version 17, was used for all analysis. The probity value was determined from the probity model developed by Finney (1971).

Table 1. Mean physicochemical parameters of the test concentrations (Cyperdicot) on *C. gariepinus*.

Parameter	pH	Temperature (°C)	Cond (µM/cm)	DO (mgl^{-1})	Alk (mg/L)	Th (mg/L)
Control	7.0±0.1[a]	26.0±0.7[a]	67.0±0.1[a]	6.3± 0.	24.2±0.1	6.2±0.2
0.08 mg/L Cyperdicot	6.9±0.2[a]	27.0±0.1[b]	66.4±0.2[b]	5.4±0.1	23.9±0.8	6.0±0.1
0.16 mg/L Cyperdicot	6.7±0.1[b]	27.2±0.1[b]	65.3±0.3[b]	5.3±0.6	23.6±0.2	5.8±0.3
Vitamin E	6.6±0.2 [c]	27.4±0.3[c]	63.8±0.6[c]	5.2±0.1	22.8±0.9	5.6±0.2
0.08 mg/L Cyperdicot + Vitamin E	6.3±0.3[c]	27.5±0.1[c]	62.1±0.2d[e]	4.9±0.1	22.6±0.5	5.4±0.6
0.16 mg/L Cyperdicot + Vitamin E	63±0.0.1[e]	27.0±0.3[d]	59.2 ±0.2[e]	4.7±0.2	22.1±0.3	4.9 ±0.4

Cond = conductivity, micro-siemens per centimeter (μscm^{-1}); DO = dissolved oxygen (mgl^{-1}); milligram per litre; Alk = total alkalinity = milligram per litre =mgl^{-1}; Th = total hardness = milligram per litre = mgl^{-1}.

RESULTS

The mean physicochemical parameters of the test concentration (Cyperdicot) on *C. gariepinus* are shown in Table 1.

Toxicity and behavioural responses

No adverse behavioural changes or any mortality were recorded in the control fish and vitamin E throughout the period of the bioassay (Table 2). The behaviour of the control fishes and their colour were normal. Symptoms of toxicosis observed in the fish behaviour with Cyperdicot include lack of balance, agitated or erratic swimming, air gulping, restlessness, sudden quick movement, excessive secretion of mucus, and swimming on the back. The juveniles of *C. gariepinus* exposed to sub-lethal concentrations (0.08 and 0.16 mg/L) of Cyperdicot, respectively exhibited fast behavioural changes even at the low concentration. Fish showed a colour fading and retardation in opercular movement. They also lost their feeding appetite. An increase in skin mucus secretion and its bioaccumulation on the gills were also observed. Vitamin E treated fish were behaviourally normal and had good appetite as the control group. Those treated with vitamin E + Cyperdicot showed abnormalities in their behaviour similar to that of the fish treated with Cyperdicot.

Mortality

The percentage mortality increased with increase in toxicant concentration. Catfish juveniles were exposed to 0.08, 0.16, vitamin E + 0.08 and vitamin E+ 0.16 had 6.67, 13.33, 9.71, and 13.33% mortalities, respectively (Table 3). The first 24 h LC_{50} at 95% confidence limit was estimated as 1100.78 mg/L. The percentage mortality at second day increased with the toxicant concentration. Catfish juveniles exposed to 0.08, 0.16, vitamin E + 0.08 and vitamin E + 0.16 mg/L had 13.33, 13.33, 9.16, 6.01, and 5.96% mortalities, respectively (Table 3). The second day LC_{50} at 95% confidence limit for toxicant concentration was estimated as 416.67 mg/L. The percentage mortality at third day increased with the toxicant concentration. Catfish juveniles exposed to 0.08, 0.16, vitamin E + 0.08 and vitamin E + 0.16 mg/L had 16.51, 26.67, 33.33, 19.61, and 24.39% mortalities, respectively (Table 3). The third day LC_{50} at 95% confidence limit for toxicant concentration was estimated at 181.76 mg/L. The percentage mortality at the fourth day increased with the toxicant concentration. Catfish juveniles exposed to 0.08, 0.16, vitamin E + 0.08 and vitamin E + 0.16 mg/L had 6.67, 13.33, 10.11 and 13.05% mortalities, respectively (Table 3). The four days LC_{50} at 95% confidence limit for toxicant concentration was estimated at 152.02 mg/L.

Morphometric results

Condition factor (CF)

Condition factor is an index of growth rate. The condition factor (mg/cm^3) of *C gariepinus* of all treated groups did not significantly change after the end of the 1st, 2nd and 4th week as compared to the control group. Condition factor recorded at the end of the 3rd week significantly decreased in the 0.16 mg/L Cyperdicot and vitamin E and 0.16 mg/L Cyperdicot treated groups, respectively compared to the control group (Table 4). The results by the three-way ANOVA revealed that, Cyperdicot concentrations and time significantly affected the CF. Vitamin E did not significantly affect the CF, while the interaction between vitamin E and time significantly ($p < 0.001$) affected the CF.

Hepatosomatic index (HSI)

The hepatosomatic index (%) of juveniles of *C. gariepinus* recorded at the end of 1st week significantly ($p < 0.05$) decreased to 0.23 ± 0.035% in 0.16 mg/L

Table 2. Behavioural and dermatological changes of *C. gariepinus* juveniles exposed to various concentrations of Cyperdicot.

Parameter	Toxicity test																							
	Exposure time (h)																							
	24						48						72						96					
	Concentration (mg/L)																							
	0	0.08	0.16	Vit. E	0.08 + Vit. E	0.16 + Vit. E	0	0.08	0.16	Vit. E	Vit. E + 0.08	Vit. E + 0.16	0	0.08	0.16	Vit. E	Vit. E + 0.08	Vit. E + 0.16	0	0.08	0.16	Vit. E	Vit. E + 0.08	Vit. E + 0.16
Behavioural changes																								
Loss of reflex	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Air gulping	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Erratic swimming	-	-	+	-	-	+	-	-	+	-	-	+	-	+	++	-	+	++	-	++	++	-	++	+++
Dermatological changes																								
Discoloration	-	+	+	-	+	+	-	++	++	-	++	++	-	++	++	-	++	++	-	+++	+++	-	+++	+++
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	++	++	-	++	++
	Sub-lethal test																							
Exposure time (week)	24						48						72						96					
Behavioural changes																								
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+
Air gulping	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	++	-	++	+++
Erratic swimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	++	++
Dermatological changes																								
Discoloration	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	++	+	-	++	++	-	++	+++
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-No significant, +low severity, ++moderate severity, +++high severity.

Cyperdicot treated group compared to 0.27 ± 0.028) of the control group. At the end of the 3rd week, a significant decrease (0.208 ± 0.015%) of the control group was also recorded (Table 5). The results of the three-way ANOVA revealed that the concentrations significantly ($p < 0.001$) affected the HSI. The interactions between Cyperdicot concentrations and time and between vitamin E and time significantly ($p < 0.01$) affected the HSI. The interaction between Cyperdicot concentrations, vitamin E, and time significantly ($p < 0.05$) affected HSI.

Histopathological observations of the gills, kidney and liver in *C. gariepinus* exposed to Cyperdicot

The histopathological changes observed in *C. gariepinus* during the experiment are presented in Tables 5 and Figures 1 and 2. No alterations were observed in the gills, kidney and liver of the control. The severity and frequency of gill, kidney and liver lesions were found to be more pronounced in fish exposed to the highest concentration of Cyperdicot. Histopathological alterations were concentrations and duration dependent.

DISCUSSION

Acute and chronic toxicity tests are mostly used to

Table 3. Percentage mortality of *C. gariepinus* juveniles exposed to various concentrations of Cyperdicot.

Toxicant concentration (mg/L)	Percentage mortality				
	24	48	72	96	Cumulative mortality (%)
0	0.00	0.00	0.00	0.00	0.00
0.08	6.67	13.33	20.00	6.67	46.67
0.16	13.33	13.33	26.67	13.33	66.67
Vitamin E	9.71	9.16	16.51	10.11	45.49
Vitamin E+0.08	6.09	6.01	19.61	6.21	41.79
Vitamin E+0.16	12.81	5.96	24.39	13.05	41.79
LC_{50} (mg/L)	1200.78	426.67	191.76	163.02	60.21
Log (concentration)	3.08	2.63	2.28	2.21	-

Table 4. Condition factor (FC) (g/cm^3) of juveniles of *C. gariepinus* daily exposed to various concentrations of Cyperdicot (0, 0.08 mg/L and 0.16 mg/L) and/or vitamin E (0.450 kg^{-1} dry weight diet).

Treatment	Time intervals			
	1st week	2nd week	3rd week	4th week
Control	0.433±0.10	0.441±0.03	0.455±0.04	1.464±0.03
0.08 mg/L Cyperdicot	0.631 ±0.05	0.31±0.03	0.334±0.035	1.278±0.06
0.16 mg/L Cyperdicot	0.769±0.04	0.39±0.05	0.268±0.04*	1.362±0.05
Vitamin E	0.441±0.08	0.449±0.09	0.525±0.06	1.568±0.05
0.08 mg/L Cyperdicot + Vitamin E	0.495±0.06	0.442±0.05	0.425±0.03	1.455±0.10
0.16 mg/L Cyperdicot + Vitamin E	0.434±0.05	0.352±0.06	0.287±0.02*	1.31±0.03

assess the toxicity of chemicals on non-target organisms (Santos et al., 2010). 96 h LC_{50} is one of the most important parameters for evaluating the toxic effects of pollutants (Nwani et al., 2015). In the present study, the 96 h LC_{50} values of Cyperdicot for the African catfish, *C. gariepinus* was found to be 0.80 mg/L. In general, toxicity of chemicals to aquatic organisms has been reported to be affected by temperature, pH, dissolved oxygen, size and age, strain of species, water quality, concentration and formulation of test chemicals (Nwani et al., 2010; Boran et al., 2012; Rauf and Arain, 2013). The magnitude of toxic effects of pesticides also depends on the length and weight, corporal surface/body weight ratio and breathing rate (Murty, 1986). Oh et al. (1991) also reported that varied inhibition of acetylcholinesterase, detoxicification and absorption are factors causing the selective toxicity of pesticides for various species of fish.

Clinical symptoms observed during acute exposure of *C. gariepinus* to Cyperdicot in the present study are consistent with the findings of other authors and may indicate Cyperdicot-induced suppressed activity acetylcholineesterase. The abnormal behavior exhibited by fish in the experimental groups, such as abnormal swimming behavior are due to inhibition of AChE activity leading to accumulation of acetylcholine in choligenic synpases, thus causing hyper stimulation. Muralidharan (2012) reported that hyperactivity of fish exposed to pollutants could be attributed to impaired gill function, and the secretion of increased amount of mucus to coat the body and gills may be an attempt to produce relief from the irritating pollutant. The secretion of copious mucus by fish could also be a defense mechanism to neutralize the effect of pesticide which gradually covers the body, gills and buccal cavity. Repeated opening and closing of the mouth and opercula covering accompanied by partially extended fin as observed in the present study could be due to clearance of the accumulated mucus debris in the gill region for proper breathing. Similar behavioral changes In *C. gariepinus* exposed to atrazine and chlorpyrifos (Nwani et al., 2013a) and *Cirrhinus mrigala* exposed to diazinon (Rauf and Arain, 2013) have been reported.

Histopathological lesions observed in gill tissues of *C. gariepinus* exposed to Cyperdicot in the present study are similar to reports in *Cyprinus carpio* (Muralidharan, 2014). Similar pathological lesions in the gill architecture were observed in *Orochromis niloticus* exposed to dimethoate (Elezaby et al., 2001), *Puntius gonionotus* exposed to paraquat (Cengiz and Unlu, 2006), *Oncorhynchus mykiss* exposed to fungicide captan (Boran et al., 2012) and in *Gobiocypris rarus* (Yang et al., 2010), *Gnathonemus petersii* (Alazemi et al., 2012) and

Table 5. Summarized histopathological effects in the kidney, gill and liver of *C. gareipinus* administered exposed to Cyperdicot and the control.

Concentration (mg/kg)	Liver						
	Duration (Days)	Lymphocytic infiltration	Cell rupture	Congested central vein	Glycogen vacuoltion	Pyknotic necrosis	Severe of infiltration of leukocytes
Control	7	0	0	0	0	0	0
0.08	7	1	3	2	0	0	0
0.16	7	0	1	1	0	0	0
Vit E	7	0	0	0	0	0	0
Vit E+0.08	7	0	0	0	0	0	0
Vit E +0.16	7						
Control	14	0	0	0	0	0	0
0.08	14	0	0	1	0	2	0
0.16	14	0	1	2	0	0	0
Vit E	14	3	0	0	0	0	0
Vit E+0.08	14	0	0	0	0	0	0
Vit E +0.16	14						
Control	28	0	0	0	0	0	0
0.08	28	0	3	3	0	0	0
0.16	28	0	0	0	0	0	0
Vit E	28	0	0	0	0	0	3
Vit E+0.08	28	0	0	0	0	0	0
Vit E 0.16	28	0	0	0	0	0	0

Concentration (mg/kg)	Gill						
	Duration (Days)	Necrotic tubules	Tubule disintegration/ degeneration	Cystic spaces	Proliferation of polymorphonuclear cells	Vacuolation	Hematopoetic tissue
Control	7	0	0	0	0	0	0
0.08	7	1	3	2	0	0	0
0.16	7	0	1	1	0	0	1
Vit E	7	0	0	0	0	0	0
Vit E+0.08	7	0	0	0	0	0	0
Vit E +0.16	7						
Control	14	0	0	0	0	0	0
0.08	14	0	0	1	0	2	0

Table 5. Contd.

0.16	14	2	1	2	0	0	1
Vit E	14	0	0	0	0	0	0
Vit E+0.08	14	0	0	0	0	0	0
Vit E +0.16	14						
Control	28	0	0	0	0	0	0
0.08	28	0	3	3	0	0	0
0.16	28	0	0	0	2	0	0
Vit E	28	0	0	0	0	0	0
Vit E+0.08	28	0	0	0	0	0	0
Vit E 0.16	28	0	0	0	0	0	0

Concentration (mg/kg)	Kidney						
	Duration (Days)	Congestion of bloodspaces	Hypertrophy	Epithelia lifting	Oedema	Obliteration of lamellaearchitecture	Necrosis of epithelia cells/damage of gills
Control	7	0	0	0	0	0	0
0.08	7	3	1	1	0	1	1
0.16	7	0	0	2	0	0	1
Vit E	7	0	2	0	0	0	0
Vit E+0.08	7	0	0	1	0	0	0
Vit E +0.16	7		0	1	0	0	0
Control	14	0	0	0	0	0	0
0.08	14	1	0	1	0	2	1
0.16	14	0	1	2	0	0	0
Vit E	14	0	0	0	0	0	0
Vit E+0.08	14	0	0	0	0	0	0
Vit E +0.16	14	0	0	0	0	0	0
Control	28	0	0	0	0	0	0
0.08	28	0	3	3	0	2	0
0.16	28	0	0	0	0	0	0
Vit E	28	0	0	0	0	0	0
Vit E+0.08	28	1	0	0	0	0	0
Vit E 0.16	28	0	0	0	0	0	0

Lesions were scored based on their severity (0 = none, 1 = mild, 2 = moderate, 3 = strong).

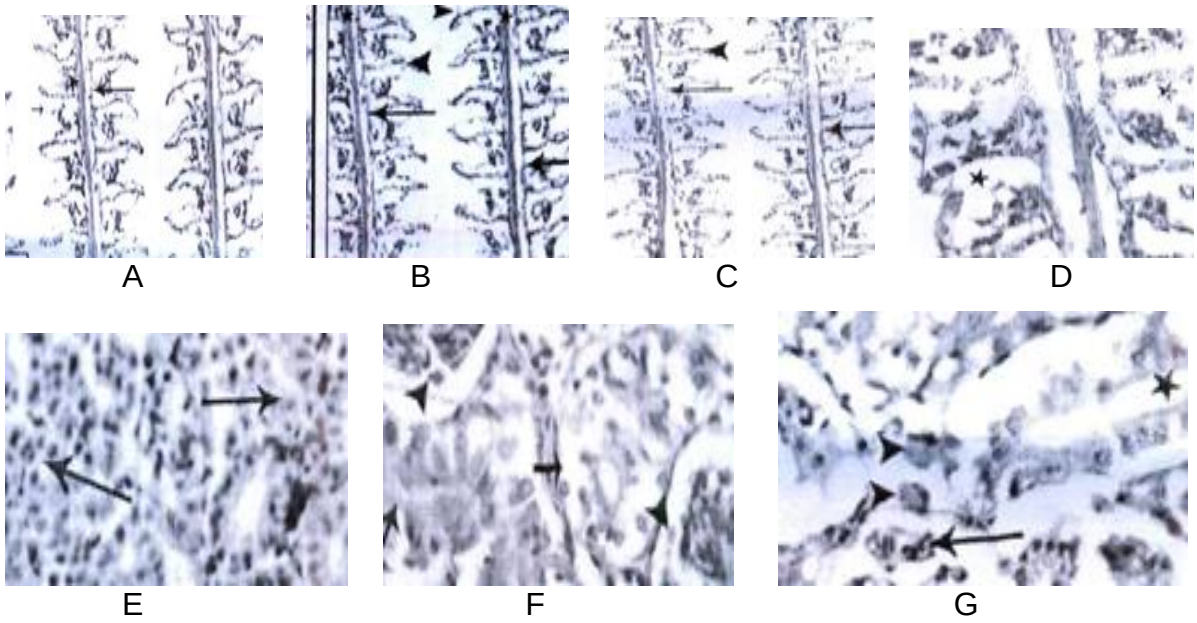

Figure 1. (A) Photomicrography of gill section of control group of juveniles' *C. gariepinus* showed no significant lesion ×400. (B) The 0.16 mg/L Cyperdioct administration on gill showed: a. Epithelia hyperplasia, lamellar aneurysm. b. Leucocytes infiltration and hyalinization of adductor muscles. c. Deformation of the cartilage, necrosis of epithelial cells, completes destruction of the gills, obliteration of lamellae architecture. (x400). (C) The Vitamin E and 0.08 mg/L Cyperdicot showed congestion of entire lamellae and epithelia hyperplasia, vacuolar degeneration pillar cells infiltration and Synechiae x400. (D) The 0.16 mg/L Cyperdicot, 1.70 mg/L hyperplasia and aneurysm, oedema intraepithelial oedema, fused epithelia hyperplasia. (E) Kidney observations: Control showed normal architecture and renal tubule x400. (F) The 0.08 mg/L Cyperdicot. a) Swelling of epithelial cells of renal tubule with diluted lumen and occlusion lumen and fragmentation of glomeruli. b) Complete destruction of tubule architecture, fragmentation of glomerulus brownish pigments x 400. (G) The 0.08 mg/L Cyperdicot. a) Swelling of epithelial cells of renal tubule with diluted lumen and occlusion lumen and fragmentation of glomeruli. b) Complete destruction of tubule architecture, fragmentation of glomerulus brownish pigments ×400.

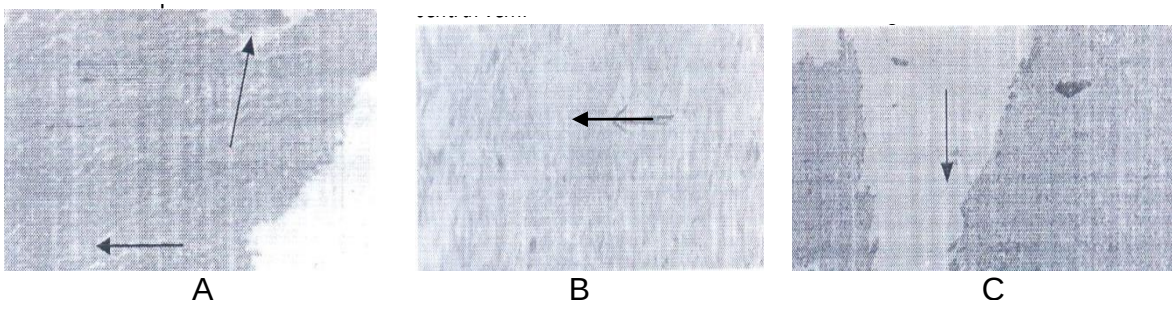

Figure 2. (A) Liver of *C. gariepinus* ×400. The 4 week exposed to 0.08 mg/L Cyperdicot showing glycogen vacuolation. (B) Liver *C.gariepinus* of ×400. The 4 week exposed to Vitamin E + 0.08 mg/L. Cyperdicot showing severe infiltration of leukocytes (arrow) pyknotic (N) and vacuoles(L). (C) Liver of *C.gariepinus* x400. The 4 week exposed to 0.16 mg/L + Vitamin E Cyperdicot showing diffused hepatic necrosis.

C. carpio (Blahova et al., 2014) exposed to atrazine. Epithelial hypertrophy could be as a result of epithelial detachment as stated by Machado and Fanta (2003). Epithelial lifting increases the distance through which the toxicant reaches the blood stream thereby causing impaired oxygen uptake (Kumar et al., 2010); it could result in dysfunction or even non-functional gills and eventually suffocate the fish. Lamella fusion could be a protective mechanism as it reduces the amount of vulnerable gill surface area. According to Olurin et al. (2006), these pathological changes may be a reaction to toxicant intake or an adaptive response to prevent the

entry of the pollutants through the gill surface and probably increase capillary permeability.

Histopathologically, this was due to proliferation of mucus cells and epithelial hyperplasia of gills. This could be as a result of coating the body so as to reduce contact with the toxic environment and get relief from the pollutant irritation (Al-Ghanim et al., 2008).

The liver of the exposed fish had slightly vacuolated cells showing evidence of fatty degeneration. Necrosis of some portions of the liver tissue observed resulted from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The degenerative changes in the hepatocytes of *C. gariepinus* were found to increase as the investigation progresses into weeks. In Sri Lanka, gills of *Rasbora caverii* collected from canals near rice fields, covering pesticide application periods during rice cultivation season showed also similar changes (De Silva and Samayawardhena, 2002; Wijeyaratne and Pathiratne, 2006) with juvenile guppics (*Poecilla reticulate* Peters) exposed to sub-lethal concentration of Cypercicot (Rao et al., 2005; Kunjamma et al., 2008).

The different concentrations of Cyperdicot used in this study under different exposure periods showed different degrees of pathological changes. Similar results were recorded in the freshwater fish (*P. gonionotus, Gambusia affinis* and *Corydoras paleatus*) exposed to pesticides Paraquat and Dimethoate (Elezaby et al., 2001; Cengiz and Unlu, 2003; Fanta et al., 2003; Jiraungkoorskul et al., 2003).

These vacuolar degenerating of glomerular tuft, shrinkage of some glomeruli and dilatation of others, increased Bowman's capsule space, cloudy swelling of some epithelial tubules and dilatation of tubules lumens and obstruction observed are in agreement with the changes in the kidney of freshwater fish *(Piaractus mesopotamicus)* exposed to organophosphate insecticide (Mataqueiro et al., 2009). The shrinkage in renal corpuscles clearly indicated that treated fish adopt some other routes of nitrogen excretion while the dilation of the renal corpuscles may be due to an increase in the filtration rate and consequently in urine volume, which may be a mechanism used by fish to overcome the toxic effect of the pesticide (Roy and Bhattacharya, 2006). The decreases in the tubular lumen may be due to the cloudy swelling of the epithelial cells of the renal tubules, which could be a reversible change; also, the dilation in the tubules lumen may be due to the marked decrease in the length of the epithelial cells as a result of epithelial tubules degeneration. In the present study, the recognized homogenous eosinophilic deposits within tubular lumen could be attributed to the protein leakage into the filtrate due to the glomerular disease (Roberts, 2001).

Cyperdicot used in the present research caused some histopathological changes in the kidney tissues and the vitamin E was not able to prevent these changes. The insecticide Cyperdicot caused kidney damage, and a combination of vitamins E and C reduced partially this damage. On a relative basis, Cyperdicot appears to be capable of producing a wider spectrum of significant histopathology impairments in fish with even sub-lethal concentrations and should be categorized as an important pollutant of the aquatic environment (Oncu et al., 2002).

The morphometric study included condition factor and hepatosomatic indices. The condition factor (mg/cm^3) of *C. gariepinus* of all treated groups showed very minor change; significant decrease in the condition factor of 0.16 mg/L Cyperdicot at the end of the 3rd week when compared with the control group and vitamin E could not prevent this decrease. The significant lower condition factor was recorded by Teh et al. (2005) in *Pogonichthys macrolepidotus* exposed to sub lethal concentrations of diazinon. The few changes in condition factor in the present experimental periods could be attributed to this factor. This factor could not be enough sensitive biomarker to measure the environmental stress in natural environments (Wiieyaratne and Pathiratne, 2006). The recorded non-significant effect of vitamin E on the condition factor throughout the experimental periods was also recorded in the freshwater fish rainbow trout (*O. mykiss*) (Al-Juary et al., 2006). This indicated that the addition of a-tocopherol to the diet did not significantly alter the palatability of the diet, its nutrient content, and the caloric values (Al-Juary et al., 2006). Tissue somatic indices, such as the hepatosomatic index are general measurement of the overall condition of fish or growth status of a specific tissue (West, 1990). The minor changes were recorded in the present study in the hepatosomatic index of fish treated with Cyperdicot. A significant decrease in the hepatosomatic index of fish treated with the high sub lethal concentration of Cyperdicot was recorded at the end of the 1st and 3rd weeks. It could be concluded from the present study that the toxic effect of Cyperdicot on fish is clear on their behavioural and histopathological aspects of gills, liver and kidney tissues while vitamin E has no amelioration effects on these parameters.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Adewolu MA, Adeniji CA, Adejobi AB (2008). Feed utilization, growth and survival of *Clarias gariepinus* fingerlings cultured under different photoperiods. J. Aquacul. 283:64-67.

ATSDR (Agency for Toxic Substances and Diseases Registry) (2005). Toxicologyic Information about Insecticides Used for Eradicating Mosquitoes (West Nile Virus Control). Department of Health and Human Services: ATSDR. Available at: http:www.astdr.cdc.gov/consultations/west _nile _ virus/fenthion.html.

Akinwole AO, Faturoti EO (2007). Biological performance of African

catfish (*Clarias gariepinus*) cultured in recirculation system in Ibadan. Aquac. Eng. 36:18-23.
Alazemi BM, Lewis JW Andrews EB (2012). Gill damage in the freshwater fish Gna thonemus petersii (family: Mormyradae) exposed to selected pollutants: an ultrastructural study. Environ. Technol. 17(2):225-238.
Al-Ghanim H, Al-Balawi A, Al-Akel F, Al-Misned Z, Ahmad H (2008). Ethological response and haematological and biochemical profiles of carp *(Cyprinus carpio)* exposed to trichlorfon. J. Food Agric. Environ. 6:473-479.
Ali D, Nagpurea N, Kumar S, Kumar, B. Kushwahaa B, LakraaW (2009). Assessment Of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem. Toxicol. 47:650-656.
Al-Juary AA, Al-Awward KH , Al-Torahi FA (2006). The effect of a-tocopherol on cholesterol and lipoproteins levels in rats. Pak. J. Nutr. 5:337-346.
Altinok I, Capkin E (2007). Histopathology of rainbow trout exposed to sublethal concentration of methiocarb, a carbmate pesticide, to rainbow trout. Toxicol. Pathol. 35:405-410.
APHA, AWWA, WPCF (2005). Standard Methods for the Examination of Water and Waste Water 21st, 401 Ed. American Public Health Associations, Washington, DC.
Bancroft J, Gamble M (2002). Theory and Practice of Histology Techniques. 5th (ed), Churchill Livingstone, London.
Blahova J, Modra H, Sevcikova M, Marsalek P, Zelinickova L, Skoric M, Svobodova Z (2014). Evaluation of biochemical, haematological and histopathological responses and recovery ability of common carp Cyprinus carpio) after acute exposure to atrazine herbicide. Biomed Res. Int. 2014:980948.
Boran H, Capkin E, Altinok I, Terzi E (2012). Assesement of acute toxicity and histopathology of the fungicide captain in rainbow trout. Exp. Toxicol. Pathol. 64:175-179.
Cengiz E, Unlu E (2003). Hisopathology of gills in mosquito fish, *Gambusia affinits* after long-term exposure to sub-lethal concentrations of Malathion. J. Environ. Sci. Health B 38:581-589.
Cengiz El, Unlu E (2006). Sublethal effects of commercial deltamethrin on the structure of the gills, liver and gut tissue of mosquito fish, Gambusia affinis: a microscopic study. Environ. Toxicol. Pharmacol. 21:246-253.
De Silva PM, Samayawardhena LA (2002). Low concentrations of lorsban in water result in far reaching behavioral and histological effects in early stages in guppy. Ecotoxicol. Environ. Saf. 53:248-254.
Elezaby M, El-Serafy S, Heckmann R, Sharf-Eldeen K, Seddek M (2001). Effect of some toxicants on the fresh water fish *Oreochromis niloticus.* J. Egypt Ger. Soc. Zool. 36:407-417.
Ervnest HA (2007). Text book of modern Toxicology (3rded).John Willy and sons, Inc. Hoboken, New Jersey ISBN 0-471-26508-X557.
Eto M (1979). Organophosphorous pesticides: Organic and Biological Chemistry. Boca Raton (FL): CRC Press.
Eyo JE, Levi CA, Asogwa CN, Odii EC, Chukwuka CO, Ivoke N, Onoja US, Onyeke CC (2013) .Toxicity and effect of Carica papaya seed aqueous extract on liver biomarkers of *Clarias gariepinus*. Int. J. Indig. Med. Plants 46:1301-1307.
Fanta E, Rios F, Romao S, Vianna A, Freiberger S (2003). Histopathology of the fish *Corydoras paleatus* contaminated with sub-lethal levels of organophosphorus in water and food. Ecotoxicol. Environ. Saf. 54:119-130
Finney DJ (1971). Probit analysis, 3rd edition Cambridge University Press. Cambridge. P 20.
Jiraungkoorskul W, Upatham E, Kruatrachue M, Sahaplong S, Vichasri-Grams S, Pokethitryook P (2003). Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromisniloticus).* Environ. Toxicol. 18(4):260-267.
Jungera P (2014). Fenthion safety summary for veterinary use of dogs, cats, cattles, sheep, goats and swine. Toxicity, poisoning, intoxication, overdose, antidote. Parasitipedia.net. Accessed 18th February 2015.
Kim K, Wang X, Choi S, Park G, Koo J, Bai S (2003). No synergistic effects by the dietary Supplementation of ascorbic acid, a-tocopherl acetate and selenium on the growth performance and challenge test of *Edwardsiellatarda*in fingerling Nile tilapia, *Oreochromis niloticus L.* Aquac. Res. 34:1053-1058.
Kumar M, Prasad MR, Srivastva K, Tripathi S, Srivastva AK (2010). Branchial histopathological study of catfish Heteropneustes fossilis following exposure to purified neem extract, Azadirachtin. World J. Zool. 5(4):239-243.
Kunjamma A, Philip B, Bhanu S, JoseJ (2008). Histopathological effects on *Oreochromis mossambicus*(Tilapia) exposed to chlorpyrifos. J. Environ. Res. Dev. 2:553-559
Machado RM, Fanta E (2003). Effects of the organophorous methyl parathion on the branchial epithelium of a freshwater fish *Melynniseoosevelti.* Braz. Arch. Biol. Technol. 46:361-372.
Mataqueiro M, Nakaghi S, de Souza J, da Cruz J, de Oliveira G, Urbinati E (2009). Histopathological changes in the gill, liver and kidney of pacu *(Piaratus mesopotamicus,* exposed to various concentrations of trichlorfon. J. Appl. Ichthyol. 25:124-127.
Miller DS (2002). Xenobiotic export pumps, endothelin signaling and tubular nephrotoxicantsa case of molecular hijacking. J. Biochem. Mol. Toxicol. 23:121-127.
Muralidharan L (2012). Haemato-Biochemical alterations induced by chronic exposure to fenthion in Cyprinus carpio. Trends Fish. Res. 1(3):19-25.
Muralidharan L (2014). Histopathological studies on carp (Cyprinus carpio) exposed to fenthion. Int. J. Adv. Res. 2:11-26.
Murty AS (1986). Toxicity of pesticides to Fish. CRC Press Inc Boca Raton.
Nwani CD, Ama UI, Okoh F,Oji UO, Ogbonyealu RC, Agha A (2013a). Acute toxicity of the chloroacetanilide herbicide butachlor and its effect on behavior of the freshwater fish Tilipia zili. Afr. J. Biotechnol. 12:499-503.
Nwani CD, Ekweme hi, Ejere VC, Onyeke CC, Chukwuka CO, Onas PS, Nwadinigwe AO (2015). Physiological effects of paraquat in juvenile African Catfish Clarias gariepinus (Burchel 1822). J. Coast. Life Med. 3(1):35-43.
Nwani CD, Ivoko N, Ugwu DO, Atama C, Onyishi GC, Echi PC, Ogbonna SA (2013b). Investigation on acute toxicity and behavioural changes in a freshwater catfish, Clarias gariepinus Burchell, 1822), exposed to organophosphorous pesticide, Termifos. Pak. J. Zool. 45(4):959-965.
Nwani CD, Lakra WS, Nagpure NS, Kumar, R, Kushwaha B, Srivastava SK (2010). Mutagenic and genotoxic effects on carbosulphan in freshwater fish in Chanaa Punctatus (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem. Toxicol. 48:202-208.
Oh HS, Lee SK, Kim YH, Roh JK (1991).Mechanism of selective toxicity of diainon to killifish (Oryzias latipes) and loach (Misgurmus anguillicaudatus).Aquat. Toxicol. Risk. Assess. 14:343-353.
Olurin K, Olojo E, Mbaka G, Akindele A (2006). Histopathological responses to gill and liver tissues of Clarias gariepinus fingerlings to the herbicide, glyphosate. Afr. J. Biotechnol. 5:2480-2487.
Oncu M, Gultekin F, Karaz E, Altunas I, Delibas N (2002). Nephrotoxicity in rats induced by chlorpryfos-ethyl and ameliorating effects of antioxidants. Hum. Exp. Toxicol. 21:223-230.
Packer L, Weber SU, Rerbach G, Weber R (2001).Molecular aspects of a-tocotrienol action and cell signaling. J. Nutr..131(2):369-446.
Peebua P, Kosiyachinda P, Pokethitiyook M, Kruatrachue M (2007). Evaluation of alachlor herbicide impacts on Nile tilapia *(Oreochromisniloticus)* using biochemical biomarkers. Bull. Environ. Contam. Toxicol.78:138-141.
Rahman MZ, Hossain Z, Mellah MFR, Ahmed GU (2002). Effect of Diazinon 60EC on *Anabustestudinus*, *Channapunctatus* and *Barbadesgomonotus*. NAGA: The ICLARM Quarterly 25:8-11.
Rao J, Begum G, Pallela R, Usman PR (2005). Changes in behavior and brain acetycholinesterase activity in mosquito fish, *Gambusia affinis*in Response to the sub-lethal exposure to chlorpyrifos. Int. J. Environ. Res. Public Health 2:478-483.
Rauf A, Arain N (2013).Acute toxicity of diazinon and its effects on haematological parameters in the Indian carp, *Cirrhinus mrigala* (Hamilton).Turk. J. Vet. Anim. Sci. 37:535-540.
Reish DJ, Oshida RS (1987). Manual of methods in aquatic environment research, Part 10. Short-term static bioassay.FAO Fish. Tech. Paper 247, 62.

Roberts RJ (2001). Fish Pathology 3rd (Ed). W.B. Sauners. New York.
Roy S, Bhattacharya S (2006). Arsenic-induced histopathology and synthesis of Stress proteins in liver and kidney of *Channa puntatus*. Ecotoxicol. Environ. Saf. 65(2):218-229.
Santos LH, Araujo AN, Fachini A, Pena A, Deleure-Matos C, Montenegro MCB (2010). Ecotixicogical aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard Mat. 175:45-95.
Scheil Vl, Zurn A, Kohler H, Triebskorn R (2009). Embryo development, stress protein (Hsp70) responses, and histopathology in Zebrafish (Daniorerio) following exposure to nickel chloride, chlorpyrifos, and binary mixtures of them. Environ. Toxicol. 25:83-93.
Teh S, Hung S, The F, Deng D, Werner I (2005). Sublethal toxicity of orchard storm water runoff in Sacramento splittail (*Pogonichthys macrolepidotus*) larvae. Mar. Environ. Res. 59(3):203-216.
Todd NE, Van Leeuwen MV (2002). Effects of Sevin (Carbaryl Insecticide) on Early Life Stages of Zebrafish (*Daniorerio*). J. Ecotoxicol. Environ. Saf. 53:267-272.
West G (1990). Methods of assessing ovarian development in fishes-a review. Aust. J. Mar. Freshw. Res. 41(12):99-222.
White A, Fietcher TC (1985). Seasonal changes in serum glucose and condition of the Plaice, Pleuronectes platessa L. J. Fish Biol. 26:755-764.
Wiieyaratne W, Pathiratne A (2006). Acetyl cholinesterase inhibition and gill lesions in *Rasbora caverii*an indigenous fish inhabiting rice field associated water bodies in Sri Lanka. Ecotoxicology 1:609-619.
Yang I, Zha J, Li Z, Wang Z (2010).Atrazaine affects Kidney and adrenal hormones (AHS) related genes expressions of rare minnow (Gobiocypris rarus). Aquat.Toxicol. 97:204-211.