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Full Length Research Paper

Effect of dimethoate on some selected metabolites in the brain, liver and muscle of *Clarias lazera*

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Insecticide is used for the control of insects both in agricultural field and household. This study evaluated the effects of dimethoate on some metabolites in the brain, muscle and liver of *Clarias lazera*. The *C. lazera* samples were purchased from a private farm in Tombia Town, Bayelsa State, Nigeria. The fishes were subjected to different concentrations of toxicant 0.00, 2.50, 3.00 and 3.50 ppm for 30 days. The fish samples were dissected and the muscle, brain and liver were collected and analyzed using standard analytical procedure for metabolites (triglyceride, cholesterol, protein and cortisol) in tissues/organs. The result showed significant variations (P<0.05) in most of the metabolites of *C. lazera* exposed to dimethoate compared to the control. However, the concentration were in the order brain> liver> muscles (cholesterol), Liver> muscles> brain (triglyceride), muscle> liver> brain (Protein), liver> brain> muscles (cortisol). The study showed that dimethoate could alters metabolites of *C. lazera* thereby hindering proper metabolism of the various organs affected. As such caution should be exercised on the use of dimethoate insecticides so that it will not contaminate surface water that harbors wide range of fish species.

Key words: Aquatic ecosystem, ecotoxicology, fish, insecticides.

INTRODUCTION

In the recent times the use of pesticides has increased especially in agriculture. The use of synthetic pesticides typically has ecological effects on the ecosystem either directly or indirectly. Generally, pesticides are materials used with the intention to control, prevent, and mitigate biological activity (Seiyaboh et al., 2013; Lawson et al., 2011; Akan et al., 2013; Ogamba et al., 2015a). These pesticides are classified according to the target organism hence they are insecticides, rodenticides, fungicides, herbicides and fumigants (Akan et al., 2013; Ajani et al., 2015; Ogamba et al., 2015a). However, unsustainable use of pesticides could have adverse effect on the ecosystem (Tak et al., 2014). Pesticides are generally recalcitrant to degradation (Ogamba et al., 2015a). These pesticides could pose a threat to biotic and abiotic environment (Inyang and Patani, 2015) especially the aquatic ecosystem (Inyang and Ollor, 2015).

Aquatic ecosystem is one of the major receiving ends of pesticides. This is because during rain fall, the pesticides are transported to nearest available water body through run off (Ogamba et al., 2015a; Tak et al., 2014; Izah and Angaye, 2016) and or through wind especially when they are careless discharged into the environment (Inyang and Thomas, 2016). Pesticides pollution often results from farmlands application, industrial pollution and careless disposal of pesticide containers (Inyang et al., 2015). As such the productivity of the aquatic ecosystem can be indirectly influenced by the concentration of toxic substances received by the water as well as its flow rate.

Different pesticides contain various types of chemicals including organochlorines, halogenates, hydrocarbons, carbamates, heterocyclic compounds, organophosphates, chlorinated phenoxy substances, amines and ureas, phenolic compounds and pyrethroids (Lawson et al., 2011; Ogamba et al., 2015b). Of these chemical components of pesticides, organophosphate, organocholorines, pyrethroids and carbamate are mainly used for production of insecticides (Banaee, 2013). Organophosphorous pesticides is one of the commonly

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used probably due to their high insecticidal activity, low toxicity to mammal, less persistence and rapid biodegradable (Tak et al., 2014). The biodegradability of the organophosphorous pesticides are hindered by low temperature and moisture, high alkalinity and absence of suitable microbial isolates (Tak et al., 2014). Most of insecticides are highly toxic to non-target organisms that inhabit in natural environments close to agricultural fields (Banaee, 2013) and even residential areas (i.e. human).

Dimethoate is a typical example of organophosphorus insecticide used to kill insects via contact. Dimethoate is mostly transported through leaching and volatilization. Dimethaote are highly volatile in water and stable. Dimethoate is used to mitigate several insect that attack a wide variety of crops, storage and livestock's house. Dimethoate is among the widely utilized insecticides. Some of the insects that dimethoate can be used against include fruit sucking aphids, mites, saw flies and boring insects on cereals, cotton, chilly, tobacco and oil seeds (Binukumari and Vasanthi, 2013a), aphids, thrips, plant hoppers and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables (Dubey et al., 2015). Dimethoate has found application house flies in farm buildings and livestock for control of botflies (Dubey et al., 2015). Dimethoate may have health effects on individuals exposed to it either in the production/ manufacturing or use.

Fish which is an aquatic dweller, is an important source of animal protein (Ineyougha et al., 2015; Izah and Angaye, 2015, Angaye et al., 2015; Banaee, 2013) and lipids for humans and domestic animals (Banaee, 2013). Like other aquatic dwellers, fish is at risk to wide range of pesticides especially insecticides in the course of their life cycle (Banaee, 2013). Insecticides on fishes causes physiological dysfunction in various biological systems (including behavioral changes, oxidative stress) changes in hematological parameters (including red blood cell counts, hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular haemoglobin concentration which may be influenced by intrinsic and extrinsic factors), blood biochemical parameter (including acid phosphatase, alkaline phosphatase, creatinine, creatinine kinase, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase, total protein, albumin, and immunoglobulin (Banaee, 2013). The blood biochemical parameters are mostly used to assess aberrations in the liver and other tissues (Banaee et al., 2011). Similarly, Rathod et al. (2009) noted that biochemical studies are good parameters for the assessment of pesticide effects on biochemical composition of vital tissue of fish. Some pesticides has the tendency to disrupted the functioning of the endocrine system. According to Ţălu et al. (2012), *Poecilia reticulata* exposed to lindane has its red pigmentation (due to inhibition of red pigment expression) and fish dimensions reduced.

In Nigeria, the genus *Clarias* is one of the commonly acceptable fish with economic importance (Adewumi et al., 2015; Ogamba et al., 2015a). Several species are found in the *Clarias* genus. Among them, *C. gariepinus* have been widely studied. Hence, this study focused of the effect of dimethoate on some selected metabolites in the brain, liver and muscle of *C. lazara*.

MATERIALS AND METHODS

Source of fish, experimental location and acclimatization

Thirty seven healthy *C. lazera* were purchased from a private fish farm in Tombia Town, Bayelsa State, Nigeria. The fish samples were transported to Fisheries Department, Niger Delta University, Wilberforce Island, Nigeria where the experiment was conducted from June to September, 2015. Thirty adult *C. lazera* (mean weight, 90.00 ± 0.10 g, mean length, 22.14 ± 0.12cm) were acclimatized individually in a rectangular aquaria for 10 days during which they were fed once a day (10.00 - 11.00 hrs). They were fed with 35% crude protein diet at 1% biomass (Ogamba et al., 2013, 2015a; Seiyaboh et al., 2013). The toxicant used in this study is dimethoate was purchased from Swali market, Bayelsa state, Nigeria.

Preparation of toxicant solution

The toxicant i.e. dimethoate was prepared with reference to the formula;
N1 V2 = N2V2 (Grinshaw, 1978 cited in Seiyaboh et al., 2013 and Ogamba et al., 2011; Ogamba et al., 2015a).

N1 = Manufacturer concentrated (40 g/l)
N2 = Concentration of test solution desired.
V1 = Volume of the original solution added.
V2 = Volume of the test solution (30 litres).

General bioassay technique

Sublethal concentrations of dimethoate for the assay (2.50, 3.00, 3.50 ppm) were determined based on the range finding test (Inyang et al., 2010). These were prepared by transferring 1.88, 2.25 and 2.63 mls respectively of the original concentration of dimethoate (40 g/l) and making it up to 30 L with borehole water in the test aquaria. The volume of diluents used for the control was 30 L. Replication of each treatment level (concentration) and control were set up by introducing fishes individually into each aquarium. The exposure

Table 1. Some physicochemical parameters of exposed aquaria water.

Treatments	Concentration, ppm	pH	Conductivity, µS/cm	Temperature, ºC	Turbidity, NTU	Dissolved oxygen, mg/ml	Alkalinity, mg/l
A	0.00	6.27±0.08b	136.12±14.04a	26.00±0.00a	0.50±0.06a	6.90±0.01a	12.30±0.75a
B	2.50	6.37±0.07a	136.12±14.04a	26.00±0.00a	0.50±0.06a	6.90±0.01a	12.35±0.20a
C	3.00	6.37±0.07a	136.12±14.04a	26.00±0.13a	0.50±0.06a	6.90±0.01a	12.60±0.80a
D	3.50	6.37±0.07a	136.12±14.04a	26.00±0.30a	0.50±0.06a	6.90±0.01a	13.30±0.20a

Data is expressed as mean ± standard error; Different letters along the column indicate significant difference according to Tukey HSD statistics.

Table 2. Metabolites in the brain exposed to dimethoate.

Treatment	Concentration, ppm	Cholesterol, mg/dl	Triglyceride, mg/dl	Protein, mg/dl	Cortisol, mg/dl
A	0.00	0.450±0.000a	0.110±0.000b	1.497±0.003c	4.760±0.001d
B	2.50	0.507±0.007b	0.083±0.003a	2.510±0.001d	2.960±0.010a
C	3.00	0.657±0.007c	0.307±0.007c	1.007±0.007b	3.360±0.010b
D	3.50	0.457±0.007a	0.293±0.003c	0.000±0.000a	4.653±0.003c

Data is expressed as mean ± standard error; Different letters along the column indicate significant difference according to Tukey HSD statistics.

period lasted for 30 days during which the exposure media were renewed daily.

On the design, Completely Randomized Design (CRD) experimental method was employed in this study. The *C. lazera* used for this study were randomly divided into four groups of three fishes each, representing one control group and three treatments i.e. A (0.00 ppm) (control group), B (2.50 ppm), C (3.00 ppm) and D (3.50 ppm).

Sample collection and laboratory analysis

At the end of the experiment i.e. 30 days, the fishes were killed and dissected and the liver, muscles and brain was obtained. Each organ/part was finely blended using ceramic pestle and mortar. Then perchloric acid was added to the samples meant for metabolites analysis. The samples were centrifuges for 10 minutes at 3000 rpm. Then the resultant supernatant was transferred into sample bottle for analysis.

Physico-chemical properties of the aquarium water

The aquarium water was collected after the introduction of the toxicants during the experiment. The physico-chemical properties such as turbidity, temperature, conductivity and pH were analyzed using in-situ meter, dissolved oxygen were analyzed using wrinkler's method and Alkalinity was analyzed by titration method. 0.1 NH_2SO_4 and methyl orange were used as titrant and indicator respectively during alkalinity determination.

Biochemical analysis

The biochemical analysis was carried out using several analytical procedures. Triglyceride was analyzed based on the method previously described by Bucolo and David (1973), cholesterol concentration was estimated by the method previously described by Amundson and Zhou (1999). The total protein concentration was estimated by the method of Lowry et al. (1951). The cortisol was determined by the method previously described by Hyams and Carey (1988).

Statistical analysis

Statistical analysis was carried out using SPSS software. The data was expressed as Mean ± standard error and one-way analysis of variance was carried out at P = 0.05, and Tukey HSD Test was used for multiple comparison.

RESULTS

Table 1 present the physicochemical water quality parameters of exposed aquaria water exposed to various concentration of the toxicant. The values ranged from 6.27 – 6.37, 136.12 µS/cm, 26.00ºC, 0.50NTU, 6.90mg/l (among the various concentration of the toxicants) and 12.30 – 13.30mg/ml for pH, conductivity, temperature, turbidity, dissolved oxygen and alkalinity respectively. Basically no significant difference (P>0.05) among the various level of toxicant with regard to the water quality parameters studied.

The metabolites i.e. (cholesterol, protein, triglyceride and cortisol) concentration in the brain, muscle and liver of *C. lazera* is presented in Tables 2 – 4. In the brain, the Cholesterol was 0.450 mg/dl (Treatment A), 0.507 mg/dl (Treatment B), 0.657 mg/dl (Treatment C), 0.457 mg/dl

Table 3. Metabolites in the muscles exposed to dimethoate.

Treatment	Concentration, ppm	Cholesterol, mg/dl	Triglyceride, mg/dl	Protein, mg/dl	Cortisol, mg/dl
A	0.00	0.450±0.000b	0.147±0.007a	5.533±0.033c	5.263±0.013d
B	2.50	0.503±0.003c	0.347±0.007c	9.007±0.007d	3.507±0.007c
C	3.00	0.400±0.000a	0.207±0.007b	2.007±0.007a	2.840±0.010b
D	3.50	0.600±0.000d	0.340±0.012c	4.003±0.003b	1.500±0.000a

Data is expressed as mean ± standard error; Different letters along the column indicate significant difference according to Tukey HSD statistics.

Table 4. Metabolites in the liver exposed to dimethoate.

Treatment	Concentration, ppm	Cholesterol, mg/dl	Triglyceride, mg/dl	Protein, mg/dl	Cortisol, mg/dl
A	0.00	0.503±0.003ab	0.160±0.000a	1.500±0.00a	3.750±0.017b
B	2.50	0.433±0.033a	0.263±0.003c	6.000±0.058d	5.750±0.012c
C	3.00	0.553±0.003b	0.523±0.003d	4.500±0.058c	3.400±0.058a
D	3.50	0.503±0.003ab	0.243±0.003c	2.003±0.003b	6.203±0.003d

Data is expressed as mean ± standard error; Different letters along the column indicate significant difference according to Tukey HSD statistics.

(Treatment D); Triglyceride was 0.110 mg/dl (Treatment A), 0.083 mg/dl (Treatment B), 0.307 mg/dl (Treatment C), 0.293 mg/dl (Treatment D); Protein was 1.497 mg/dl (Treatment A), 2.510 mg/dl (Treatment B), 1.007 mg/dl (Treatment C), 0.000 mg/dl (Treatment D); and Cortisol was 4.760 mg/dl (Treatment A), 2.960 mg/dl (Treatment B), 3.360 mg/dl (Treatment C), 4.653 mg/dl (Treatment D). There was significance difference ($P < 0.05$) among the various concentrations (Table 2).

The cholesterol increased up to 3.00 ppm before beginning to decline. The triglyceride concentration declined at 2.50 ppm before increasing at the higher concentration. Similarly the protein concentration increased at 2.50 ppm and finally decline at higher concentration while Cortisol concentration showed complete decline as the toxicant level increases.

The concentration of the various metabolites in the muscles showed un-uniform pattern just as in the brain. However the control value (i.e. Treatment A) for Cholesterol were significantly ($P < 0.05$) lower than the concentration of Treatment B and D, but significantly ($P < 0.05$) higher than Treatment C. The triglyceride level was significantly ($P < 0.05$) lower in the control than other treatments, while cortisol decreases as the toxicant level increases. The protein showed sudden increase as the toxicant was introduced (Treatment B), and suddenly decline in Treatment C before increasing again in Treatment C) (Table 3).

Based on the concentration of the metabolites in the liver, the cholesterol level ranged between 0.433 – 0.553 mg/dl being not significantly different ($P>0.05$) between the control and other concentration. However, significance difference ($P<0.05$) exist between Treatment B and C. The triglyceride concentration ranged from 0.160 – 0.263 mg/dl, being significantly different among the various concentrations. However, the highest and least concentration were observed at treatment C and treatment A respectively. Like triglyceride, protein showed a significant ($P < 0.05$) increase as the toxicant concentration increases with a range of 1.500 – 6.000 mg/dl. While the cortisol concentration were 3.750 mg/dl, 3.400 mg/dl and 6.203 mg/dl in Treatment A, Treatment C and Treatment D respectively, being significant different ($P < 0.05$) among the various concentration. However, the highest and least concentration was observed in treatment B and Treatment C respectively (Table 4).

Discussion

Generally influence of water quality parameter could result to change in the metabolites especially when the water is toxic. In this study the metabolites alteration and fluctuation is not connected to the water used in the experiment after the introduction of the toxicant. This is because there were no significant difference ($P > 0.05$) between the control and the various concentration of the toxicant with regard to conductivity, temperature, turbidity, dissolved oxygen and alkalinity but significant variation ($P>0.05$) exist in pH (Table 1). The trend in the water quality parameter is similar to the result of previous authors on the toxicity of some pesticides in fisheries including diazinon (Inyang et al., 2008), dimethyl 2, 2-dichlorovinyl phosphate (Ogamba et al., 2015b) and Paraquat dichloride (Seiyaboh et al., 2013).

The variation in the various metabolites could be due to changes in the rate of synthesis and metabolism resulting from the toxicants. The trend in this study have been reported by Shreni (1979) who observed decline before

increase in cholesterol level in liver and brain of *H. fossilis* when starved. Ogamba et al. (2015b) noted fluctuation in electrolytes of *C. gariepinus* when exposed to dimethyl 2, 2-dichlorovinyl phosphate. Similarly Ogamba et al. (2015a) also reported fluctuation in electrolytes of C*. gariepinus* when exposed to various concentrations of 2, 4-Dichlorophenoxyacetic acid. Ganeshwade (2012) reported fluctuation in the cholesterol level in the muscle, gills and kidney of *Puntius ticto* exposed to dimethoate.

The metabolites concentration were in the order brain> liver> muscles (cholesterol), Liver> muscles> brain (Triglyceride), muscle> liver> brain (Protein), liver> brain> muscles (cortisol). This trend could be due to variation in the biochemical constituents of each of the sites with regard to accumulation. The bioaccumulation of the toxicant in the different part of the fish i.e. brain, muscle and liver could be connected to their role in metabolism. For instance, the liver protect the body from potentially harmful ingredients through absorption of substances from the intestinal tract, gills, and skin (Banaee, 2013).

An increase in cortisol concentration could trigger the activation of specific intracellular responses through glucocorticoid receptors (Teles et al., 2012). Fluctuation exists in protein content among the various parts of the fish. Protein is a major organic compound found in the living cell and they play essential role in the process of interaction of cellular medium (Magar and Shaikh, 2012). The occasional decline in protein content could be due to decline in protein synthesis and increased proteolysis while an increase may be associated with increase in protein synthesis due to increase in enzyme activity involved in protein synthesis (Magar and Shaikh, 2012). However authors have reported decline in protein content when exposed to insecticides. Cholesterol is typically higher in fish liver oils but in the present investigation the consumable part of fish, muscle and brain also contain cholesterol (Sujatha et al., 2013). Cholesterol play a role in defensive and transports fat to liver in the form of cholesterol ester for oxidation, granulation of cell division and acts as an antagonist to phospholipids, formation of bile acids and bile salts, 7-dehydrocholestrol and vitamin D3, corticosteroid hormone, androgens, estrogens and progesterone (Sujatha et al., 2013). Alteration in the cholesterol in the liver of fish exposed to dimethoate could be associated toxicity stress which suppresses the activity some enzymes responsible for lipid transformation thereby disrupting lipid metabolism (Ganeshwade, 2012).

The findings of this study have shown that different concentration of dimethoate significantly affects the metabolites of *C. lazera*. This is accordance with previous studies that have reported alteration in function of fishes parts when exposed to toxicants. But the trend in the previous study with the result of thie work is farm apart. For instance, Dubey et al. (2014) reported that dimethoate can alters morphological and physiological functions in hepatic tissues of *C. batrachus* modulating the enzymological and biochemical changes. The authors further reported an increase in hepatic glutamate oxaloacetate transaminase, glutamate pyrophosphate transaminase, acid phosphatase, alkaline phosphatase (i.e. increases at only 30 days and decline compared to control after 60 days), protein estimation (i.e. decreases at 60 days but do not show significant difference with the control at 30 days) in the liver of fresh water fish *C. batrachus* exposed to dimethoate. Dubey et al. (2015) reported increase in acid phosphatase, alkaline phosphatase, renal glutamate oxaloacetate transaminase, glutamate pyrophosphate transaminase and decrease in protein metabolism in the kidney of *C. batrachus* exposed to dimethoate. Ganeshwade (2012) reported that Cholesterol content in *Puntius ticto* exposed to various concentration dimethoate increases in the ovary and testis as the concentration of the toxicant increases; decreases in the intestine, brain and liver as the concentration of the toxicant increases; and fluctuation exist in the muscle, gills and kidney as the concentration of the toxicant increases. Binukumari and Vasanthi (2013b) reported that dimethoate 30% could affect some haematological parameters of fresh water fish, *Labeo rohita.* The authors reported decrease in red blood cell, haemoglobin, platelets, polymorphs, pack cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration and an increase in white blood cells, lymphocytes, eosinophil in the blood of *L. rohita* exposed to dimethoate. Binukumari and Vasanthi (2013a) reported decline in protein value (activity) on the gill, liver, kidney, muscle of *L. rohita* exposed to dimethoate. Singh (2013) reported that dimethoate is a strong hepatotoxic and can affect histology, carbohydrate and protein metabolism and histopthalogical changes. The authors reported that on exposure of common carp to dimethoate, the blood glucose increases, liver glycogen decreases and liver protein increases up to 48 hours (exceeding the control) and begin to decline (less than control) at 96 hours. As such dimethoate can induce alteration in biochemical composition of fish tissue under pesticides stress (Rathod et al., 2009).

Conclusion

Pesticides in an integral part of modern agriculture and for the eradication of household pest especially in developing countries. Pesticides are classified based on their functions including herbicides, insecticides, fungicides etc. This study assessed the effect of dimethoate, an insecticide on some metabolites of *C. lazera.* The study found that the metabolites in *C. lazera* are affected by pesticides (dimethoate). However, triglycerides, cholesterol, protein and cortisol are altered in the brain, liver and muscles of *C. lazera* could affect

the metabolism processes in fish in general. As such caution should be exercised on the use of this dimethoate in agricultural field close to water bodies, to avoid disposition in the water body via runoff.

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