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The Influence of Cypermethrin on the Biochemical Parameters of *Clarias gariepinus*

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Abstract

The experiment was conducted to evaluate the effect of cypermethrin, a synthetic pyrethroids on juveniles *Clarias gariepinus* (catfish). The effect was assessed based on the comparing the results of biochemical examinations (glucose, Aspartate and alanine transaminase enzymes, total protein, albumin, globulin, and cholesterol) in the blood of control and experimental group exposed to three nominal concentrations of cyperking, 10EC Pesticide Preparation (active substance 100mg/l) of cypermethrin in a static non-renewal bioassay for 96 hours. Fish exhibited progressive change in behaviours. Biochemical evaluation showed significant higher values ($P \leq 0.05$) for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Albumin and glucose, Total protein, globulin and cholesterol showed a significant reduction ($P \leq 0.05$) with significant difference ($P \leq 0.05$) as compared to the control. Thus, concluding that cypermethrin is toxic to juveniles of *Clarias gariepinus*. It is therefore recommended that awareness of the effect of pollution in aquatic ecosystem should be conducted.

Keywords: Effect, Biochemical, Cypermetrin, Juvenile, Catfish

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Introduction

Water covers about 70% of the earth, and happens to be the most essential natural resource (Terry, 1996). Despite this awareness of the essentiality of water, humans have ignored its importance by polluting it (Meena *et al.,* 2005); thereby posing great risk to its aquatic biota. The use of mechanical and biological means of pest control has been abandoned for an easier and faster use of agricultural pesticides for control of pest, in order to generate massive crop yield, so as to meet-up with the ever-growing human population (Pal *et al.*, 2009). The careless and indiscriminate use of these synthetic pesticides through run-off and spray drift, has led to the global pollution of water bodies (Ngidlo, 2013), leading to mortality of aquatic organisms and a general deterioration of aquatic ecosystem (Gupta *et al.,* 2013). Residues of these toxic chemicals found in water, sediments, fish and other aquatic biota can pose a risk to organisms, predators and human being. 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).

Cypermethrin is globally used for the control of pest, in order to improve food productivity (Usmani and Knowles, 2001), but their use could create a risk of food contamination as well as affects the non-target aquatic species like vertebrates and invertebrates (Das and Mukherjee, 2003). It is a synthetic pyrethroid, with a very high activity and stability (Khan *et al.,* 2006). Of all the pesticides available in the market, pyrethroids make about 25% of global pesticide sale (Zhang *et al*., 2011). They contain heavy metals which enters the water bodies, thereby affecting the growth, physiology, reproduction and survival of fish (Hayat *et al.,* 2007). Agricultural run-off happens to be the main route of entry of cypermethrin into the aquatic eco-system, and this affects the non-target species (Arjmandi *et al.*, 2010).

Clarias gariepinus or African sharp tooth catfish is a species of catfish of the family *Clariidae,* the air breathing catfishes. They are indigenous to the inland waters of most of African countries and also an ecological important and commercially valued fish for the Nigerian fishing industry (Ita, 1980). Pesticides are substances used to control organisms, including insects, water weeds, and plant diseases. Pesticides usage in agricultural fields to control pests is extremely toxic to non-target organisms like fish and affect fish health through impairment of metabolism, sometimes leading to mortality (Shankar *et al.,* 2013). Increased human population with rapid pace of industrialization induced problem of disposal of waste waters. The domestic wastes and untreated or partially treated industrial effluents, supplemented with pollutants like heavy metals, agrochemicals and many organic compounds, have greatly contributed to massive fish death of aquatic ecosystems (Pazhanisamy and Indra, 2007).

Studies on pesticides toxicity in fish has shown that at chronic level, it causes diverse effects including oxidative damage, inhibition of acetyl cholinesterase (AChE) activity, histopathological changes as well as developmental changes, mutagenesis and carcinogenicity. Agrochemicals are categorized according to their target use to many groups such as Insecticides, Fungicides, Herbicides, Rodenticide, Nematicides, Acaricides, Molluscicides, Homicides, Ovicides and so. The three major agrochemicals are herbicides (weed control), insecticides (insect control), and fungicide (mycotic control). Nematicides are agrochemicals used to control soil, leaf and stem-dwelling nematodes (round worms). Acaricide is an agrochemical that control mites and ticks (Louis and Diana, 2013).

Agrochemicals capacity to harm fish and aquatic animals is largely a function of its toxicity, exposure time, dose rate, and persistence in the environment. A lethal dose is the amount of agrochemical necessary to cause death because not all animals of a species die at the same dose, a standard toxicity dose measurement called a lethal concentration 50 (LC50) is used. This concentration of agrochemical that kills 50% of a test population of fish within a set period of time is usually determined after 24 to 96 hours. Exposure of fish and other aquatic animals to agrochemicals depends on its biological availability (Bioavailability), bioconcentration, biomagnifications, and persistence in the environment.

Fish and aquatic animals are exposed to agrochemicals in three primary ways. Dermal, direct absorption through the skin by swimming in pesticide-contaminated waters, Inhalation, by direct uptake of agrochemicals through the gills during respiration, and orally, by drinking agrochemicals contaminated water or feeding on contaminated prey.

The remarkable increase in the use of Cypermethrin either for house-holds or for agricultural purposes has resulted in higher concentration of these substance in a variety of environments, thus causing unintended damage to the aquatic life. Population of aquatic organism are affected by exposure to insecticides that find their way to the rivers, ponds and other water bodies through spray drift or through runoff from agricultural fields. Therefore, the aim of the study was to investigate the effect of cypermethrin on the behavioral and biochemical parameters of blood in juveniles *Clarias gariepinus.*

Materials and Methods

Sample Collection

Live specimens of 60 juveniles *Clarias gariepinus* with average length 11.19cm (9.0 - 13.9cm) and an average weight 15.02g (3.65 - 28.96g) were purchased from Excel fish farm, Kalshingi, Akko Local Government, Gombe State. Thereafter, the samples were transferred to the Hydrobiology and Fishery Laboratory, Zoology Department, Faculty of Science Complex, Gombe State University, Gombe. The fish were acclimatized in laboratory conditions for seven days during which they were fed with commercial floating pellets at 10% of their body weight. Unconsumed feed and faecal were removed and water replenished regularly as recommended by Oyelese and Faturoti, (1995).

Test Pesticide used

Agricultural grade cypermethrin, Trade name cyperking (10% E.C) was used for the experiment.

Experimental Design

Five *Clarias gariepinus* juvenile per concentration of toxicant were used with 3 replicates each for 96h. Based on this, four concentrations (0.0, 0.02, 0.04, 0.06mg/l) of the insecticide were prepared and tested on the *Clarias gariepinus* juveniles for the definitive test. Five acclimated fish were used in each aquarium containing different concentrations of cypermethrin as well as in the control (Rahman *et al*, 2002). At the beginning of the tests and every one-hour behavioural changes and the number of dead fishes were recorded. Other external changes in the body of the fish were observed accordingly.

Sampling Techniques

Blood sampling

Blood samples were taken from fish by cardiac puncture using plastic disposable syringes. Blood of two fishes from each aquarium was used for obtaining 16 blood sample which was used for biochemical analysis using the following principles.

Albumin test principle

Colometric assay

At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye to form a blue-green complex.

Albumin +BCG $\xrightarrow{pH}{}^{4.1}$ Albumin-BCG complex

The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically.

Aspartate aminotransferase (AST) test principle

This assay follows the recommendations of the international federation for clinical chemistry (IFCC), but was optimized for performance and stability.

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^{+}.

Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

L-Aspartate + 2-oxoglutarate $\xrightarrow{AST}$ oxaloacetate + L-glutamate

Oxaloacetate + NADH + H^{+} $\xrightarrow{MDH}$ L-malate + NAD^{+}

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Alanine aminotransferase (ALT) test principle

This assay follows the recommendations of the International federation for clinical chemistry (IFCC), but was optimized for performance and stability.

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^{+}. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

L-alanine + 2-oxoglutarate $\xrightarrow{ALT}$ pyruvate + L-glutamate

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

Total protein (TP) test principle

Colorimetric assay

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.

$$\text{Protein} + \text{Cu}^{2+} \xrightarrow{\text{Alkaline solution}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration which can be determined photometrically.

Statistical Analysis

All the statistical analyses were conducted via SPSS program version 22. The obtained data were subjected to one-way analysis of variance (ANOVA) to evaluate the effect of cypermetrin administered at different concentrations. Differences between means were tested at a 5% probability level using Tukey test as a post-hoc test.

Results

Behavioral changes were observed in Juvenile *Clarias gariepinus* exposed to different concentrations of cypermethrin. The control fish behaved in a natural manner (that is, they were active with well-coordinated movements) and were observed to respond to alert of the slightest disturbance, while those exposed to cypermethrin exhibited abnormal behaviours such as erratic swimming movements, loss of balance, restless, secrete excess mucus all over their bodies, gulping air at the surface, swimming on the water surface, rolling movement, back swimming, sudden quick movement, loss of appetite, reddish vent and finally the fish sank to the bottom of the water and died with their mouths open.

Table 1: The table below shows the effect of cypermethrin on the behaviors of juveniles *Clarias gariepinus* at different time intervals

BEHAVIORAL CHANGES	8hr	16hr	24hr	32hr	40hr	48hr	56hr	64hr	72hr	80hr	88hr	96hr
Loss balance	-	-	-	-	-	+	+	-	-	-	-	-
Erratic swimming	-	-	-	-	-	-	+	-	-	-	-	-
Air gulping	-	-	-	+	-	-	-	-	-	-	-	-
Restlessness	-	-	-	-	+	-	-	-	-	-	-	-
Mortality	-	+	-	-	-	-	+	+	-	-	-	-
Sudden quick movement	-	-	-	-	-	+	+	-	-	-	-	-
Excessive mucus secretion	-	-	-	-	+	+	+	-	-	-	-	-
Rolling movement	-	-	-	-	-	+	-	-	-	-	-	-
Back swimming	-	-	-	-	-	-	+	-	-	+	-	-
Discoloration	-	-	+	-	-	-	-	-	-	-	-	-
Abnormal behavior	-	+	+	+	+	+	+	+	+	-	-	-

Key: + = Shows the behavioral changes occurring at different hours, hr = hours

Table 2: Effects of Cypermethrin on Biochemical Parameters of Blood in Juveniles of *Clarias gariepinus*

	Mean ± SE of blood parameters (mg/L)						
	Glucose	AST	ALT	TP	ALB	Globulin	Cholesterol
0.00mg/L	2.10 $\pm0.10^{b}$	391.03 $\pm0.23^{b}$	131.20 $\pm0.23^{c}$	23.27 $\pm0.26^{a}$	2.03 $\pm0.04^{c}$	20.97 $\pm0.15^{a}$	130.33 $\pm1.45^{a}$
0.02mg/L	0.98 $\pm0.06^{c}$	372.40 $\pm0.17^{c}$	151.13 $\pm0.19^{b}$	17.10 $\pm0.25^{c}$	5.20 $\pm0.01^{b}$	11.97 $\pm0.15^{b}$	97.33 $\pm0.88^{c}$
0.04mg/L	2.73 $\pm0.06^{a}$	751.73 $\pm0.12^{a}$	184.07 $\pm0.20^{a}$	20.23 $\pm0.38^{b}$	7.50 $\pm0.01^{a}$	12.00 $\pm0.06^{b}$	117.00 $\pm1.15^{b}$
p-value	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05

The Table 2 presents the results of the effects of different concentrations of cypermethrin on some blood biochemical parameters of juveniles *Clarias gariepinus*. Means with different superscripts down the row are significantly different from each other at $\alpha = 0.05$.

The result on table 2 shows that the fish treated with 0.04Mg/L concentration of cypermethrin has increase the glucose level (2.73 ±0.06^{a}) compared to the control (2.10 ±0.10^{b}) and 0.02Mg/L of cypermethrin concentration has dirastically decrease the glucose level (0.98 ±0.06^{c}) compared to the control (2.10 ±0.10^{b}). Therefore there is a significant difference in the glucose level of fish blood between the three blood samples.

The results on AST showed that the sample of fish blood treated with 0.04Mg/L concentration of cypermethrin has greatly increased the AST level (751.73 ±0.12^{a}) compared to the control (391.03 ±0.23^{b}) but there is no significant difference in the AST level between 0.02Mg/L of cypermethrin concentration and the control. However, there was a significant increase of ALT in treatments with 0.02mg/l and 0.04mg/l concentration of cypermethrin. The ALT level in blood increases as the concentration of cypermethrin increases.

The results of total protein in (Table 2) showed that treatment with 0.02mg/l and 0.04mg/l shows significant decrease in the total protein with mean values (17.10 ±0.25^{c} and 20.23 ±0.38^{b}) IU/L compared to the control with mean value (23.27 ±0.26^{a}) IU/L respectively. The result of Globolin in (Table 2) shows that there is no significant difference in treatments with 0.02mg/l and 0.04mg/l concentrations of cypermethrin with mean values (11.97 ±0.15^{b} and 12.00 ±0.06^{b}) IU/L respectively but there is high significant decrease in both samples compared to the control with (20.97 ±0.15^{a}) IU/L as the mean value. The result of cholesterol concentration in (Table 2) shows that there is a significant decrease between treatments with 0.02mg/l and 0.04mg/l concentration of cypermethrin with mean values (97.33 ±0.88^{c} and 117.00 ±1.15^{b}) IU/L compared to the control which has a mean value of (130.33 ±1.45^{a}) IU/L.

Discussion

Experimental studies using live organisms plays an important role in developing knowledge and better understanding of life processes, habitats and life forms. Fishes exist in large numbers and have diverse physiological, behavioral, and ecological importance, occupying virtually many aquatic habitats and serves as source of food for both humans and other aquatic animals and also serves as important indicators of environmental quality. Thus, pesticides from Agricultural run-offs, spray drift or direct application are known to reduce the survival, growth and reproduction of fish producing many effects on them. Thus, this research will provide a baseline data concerning the effect of cypermethrin on *Clarias gariepinus* in Gombe.

In the present study the control fish behaved in a natural manner (they were active with well-coordinated movements). They were alert to the slightest disturbance, but the fish exposed to cypermethrin exhibited erratic swimming movements and loss of balance due to inhibition of AchE activity, leading to accumulation of acetylcholine in the cholinergic synapses, leading to hyperstimulation (Mushigeri and David, 2005). They slowly became restless and secreted excess mucus all over their bodies. Mucus secretion in fish forms a barrier between the body and toxic media thereby probably reduces contact with the toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by Rao (2006) and Parma de croux *et al*. (2002) in *Prochilodus lineatus* under monocrotophos stress. Gulping air at the surface, swimming on the water surface, was seen on the first day exposure periods and continued the same more intensely, which is in accordance with the observations made by Ural and Simsek (2006). Gulping of air may help to avoid contact with the toxic medium. Surfacing phenomenon (significant preference) of upper layers in the exposed group might be the result of the need for higher oxygen levels during the exposure period (Katja *et al*., 2005). Other behavioural changes such as loss of appetite, rolling movement, sudden quick movement, back swimming was also recorded and finally the fish sank to the bottom with the least opercula movements and died with their mouths open. Compared to the control they were found to be under stress and the fish's bodies became lean towards the abdomen position but this was not fatal, Leanness in fish indicates a reduced amount of dietary protein consumed by the fish under pesticide stress which is immediately utilized and not stored as body mass (Kalavathy *et al*., 2001).

Biochemical parameters are sensitive index to change due to pesticide toxicity and can constitute important tools in toxicological studies (Siddiqui, 2004) blood biochemical parameters with significant variations ($P \leq 0.05$) were observed for the glucose, Aspartate Aminotransferase, Alanine Aminotransferase, Total protein, Albumin, globulin and cholesterol.

Serum glucose level was discovered to significantly ($P \leq 0.05$) increase in 0.04mg/l and decreases in 0.02mg/l compared to the control. The reduction in serum glucose level was mainly due to rapid utilization by the organism as a consequence of metabolic stress. Associated finding was reported by Singh *et al.*, (2010). The authors studied the effect of Phorate on serum biochemical parameter of snake head fish (*Channa punctatus*), it was discovered that serum glucose decreased significantly ($P \leq 0.05$) as compared to the control. It has been reported that the increased blood glucose is usually observed in fish under undesirable conditions and it helps the animal by providing energy substrates to vital organs to cope with the increased energy demand (Banaee *et al*., 2008). Elevation of blood glucose levels was widely used as a secondary marker of a stress response (Saha and Kaviraj, 2009). On the basis of our results it is clear that 0.04mg/l of cypermethrin acts as a stressor in fish. Increase in blood glucose levels have been reported in *Heteropneustes fossilis* (Banaee *et al*., 2008) and *Cyprinus carpio* (Varanka *et al*., 2001) after exposure to cypermethrin, copper sulfate and tannic acid, and diazinon, respectively. Also, changes in blood glucose have been suggested as useful general indicator of stress in fish (Nemcsok and Boross, 1981) reported that blood glucose appeared to be a sensitive indicator of environmental stress in fish. The stress related hyperglycemia reported in many species of *teleosts* is mediated mainly by the effects of catecholamine's (CAs) on glucose release from the liver, the main carbohydrate store in fish with epinephrine being more potent than norepinephrine (Van *et al*., 1995). Results shows that the activities of transaminases, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) increased significantly ($P \leq 0.05$) at higher concentrations compared to the control. This indicated stressed based tissue impairment (Svoboda *et al*., 2001). Increased activities of both transaminases indicated amplified transamination processes. An increase in transamination occurs due to amino acid input into the TCA cycle, in order to cope with the energy crisis during pyrethroid-based stress (Philip *et al.,* 1995). Increase in transaminase activities was also reported by Wegwu and Omeodu *et al.,* (2010). It was discovered that *Clarias gariepinus* exposed to aqueous extract of Nigeria crude oil experienced increased transaminase activities of both AST and ALT in the blood plasma as compared with control fish. However, a few studies have suggested that plasma enzyme evaluation in fishes may not be as straightforward as it is in mammals. For example, the high ammonia levels of fish may lead to high transaminase activities; therefore, the increase in activities may be associated with liver disease or changes in plasma ammonia concentration. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are found in the liver (Srivastava *et al*., 2004) heart, skeletal muscle (Petrovic *et al*., 1996), kidney, pancreas, spleen, erythrocyte, brain and gills (Bhattacharya *et al*., 2008). When diseases or injuries affect these tissues, the cells are destroyed and these enzymes are released into plasma. In this sense, if the cellular injury is chronic AST and ALT levels will remain elevated (Rao, 2006). Increased activities of AST and ALT were observed in plasma of *Channa punctatus* (Agrahari *et al*., 2007) exposed to organophosphorus pesticides. Banaee *et al*. (2008) have reported increased levels of AST and ALT followed by the exposure of *Common carp* to diazinon. Results shows that total protein (TP) and cholesterol (CHOL) decreases significantly ($P \leq 0.05$) in the exposed groups compared to the control. In contrast to this study, increase in total protein and cholesterol could be attributed to necrosis of the liver by the toxicant which leads to the impairment in the metabolism of this parameter thus resulting in significant increase in the concentration of the parameter. Similar result was recorded by Yaji *et al.,* (2011) which discovered a significant increase in total protein and cholesterol level in the blood of *Oreochromis niloticus* exposed to cypermethrin insecticide. This result was also corroborated by the findings of Faisal, (2003). The result of this research also shows that Albumin increases significantly ($P \leq 0.05$) as the concentrations of cypermethrin increases. Albumin helps in transportation of lipid in fishes (Andreeva, 1999) and also helps in the general metabolism of fishes. The rise in albumin concentration in animals due to loss through urine or faeces or through break down may result in impaired synthesis this is in accordance to the findings of Nguyen, (1999). On the other hand, Globulin decreases significantly ($P \leq 0.05$) between the control and that of the exposed but there is no significant difference between the globulin exposed at different concentrations. Decrease in Globulin levels have been reported in immune deficiency. In fact, the effect of diazinon on the immune system of tilapia (*Oreochromis niloticu s*) and beluga sturgeon (*Huso huso*) as immunomodulator has been studied by other authors (Khoshbavar-Rostami *et al*., 2006). It is therefore recommended that: greater awareness of the effect of pollution in aquatic ecosystem, adopt biological means of pest control, creation of channels in farms and Recycling of toxicated waste waters should also be conducted regularly.

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