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Effect of profenofos-pesticide on biochemical indices of *Clariasgariepinus* juveniles

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Abstract
Pesticides are essential in the safe-guarding of different Agricultural products in the world.TheProfenofos is a pesticide with anticholinesterase activity and has wide application against pests that evade farm produce. In this work the effect of Profenofos a commonly used insecticide on the biochemical parameters of Catfish is studied. The study investigated the toxicity and effects of profenofos (O-4-bromo-2- chlorophenylO-ethylS-propyl phosphorothioate) on the biochemical biomarkers of Clariasgariepinus juveniles. Based on the 96h LC50, fish were exposed to two sublethal concentrations of profenofos (1/10th of 96h LC50 = 0.3 ug /L) and 1/5th of 96h LC50 = 0.6 ug/L) in three replicates and their serum samples were analyzed for Total Protein (TP), Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) concentrations on days 1, 7, 14 and 21. The results show that biochemical parameters viz; Total Protein, Alkaline Phosphatase, Alanine aminotransferase and Aspartate aminotransferase concentrations increased significantly with time and concentration. The TP increased from (7.35 ± 0.10^{3b} -8.67 ± 46.18^{1a}) at 0.30 ug/L and (7.40 ± 0.10^{2c} –8.90 ± 0.00^{3b}) at 0.60 ug/L of profenofos when compared with the control (7.30 ± 0.93^{1d} – 7.47 ± 0.76^{2c}); the ALP increased from (59.00 ± 0.00^{1a} -88.00 ± 1.00^{1b}) at0.30 ug/L and (60.00 ± 1.00^{2a} - 89.00 ± 1.00^{1b}) at 0.60ug/L of profenofos when compared with the control (42.00 ± 1.00^{2a}-54.00 ± 6.00^{1a}); the ALT increased from (37.00 ± 1.00^{2a} -37.00 ± 2.65^{2a}) at 0.30ug/L and (40.00 ± 1.00^{2a} –67.33 ± 3.06^{1a}) at 0.60 ug/L of profenofos when compared with the control (30.33 ± 3.06^{3b} –37.00 ± 2.65^{2a}) respectively and AST activity also increased from (61.00 ± 1.00^{2a} -68.00 ± 1.00^{1a}) at 0.30 ug/L and (59.00 ± 1.00^{2a}- 77.00 ± 1.00^{1a}) at 0.60 ug/L of profenofos when compared with the control (51.33 ± 2.52^{3a} –51.33 ± 2.52^{3a}). In this work, the concentrations of the biochemical parameters were significantly higher in treated samples than control. The result suggests that profenofos may alter the liver functions or damage the liver cells and other vital organs like the kidney, heart and muscle in the Catfish juveniles.

Keywords: *Clariasgariepinus, Profenofos, Toxic effect, Biochemical parameters.*

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I. Introduction

Profenofos[O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is a broad -spectrum insecticide used in agriculture for treatment of ectoparasitic disease and pests[1]. According to[2], it is fairly lethal and was detected in vegetables. When a farm is treated with profenofos, its reported to disperse into the air, water and soil[3,4].Profenofos mainly affect humans through food ingestion[5]. Due to affordability, availability and solubility of Profenofos pesticide, its utilization has increased in recent years in Africa to increase food production to meet up with the over growing population [6,7,8,9,10,11].Water lives can absorb pesticides leading to deleterious changes in their shape, physiology and chemical reactions[12].[13,14,15,16,17,18]reported that enzyme activities are different biological indicators that can be used to access the effect of environmental pollutants in living organisms.The exposure to xenobiotics may increase the biological transformation of the toxicants to less harmful compounds by increasing the enzyme activities as a protective measure[19].African Catfishes are highly esteemed group of fishes, they have high growth rate, commands high market value as they form part of food chain, hardy in nature as they possess air breathing organs that enables them tolerate difficult aquatic and laboratory conditions.[20,21].

It then became necessary to study the biochemical response of juveniles of *Clariasgariepinus* to profenofos pesticide with time and concentration.

II. Materials and method

Experimental fish and acclimatization

450 specimens of *Clariasgariepinus* (Family: Clariidae) juveniles were bought from Sacem fish farm and transported to Applied Biology Special Laboratory Agbani,ESUT,EnuguState,Nigeria The juvenile fish with mean body weight and the length of 198.35±2.42(g) and 28.5±1.27(cm) respectively were acclimated for fourteen days in plastic tanks and fed twice daily with Coppens feed (2mm) at 3% body weight.The fish were not fed for 48 hours before and during the exposure time.Sets of 10 fish in triplicate was randomly put into 10 liter solution of tap water (dechlorinated and aerated) and different concentrations of profenofos to determine the 96hour lethal concentration (96h LC50) value. With the help of the 96h LC50, the fish were exposed to two sub lethal concentrations of profenofos (1/10th of 96h LC50 = 0.3 ug/L) and 1/5th of 96h LC50 = 0.6 ug/L) in three replicates with sets of 10 fishes and their serum samples were analyzed for Total Protein, Alkaline Phosphatase, Alanine Aminotransferase and Aspartate Aminotransferase concentrations on day 1, 7, 14 and 21 exposure periods.

Biochemical assay

Blood serum was used throughout the biochemical assay to determine;Total Protein by folin phenol reaction method as described by[22]. The method is based on the reaction of Cu^{+}, produced by the oxidation of peptide bonds, with Folin–Ciocalteu reagent, resulting in an intense blue colored molecule measured at 660nm using colorimetric techniques.

Alkaline Phosphatase (ALP) was determined using Continuous Assay Method; Alkaline phosphatase catalyzes the cleavage of phosphate group from p-nitrophenyl phosphate and liberates p-nitrophenol (PNP) and inorganic phosphate (Pi) in an alkaline medium. The phenol liberated formed a color complex in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate stops the enzymatic reaction. The catalytic activity of ALP is measured at 510nm [23,24].

The aminotransaminase enzymes,Alanine aminotransaminase (ALT) or serum glutamate pyruvate (GPT) and Aspartate aminotransaminase (AST) or serum glutamate oxaloacetate (GOT) catalyze the transfer of the amino group of glutamic acid to pyruvic acid and oxaloacetic acid in reversible reactions. The transaminase activity is proportional to the amount of oxaloacetate pyruvate formed over a definite period of time and is measured by a reaction with 2.4-dinitrophenyl hydrazine(DNPH) in alkaline solution with colorimeter at 505nm according to [25] using Randox kit.

Statistical Analysis

The data obtained were statistically analyzed by statistical package SPSS (Version 17). The data were subjected to one- way Analysis of Variance (ANOVA) and Duncan's multiple range test to determine the significance difference at 5% probability level.

III. Results

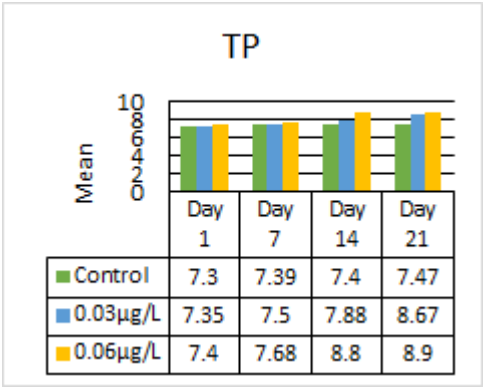
In this investigation, the exposure of Catfish juveniles to sublethal concentrations of Profenofos (0.30 ug/L and 0.60 ug/L) for days 1,7,14 and 21 respectively induced increase in concentration of TP and the activities of ALP, ALT and AST in time and concentration dependent manner.

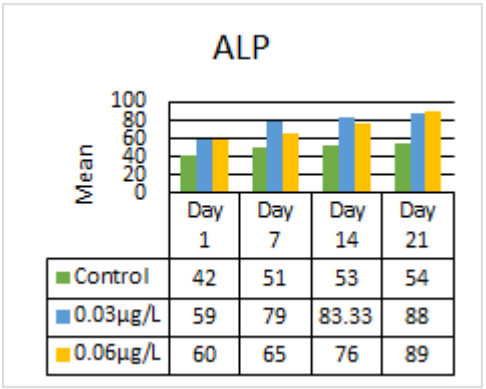
Table 1 show that Total Protein increased from (7.35 ± 0.10^{3b} -8.67 ± 46.18^{1a}) at 0.30 ug/L, and (7.40 ± 0.10^{2c} –8.90 ± 0.00^{3b}) at 0.60 ug/L of profenofos when compared with the control (7.30 ± 0.93^{1d} – 7.47 ± 0.76^{2c}); the Alkaline Phosphatase (ALP) increased from (59.00 ± 0.00^{1a} -88.00 ± 1.00^{1b}) at 0.30ug/Land (60.00 ± 1.00^{2a} 89.00 ± 1.00^{1b}) at 0.60ug/L of profenofos when compared with the control 42.00 ± 1.00^{2a} $\pm54.00\pm6.00^{1a}$; theALT increased from (37.00 ± 1.00^{2a} -37.00 ± 2.65^{2a}) at 0.30ug/L and (40.00 ± 1.00^{2a} – 67.33 ± 3.06^{1a}) at 0.60 ug/L of profenofos when compared with the control (30.33 ± 3.06^{3b} –37.00 ± 2.65^{2a}) respectively and (AST) activity also increased from (61.00 ± 1.00^{2a} -68.00 ± 1.00^{1a}) at 0.30ug/L and (59.00 ± 1.00^{2a} 77.00 ± 1.00^{1a}) at 0.60 ug/L of profenofos when compared with the control (51.33 ± 2.52^{3a} –51.33 ± 2.52^{3a}**)**.The increase in total protein and the enzyme activities (Alkaline phosphatase, Alanine aminotransaminase, Aspartate aminotransaminase) were statistically significant when compared with the control ($P \leq 0.05$).

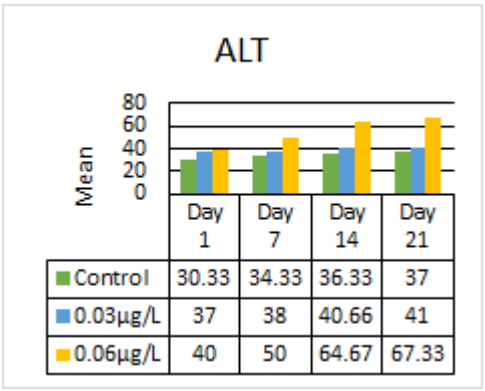
Table 1: Effect of sublethal concentrations of profenofos on biochemical indices of *C.gariepinus*at day 1, 7,14 and 21

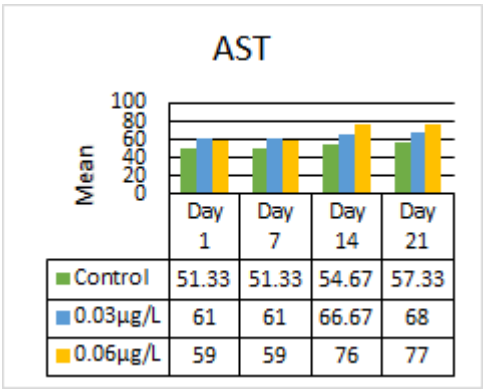
Biochemical Parameters	Concentrations ug/L	Day 1	Day7	Day14	Day21
Total Protein (TP) (g/dL)	**0.00**	7.30 ± 0.93^{1d}	7.39 ± 0.93^{1d}	7.40 ± 1.14^{1d}	7.47 ± 0.76^{2c}
	0.03	7.35 ± 0.10^{3b}	7.50 ± 0.10^{3b}	7.88 ± 0.21^{2c}	8.67 ± 46.18^{1a}
	0.06	7.40 ± 0.10^{2c}	7.68 ± 0.10^{2c}	8.80 ± 0.26^{1b}	8.90 ± 0.00^{3b}
Alkaline Phosphatase (ALP) (IU/L)	**0.00**	42.00 ± 1.00^{2a}	51.00 ± 1.00^{2a}	53.00 ± 2.00^{2a}	54.00 ± 6.00^{1a}
	0.03	59.00 ± 0.00^{1a}	79.00 ± 0.00^{1a}	83.33 ± 4.16^{2b}	88.00 ± 1.00^{1b}

	0.06	60.00±1.00^{2a}	65.00±1.00^{2a}	76.00±3.61^{3b}	89.00±1.00^{1b}
Alanine aminotransaminase (ALT) (IU/L)	**0.00**	30.33±3.06^{3b}	34.33±3.06^{3b}	36.33±3.05^{1a}	37.00±2.65^{2a}
	0.03	37.00±1.00^{2a}	38.00±1.00^{2a}	40.66±2.08^{2b}	41.00±1.00^{1b}
	0.06	40.00±1.00^{2a}	50.00±1.00^{2a}	64.67±3.51^{3c}	67.33±3.06^{1a}
Aspartate Transaminase (AST) (IU/L)	**0.00**	51.33±2.52^{3a}	51.33±2.52^{3a}	54.67±3.06^{2a}	57.33±1.15^{1a}
	0.03	61.00±1.00^{2a}	61.00±1.00^{2a}	66.67±3.21^{3b}	68.00±1.00^{1a}
	0.06	59.00±1.00^{2a}	59.00±1.00^{2a}	76.00±3.00^{3c}	77.00±1.00^{1a}









IV. Discussion

Basically, the presence of pollutants in the environment from drug factories and agricultural practices can be determined using biochemical parameters and fish as bioindicators even at very low concentration.The concentrations of the biomarkers can be used to determine the general health status of vital organs. Alteration in the concentrations would indicate detrimental effects on the physiological and structural functions of the organs [21,26]. All these parameters play vital roles in metabolic activities of the fish.

In this work, the total protein increased significantly at the two sublethal concentrations of profenofos exposed to Catfish with time. Proteins provide the framework of organs, form enzymes and hormones for controlling body functions. They are unit forms of all cells and tissues and play criticalroles in growth, development, and health of the body. Albumin and globulin are two classes of proteins found in the blood.Albumin produced by the liver play critical role in preventing fluid from escaping from blood vessels, feed tissues, and move hormones, vitamins, drugs, and substances like calcium throughout the body. The globulins which are synthesized partly by the liver and immune systems fight infection and distribute nutrients[27]. Increased total protein is associated with Addison disease, diabetic acidosis and increased production of proteins. Protein synthesis is elevated in Inflammation, hematopoietic neoplasms, late-stage liver disease and infections [28,29].

The ALP increased significantly with increasing concentration and time in Catfish exposed to profenofos in this study. The increase in serum ALP is implicated with the bone, liver, and other diseases[30]. There is elevated synthesis and release of ALP by bile duct cells into the serum when the flow of bile is blocked or there is weakened production of bile. Also, ALP is suggestive of Paget's disease of bone, blood calcium level disease, lack of Vitamin D and damaged liver cells or diseased conditions [31,32,33].

The liver majorly is involved in converting different harmful compounds including toxicants to harmless excretory form in the body,making new biomolecules like proteins, and enzymes involved in digestion. It is also central in body metabolism, coordinating red blood cells (RBCs),producing glucose and its storage[34]. Transaminases which are aminotransferases (AST and ALT) are found in the liver, kidney,heart, muscles and other tissues. When there is injury in the liver or other organs, they are released into the blood circulation[35,34,36].In this work the activities of ALT and AST were elevated significantly.[37,19,21] showed that toxicants increased activities of metabolic enzyme such as aspartate aminotransferases (AST), alanine aminotransferases (ALT).Any injury in the liver can cause a rise in ALT and AST. Elevated values have been reported in hepatitis, ischemic liver injury, toxins that cause liver damage and liver cirrhosis. Also, the damage of other organs such as the kidneys, heart or muscles can also lead to increased ALT and AST activities [35,36].

V. Conclusion:

The presence of sub-lethal concentrations of profenofos altered the biochemical parameters in the Catfish.The significant increase in the parameters indicate liver injury and suggests that the kidney or heart or muscle tissues would also be harmed due to the exposure to profenofos.

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