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Research Article

EFFECTS OF SHORT OR LONG-TERM EXPOSURE OF DITHIOPYR ON CERTAINE BLOOD, GROWTH AND TISSUE BIOCHEMICAL PARAMETERS IN CATFISH (*CLARIAS GARIPINUS*)

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Abstract

In the current study the effects of acute or chronic exposure of dithiopyr herbicide (weed controllers) in Catfish (*Clarias garipinus*) was studied. LC_{50} was determined, and recorded as 460 μgl^{-1}. Acute and chronic effects on certain blood, growth and tissue biochemical parameters were studied as a function of exposure time. Exposure of *Clarias garipinus* to acute or chronic toxicity of the herbicide resulted in significant increase in glucose, total cholesterol, creatinine, uric acid and Lactate dehydrogenase (LDH) in the herbicide-treated group, while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed a fluctuating activities in both exposures. In contrast, there was significantly ($P<0.01$) lower values for liver and muscle glycogen in acute toxicity as compared with the control group. Also, in herbicide exposed fish a significant reduction in growth parameters was recorded. These alterations could be attributed to the changes in the metabolic pathways of the studied fish that meight be takes place as a secondary response in the fish to provide energy for the "fight-or-flight" reaction and to compensate the high energy demand. This study implies the importance of previous knowledge about the susceptibility of cultured fish species before using herbicides.

Key words: Toxicity; dithiopyr; biochemical; growth; *Clarias garipinus*.

Introduction

Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish (Josef *et al*., 2011).

Pesticides affect fish directly by accumulation in their body. They cause serious impairment in metabolic, physiological and structural changes in different organs. It may affect fish indirectly by transfer to the next tropic level of food chain. The accumulation of pesticides in the tissues of fishes can result in chronic illness and cause potential damage of population. Fish are able to accumulate and retain pesticides and other pollutants from their environment. Accumulation of pesticides in the tissue of fish is dependent upon exposure concentration as well as other factors such as salinity, temperature, hardness and metabolism of fish (Mukesh, 2013).

Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to different toxic substances (De la Torre *et al.*, 1999). Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival (Abou El-Naga *et al.*, 2005).

Fish are able to uptake and retain different xenobiotics dissolved in water via active or passive processes. They can be used to detect and document pollutants released into their environment. The interest in understanding the physiological mechanisms associated with fish responding to environmental stresses has been growing (Parvez *et al.*, 2006).

Studying the biological responses to environmental chemicals through the use of biomarkers provides means to understand environmental levels of pollutants in biological terms, and more importantly, it can be used for the assessment of environmental quality in specific situations (Praveen *et al.*, 2012).

The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman *et al*., 2002). Aquatic distribution of pesticides affect a wide range of non-target organisms like invertebrates, mammals, birds and fishes especially those inhibiting the aquatic environment (Burkepile *et al*.,2000).

Teleost fish like *Clarias sp.* are good indicators of contamination by pollutants because their biochemical responses are quite similar to mammals (Wasu *et al*.,2009). Dithiopyr is herbicide weed controller that are heavily used in paddy rice fields in different countries. There are few reports on long-term effects of this herbicide (Abbas *et al*., 2007). Data about the effects of dithiopyr on biochemical parameters in fish is still scarce. The sublethal effects of herbicides on physiological and biochemical levels is very important for delineating fish health status, for understanding future ecological impact, and for the protection of human health.

For that purpose, certaine blood and tissue biochemical alterations as well as growth indices in Catfish (*Clarias garipinus*) were monitored in response to short (4 days) and prolonged (60 days) exposure to acute and sublethal concentrations of dithiopyr.

Material and methods

Fish

The present study was carried out on the Catfish (*Clarias garipinus*). A total of 225 healthy fish of both sexes were collected from the nursery ponds of El-Jomoom Fish Farm, Jeddah, Kingdom of Saudi Arabia. The average body weight and length were 38.2±1.2 gm and 14.25±0.5 cm respectively. Fish were immediately transferred to the laboratory in glass tanks (2000 liter) with aerated dechlorinated tap water at 25±0.9°C. The fish were acclimatized for one week perior to the beginning of the experiment.Water quality characteristics were measured every day by the methods described by APHA (1992).

For the LC_{50} and acute experiments fish were not fed for 48 hours prior to and during the experiments. For the chronic experiments fish were fed 25% commercial fish diet at a ratio of 5% of their body weight/day.

Herbicide

Dithiopyr is a pyridine herbicide used in this study as a commercial preparation that contains 25% active ingredient.

Determination of 96 hours lethal concentration dose (LC_{50}) for dithiopyr

Preliminary screenings were carried out to estimate the concentration of the used herbicide, which is most likely to cause 50% mortality as (LC_{50}) for 96 hours exposure to determine the appropriate testing range of concentrations. This task was done according to the procedure described by Johnson and Finley (1980) and EPA (1985). Toxicity test showed that the 96-hr LC_{50} values of *Clarias garipinus* were (460) μgl^{-1} for the herbicide dithiopyr.

Experimental design

Fish were divided into three groups each represented in a triplicate manner. Fish were distributed in glass aquaria (40X80X60 cm, capacity 100L) at a rate of 25 specimens of each fish species/aquarium and treated as follows:

Group I: Control group, fish were reared in dechlorinated tap water (same conditions without herbicide) and sampled at each time.

Group II: fish were exposed to 100% of 96 hours LC_{50} of dithiopyr for 4 days (Acute / short term exposure).

Group III: fish were exposed to (1/20) of 96 hours LC_{50} of dithiopyr for 60 days (chronic / Long term exposure).

Water for each group was periodically changed every 3 days. The duration of the experiment was 12 weeks. The exposed fish were kept under proper observation during the period of experiment for any external clinical signs, PM lesions or deaths according to Amlacher (1970).

Blood and tissue sampling

Blood samples were collected from the fish heart through cardiac puncturing with a needle and syringe, spun in a centrifuge for 5 minutes at 5000 rpm. Samples were taken daily in case of acute exposure (Group II) and every two weeks intervals in chronic exposure (Group III). Serum was obtained by centrifugation and stored at -20 °C for analysis. After decapitation of fish a piece of white epiaxial muscle and liver were taken for further analysis.

Biochemical analysis

The concentration of serum glucose was measured using the GOD-PAP method (Enzymatic Colorimetric method) according to Barham and Trinder (1972). Serum total cholesterol level was measured colorimetrically according to Watson (1960). Activities of Serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined colorimetrically by transaminases kits according to the method described by Reitman and Frankel (1957). Serum Creatinine level was measured colorimetrically as described by Henry (1974). Uric acid concentration was measured using enzymatic determination (after deproteinization) according to Barham and Trinder (1972). Activity of lactate dehydrogenase (LDH) was determined by the enzymatic reaction described by Bergmyer (1974).

Liver and muscle glycogen

Glycogen levels in the muscles and liver were analysed using anthrone technique (Wedemeyer and Yasutake, 1977).

Growth indices

Weight gain and growth rate

The body length and weight was recorded every two weeks and weight gain was determined as the difference between the initial and final weights of fishes at the end of the experimental period (12 weeks).

Condition factor (k)

"K" factor was calculated for individual fish, every two weeks, from the formula recommended by Schreck and Moyle (1990): $K = W/ L^3 X 100$

Where: W: is the wet weight in g. L: is the total length in cm.

Statistical analysis

The results were statistically analyzed using analysis of variance (F-test) and Duncan's multiple comparison tests to evaluate the comparison between means at $P< 0.05$ (Duncan, 1955). Statistical analyses were performed using a computer program SPSS, version 14 for Windows.

Results

General observations

Fish were apparently negative to any parasites and bacterial or viral diseases. No pathological alterations were detected. Toxicity signs in treated fish were more or less similar in both short-term and long-term herbicide exposed groups. Severity was confirmed with the long-term exposure in chronic case. The initial clinical signs were difficulties in respiration manifested by increasing mouth movement and surfacing, in addition to nervous manifestations as vigorous erratic swimming abnormalities, surface to bottom movement, vigorous jerks and restlessness. The fish started showing gradual loss of appetite.

The effective dose (LC_{50})

Toxicity tests showed that the 96-hr LC_{50} values for *C. garipinus* were (460) μgl^{-1} for the herbicide dithiopyr. Controls did not have any fish deaths.

Biochemical analysis

Table (1) shows the acute or chronic effects of dithiopyr on biochemical parameters in exposed fish. A highly significant ($P<0.01$) increase in serum glucose level during the exposure time was recorded. Total cholesterol level was increased significantly ($P<0.01$) till reach to higher values at the end of acute and chronic exposure time in case of treatment. AST activity showed a general trend to increase significantly ($P<0.01$) during the acute and chronic exposure time of treatment but at the end of the two experiments it showed a general trend to decrease as compared to the control values. On the other hand, ALT activity showed a similar pattern like AST in case of acute exposure, while showed a highly significant ($P<0.01$) increase in fish exposed to chronic concentration of pesticide. Also, a significant increase ($P<0.01$) in creatinine, uric acid concentrations and Lactate dehydrogenase activity (LDH) were recorded with increasing the time of exposure as compared to control group .

Growth indices

Figure (1) indicates that fish exposed to acute or chronic toxicity had a reduction in body weight gain comparing to the control group during the entire experimental period. On the other hand, averages of condition factor (K) values had no significant difference between treatments.

Liver and muscle glycogen

Figures (2) shows that Liver and muscle glycogen content was significantly decreased in fish exposed to short term toxicity as compared to control fish ($P<0.01$). On other hand, their content did not show any significant differences in long term exposure as compared to that of control fish (Fig.3).

Discussion

In the current study, the most important behavioral changes observed in *C. garipinus* were similar to those reported by Mai (2012) with the catfish *Clarias Gariepinus* exposed to phenol. The intoxicated fish exhibited abnormal behavioral changes in the form of rapid operculum movement, swimming at the water surface and gasping from the water as a respiratory distress. Later, they showed signs of nervous manifestations like less activity and remaining motionless on the aquarium bottom ascribed to the effect of Diazinone 60 on *Oreochromis niloticus* (Talaat *et al*., 2014).

Blood biochemical parameters

Blood glucose is a sensitive and reliable indicator of pollutants causing environmental stress in fish (Mekkawy *et al*., 2011). At the current study, fish exposed to acute and chronic concentrations of herbicide showed a significant increase ($P<0.05$) in serum glucose level during the exposure time than that of the control.

Increase in serum glucose levels in fish under stress was reported by Cicik and Engin (2005).

The stress hormone cortisol has been shown to increase glucose production in fish, by both gluconeogenesis and glycogenolysis, and likely play an important role in the stress-associated increase in plasma glucose concentration (Sweilum, 2006; El-Sayed and Saad, 2008).

Also, Matos *et al*. (2007) reported that hyperglycemic was indication of a disruption in carbohydrate metabolism, possibly due to enhanced glucose 6-phosphatase activity in liver, elevated breakdown of liver glycogen, or the synthesis of glucose from extrahepatic tissue proteins and amino acids. Glucose level might elevate to cope with the increased energy demand during pesticide-induced which is important pathway for the recovery from stress (Özgür *et al.*, 2011). Aditionally, The increase of serum glucose could be attributed to the decrease of oxygen consumption which

is reflected by the rapid respiration of fish and floating at the surface of water gasping for more oxygen (Mekkawy *et al*., 1996).

Cholesterol content in the blood is linked to lipid metabolism and depends on the calorific value of the feed. Yousef *et al*. (2003) reported that changes in blood cholesterol levels are related to changes caused by pesticides in the permeability of hepatic cells and that accumulation of pesticides in the liver disrupt lipid metabolism and increase serum cholesterol levels.

Table (1): Blood biochemical parameters of Catfish (*Clarias garipinus*) after short and long-term exposure to dithiopyr.

Short-term exposure							
parameters / Time	**Glucose (mg/dl)**	**Cholesterol (mg/dl)**	**AST (U/l)**	**ALT (U/l)**	**Creatinine (mg/dl)**	**Uric acid (mg%)**	**LDH (U/l)**
Control	70.75±4.86^{a}	150.20±4.11^{a}	33.27±1.23^{a}	13.83±0.71^{a}	0.875±0.02^{a}	22.67±1.05^{a}	724.78±33.76^{a}
1 day	78.85±3.91^{ab}	163.15±3.05^{b}	39.28±1.22^{b}	16.05±0.45^{b}	0.941±0.03^{b}	24.01±0.82^{a}	785.81±23.11^{ab}
2 days	84.20±2.26^{b}	167.91±2.16^{b}	49.19±2.19^{c}	17.46±0.38^{b}	0.973±0.09^{b}	28.91±0.69^{b}	825.60±14.88^{bc}
3 days	106.41±2.89^{c}	181.08±2.31^{c}	42.07±1.03^{b}	18.31±0.31^{b}	1.023±0.29^{c}	32.12±1.81^{c}	879.91±6.76^{cd}
4 days	124.15±4.23^{d}	197.14±4.10^{d}	28.79±1.35^{a}	12.98±0.39^{a}	1.074±0.15^{d}	33.16±1.56^{c}	946.31±8.97^{d}
F-value	27.17**	25.90**	17.98**	11.04**	26.00**	28.49**	13.51**
Long-term exposure							
Parameters / Time	**Glucose (mg/dl)**	**Cholesterol (mg/dl)**	**AST (U/l)**	**ALT (U/l)**	**Creatinine (mg/dl)**	**Uric acid (mg%)**	**LDH (U/l)**
Control	70.75±4.86^{a}	150.20±4.11^{a}	33.27±1.23^{a}	13.83±0.71^{a}	0.875±0.02^{a}	22.67±1.05^{a}	724.78±33.76^{a}
2 weeks	78.12±2.89^{ab}	156.32±4.19^{a}	37.15±0.98^{b}	15.89±0.38^{b}	0.919±0.08^{ab}	26.09±0.69^{b}	775.72±24.75^{ab}
4 weeks	88.57±1.74^{b}	158.21±2.88^{a}	44.79±2.03^{c}	17.11±0.29^{b}	0.956±0.01^{b}	29.22±0.46^{c}	821.53±12.89^{bc}
6 weeks	99.51±2.35^{c}	170.35±2.90^{b}	38.77±0.88^{b}	16.83±0.17^{b}	1.090±0.04^{c}	32.96±0.75^{d}	862.23±10.91^{c}
8 weeks	109.15±3.09^{c}	185.09±3.81^{c}	31.25±1.97^{ad}	19.07±0.59^{c}	1.016±0.01^{c}	34.21±0.34^{e}	938.63±19.87^{d}
F-value	19.92**	9.74**	13.52**	10.77**	11.83**	33.72**	13.25**

- Data are means ± SE. (**) highly significant (P<0.01). Number of fish for each group= 5. Means with the same letter in the same column for each parameter are not significantly different (P>0.05); otherwise, they do (Duncan's multiple range test, 1955).

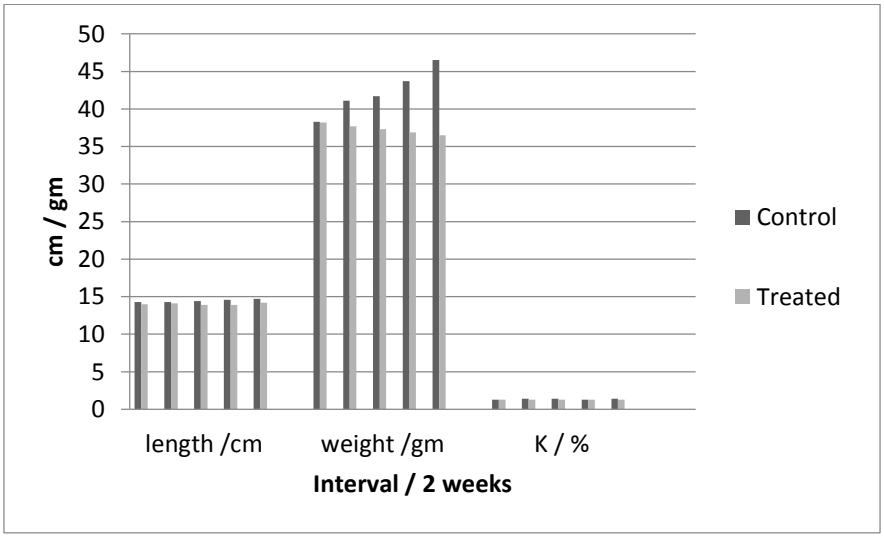

Fig. 1: Growth parameters and condition coeffecient in catfish (Clarias garipinus) after long-term exposure to dithiopyr

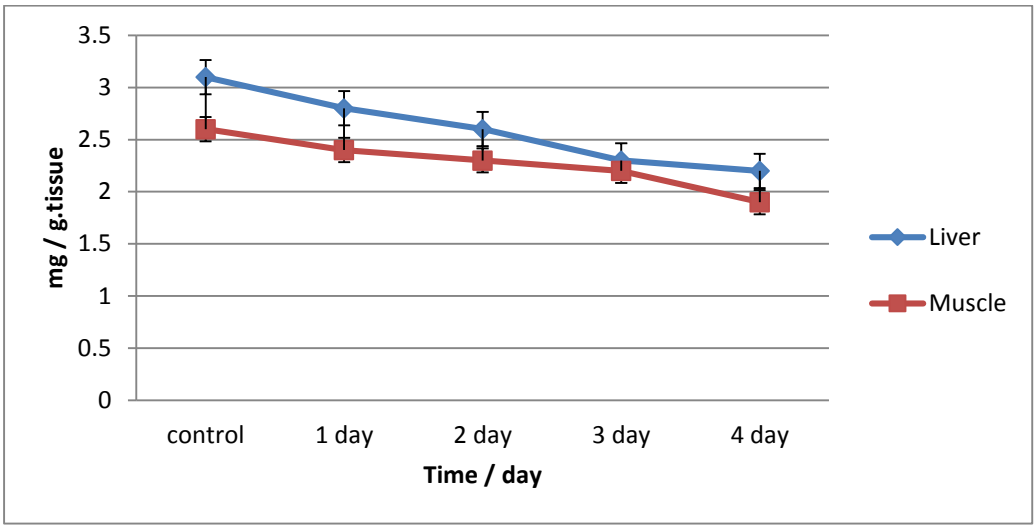

Fig. 2: Liver and muscle glycogen (mg/g) in catfish (Clarias garipinus) after short-term exposure to dithiopyr

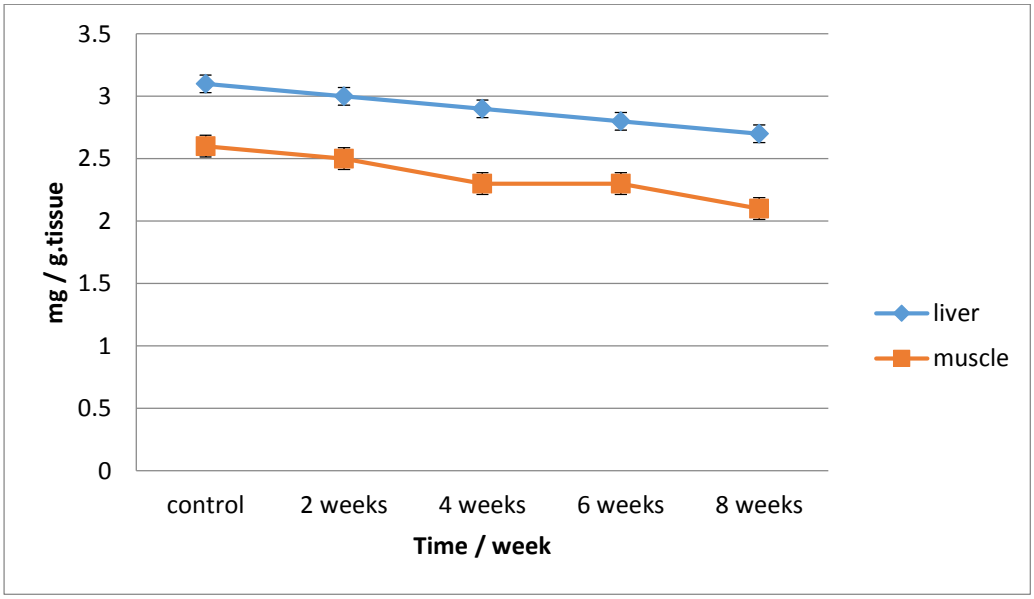

Fig.3: Liver and muscle glycogen (mg/g) in catfish (Clarias garipinus) after long-term exposure to dithiopyr

In the present study, the cholesterol levels increased significantly till attained its higher values at the end of acute and chronic exposure time. Oner *et al.* (2008) concluded that the concentrations of cholesterol, an essential structural component of membranes and the precursor of all steroid hormones, may increase due to liver and kidney failure causing the release of cholesterol into the blood. The present results are in agreement with the findings of Ozgur *et al.* (2011) who found that hypercholesteremia in the serum of cypermethrin exposed *Oreochromis niloticus*, which may be due to the stress induced by toxicants. The hypercholesteremia following cypermethrin exposure was observed in *R. quelen* (Borges *et al.*, 2007).

Moreover, the hypercholesterolemia could be attributed to the large amount of cholesterol produced by liver or less excretion of cholesterol to the bile duct as a result of stress. Simillar rises in serum cholesterol were reported in fish following exposure to different pollutants (Ghazaly, 1992).

Many soluble serum enzymes have been considered as relevant stress indicator. Therefore, activities of serum ALT, AST and LDH have been commonly used in the detection of tissue damage caused by environmental pollution. LDH is one of the important metabolic requirements for tissue and involved in energy production. It mediates the inter-conversion of lactate and pyruvate depending on the availability of NAD (Sivaperumal and Sankar, 2013).

In the present study, fish that exposed to herbicide showed a significant ($P<0.01$) elevation in ALT, AST and LDH activities. An increase of these enzyme activities in serum is a sensitive indicator of even minor cellular damage (Palanivelu *et al.*, 2005) and indicates stress-based tissue impairment. Increase in ALT, AST and LDH indicate tissue damage in liver, kidney or gill (Venkateswara Rao, 2006; Ozgur *et al.*; 2011; Sivaperumal and Sankar, 2013).

This elevation could be indicated tissue damage which may be due to disturbance in normal physiological and biochemical processes such as Kreb's cycle, TCA cycle and subsequent leakage of this enzyme from the liver cytosol through membrane into the blood stream (Palas, 2014). The present result was in agreement with the results of Jee *et al*.(2005) in fish *Sebastes schlegeli*, of Borges *et al*. (2007) in fish *Rhamdia quelen* and of El-Sayed and Saad (2008) in Tilapia *Oreochromis niloticus* who observed increased activity of serum ALT, AST and LDH in fish, exposed to pesticides.

Creatinine is a waste product largely from the muscle breakdown. Increasing levels of creatinine and uric acid above normal values are considered as kidney dysfunction indicators (Khaled *et al*., 2015).

In the present study, *C. garipinus* showed a significant increase in creatinine and uric acid concentrations with increasing the time of exposure in both acute and chronic experiment as compared to control group, these results were agree with many authors (El-Amin, 2002; Fouda, 2004). The creatinine execretion is dependent almost on the process of glomerular filtration, thus, the significant rise in the serum creatinine level meight be due to the impairment of glomerular function, tubular damage in the kidney **(**Mansour and Mossa, 2010) or due to deficiency of oxygen on the glomerular filtration rate which cause pathological changes in kidneys (Shalaby *et al*., 2005).

Growth parameters

Concerning the growth rates of the *C. garipinus* exposed to herbicide concentrations revealed that the control group showed the highest body weight gain which decreased with increasing the concentration of toxicants in water. Reduction in growth rate may be due to a reduction in oxygen carrying protein levels and red blood cells, while increase occurred in the number of white blood cells (Arellano *et al.*, 1999). Ariweriokuma *et al.*(2011) reported that the decrease in condition factor may be due to the impairment of the olfactory systems which might have affected feeding, resulting in alterations of metabolic activities and energy allocation of the fish systems.

The decrease in body weight gain may be attributed to the reduction in food consumption and/or the decrease in food conversion rate which resulted in inhibition of growth as previously reported by Sanchez *et al*. (2005).

Recently, Organosomatic indices and fish condition factor have been used to determine the sub lethal effects of these pollutants during the clinic diagnosis of fish physiology (Yi *et al.*, 2007; Ozer *et al.*, 2008; Mlamboo *et al.*, 2009).

The condition factor, asomatic biomarker,is indicative of health and reflects feeding conditions as well as energy consumption and metabolism (Schulz and Martins-Junior, 2001; Alberto *et al*., 2005). Condition factor (CF) has been used to compare growth conditions of fish. A high condition factor reflects good environmental quality; while a low condition factor reflects poor environmental quality. Toxic substances in the water may affect the growth of fish by directly changing metabolism and increasing the energy required to maintain homeostasis, or they can indirectly impact growth by reducing food availability (Sadauskas *et al*., 2011).

Exposured fish showed lower "k" values and decrease in percentage of condition factor change than the control group. The lower values of condition factor of exposed fish may reflect the changes in body protein and lipid contents and consequently decrease in the overall weight gain (Weatherley and Gill, 1983).

Liver and muscle glycogen

Decreased glycogen level in the tissues of *C. gariepinus* reared in dithiopyr-contaminated water is suggestive of impairment of oxidative metabolism. Pesticides had been reported to induce stress condition resulting in less availability of oxygen, which in turn led to less ATP production in tissues and thus adversely affecting oxidative metabolism (Olalekan, 2014). Fishes needed more energy to detoxify toxicants and to overcome stress induced by the toxicants. Carbohydrates are the primary and immediate source of energy. In stress condition, there is depleting in the carbohydrates reserve to meet energy demand. Depletion of glycogen may be due to its direct utilization for energy generation, a demand caused by pollution induced hypoxia (Tripathi and Singh, 2004a). Accompanied to recorded hyperglycemia, a decrease of both liver and muscle glycogen content were recorded. This may be attributed to the blocking transfer of glucose into the cells which may lead to increased level of glucose in blood and decreased glycogenesis in liver and muscle cells (Khaled *et al*., 2015).

Hence, it could be concluded that herbicide used in rice fields have negative impacts on the physiology and biochemistry of fish which produce a hazardous influences on the aquatic organisms and in turn affect the growth rate as well as the deterioration in meat quality to the point that it could be hazardous to humans.

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