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Hydrobiological and Behavioral Parameters of Fresh *Clarias gariepinus* Exposed to Dichlorvos Concentrations

[1]Cyril O. Usifoh*, [2]Anietie P. Effiong, [2]Emmanuel I. Etim, [2]Aniefiok S. Udobre and [3]Stella F. Usifoh

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
[3]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

ABSTRACT

The use of dichlorvos (organometallic pesticide) in public health and agriculture to control pests has led to the accumulation of dichlorvos residues in the aquatic environment. The objective is to establish the potential diagnostic tools (hydrobiological and behavioural parameters) in assessing the lethal and sublethal effects of dichlorvos on fish. Cultured *Clarias gariepinus* were sourced from two fish farms in Uyo metropolis, Akwa Ibom State, Nigeria. All cultured samples for acute toxicological studies were fortified with 2%, 5%, 10%, 20% dichlorvos and exposed for 24, 48, 72, 96 h, and above 96 h intervals respectively while 2%, 5% concentrations of dichlorvos at the same periods of exposure were used for chronic studies. Dichlorvos residues were present in all cultured fresh post-fingerlings exposed to the pesticide. This revealed significant different variations ($p<0.05$) and positive correlation in behaviour, mortality rate, survival rate, weight loss of *Clarias gariepinus* treated with dichlorvos concentrations as well as changes in pond water quality (hydrobiological) parameters ranging from temperature, pH to dissolved oxygen. Dichlorvos, therefore, constituted a major constraint to hydrobiological and behavioural cycles of fresh fish and aquacultures under study, and these parameters can be useful biomarkers in environmental bio-monitoring of pesticides contamination.

KEYWORDS: Acute and Chronic Toxicity, Aquatic Environment, *Clarias gariepinus*, Dichlorvos.

INTRODUCTION

In marine habitat, dichlorvos constitutes a major constraint to hydrobiological and behavioural cycles of fresh fish and aquacultures (Espeland *et al.*, 2010; Das, 2013). Dichlorvos (2,2-dichlorovinyl dimethyl phosphate or DDVP) is an organophosphate pesticide (Isa sand Majid, 1996; Das, 2013), an acetylcholinesterase inhibitor used as contact and stomach poison against household and store product pests (Ural and Köprücü, 2006; Lukaszewicz-Hussain, 2010), and in public health to treat ectoparasites and *Pediculosis capitis* (head lice) (Das, 2013). It is short live, biodegradable, volatile and hydrophobic compound classified by the WHO as a Class 1B, 'highly hazardous' chemical (Das, 2013; NIOSH, 2014). DDVP still accounts for two third of the total consumption in India, China and Nigeria for agriculture and public health purposes respectively (Musa, Hati, Adama and Mustapha, 2010; Hasan *et al.*, 2013). DDVP poses potential threat to the fatty acid profile, the nutritional value, the texture and the organoleptic properties (anti-inflammatory, antithrombotic, antiarrhythmic and vasodilatory properties) of essential polyunsaturated fatty acids (PUFA) especially omega-3 (ω^3) PUFA (DHA-docosahexaenoic acid and EPA-eicosapentaenoic acid) that are found in fish oil (Fournier *et al.*, 2006; Rubio-Rodriguez *et al.*, 2010). The compound can cause histopathological alterations in fish kidney, liver, gills, muscles and other organs (Russo, 2009). A variety of histopathological toxicant effects and disorders are caused by a long-term effect of water contaminated by the pesticide (Das, 2013). In humans, dichlorvos affects the nervous system, liver and kidneys, colon, breast and prostate among others (Russo, 2009; Das, 2013) and its toxicity could be studied as chronic, acute, reproductive or developmental or cancer risk (Luty *et al.*, 1998; Espeland *et al.*, 2010). Fish absorbs the toxic compound directly from water or by ingesting contaminated food, and different organs of fish, on the other hand, react in different ways or with different intensity to the presence of acute and chronic toxicity of organophosphate pesticide (Isa and Majid, 1996). Fish, therefore, becomes an "indicator" for the evaluation of the effects of these noxious compounds on human and marine environment (Das, 2013). The aim of the research work is to establish the potential diagnostic tools (hydrobiological and behavioural parameters) in assessing the lethal and sub-lethal effects of dichlorvos on fish with a view of setting up standards for the safe harvesting of fresh fish to ensure food safety.

MATERIALS AND METHODS

Equipment, Apparatus and Reagents

GC-MS (Shimadzu, Japan), aerator pressure pump (AC-9904, China), pH meter, thermometer, dissolved oxygen

*Corresponding Author: usifoh@uniben.edu 08032567723

meter, analytical balance (Merck Company, Germany), white transparent plastic fish tanks (32 x 30 Litres), 20-point and 12-point air circulating plastic connectors, connecting rubber tubules, air stones (China), 15 x 15 cm fishnet, tarpaulin fish tank -1000 litres (Faculty of Agriculture, University of Uyo) were used for the study. All reagents were sourced from Sigma Aldrich Chemicals (USA), Merck Company (Germany), Central Research Laboratory, Faculty of Pharmacy, University of Uyo.

Collection of Cultured *Clarias gariepinus* Post-fingerlings

Clarias gariepinus post-fingerlings (shooters) were sourced from Fish Culture in Vika farms Ltd, Mbiabong Etoi and Fadama Farms, Federal Housing Estate, Abak Road, all in Uyo Metropolis, Akwa Ibom State, Nigeria. The post-fingerlings were transferred to the Nursery Fish Pond (Hatchery) at the Department of Fisheries and Aquatic Environmental Management, Faculty of Agriculture, University of Uyo, Uyo. The post-fingerlings were disinfected with 0.1% potassium permanganate as described by Joshi *et al.* (2002) and acclimatized for 14 days in the Hatchery.

Acclimatization of Cultured Post-Fingerling (Shooter) in the Hatchery

A collection of post-fingerlings (shooters) brought into the hatchery, was acclimatized for 14 days in a tarpaulin tank and kept unfed on the first day (24 h) before subjected to Weight and Length Analysis (Fishlore, 2011). The standard pond water quality was maintained and monitored during acclimatization of the post-fingerlings in the hatchery (Joshi *et al.*, 2002; Fishlore, 2011).

Grouping of Cultured Post-Fingerlings in the Hatchery

Selection of the post-fingerlings (shooter) based on the average weight (24.5 ± 6.5 g) and average length (12.5 ± 2.5 cm) according to Lawrence and Temiota (2010) was carried out.

Feeding and Inoculation of the Post-Fingerlings with Dichlorvos

The post-fingerlings were acclimatized to laboratory condition for 24 h before fed with 40% body mass of crude protein commercial feed (multi-feed) once daily (Fishlore, 2011). Feeding was stopped 24 h prior to the commencement of the experiment with a view to avoiding any possible change *in situ* in the toxicity of dichlorvos (Kovendan, Vincent, Janarthanan, Saravanan, 2013). On the 11th day, ten (10) healthy specimens were selected and kept in each of the aquaria containing 20 litres of pond water before the beginning of acute and chronic exposure studies. The acute and chronic toxicity studies of cultured African catfish (*Clarias gariepinus*) post-fingerlings exposed to DDVP concentrations were assessed in a static renewal bioassay using transparent plastic fish aquaria for 24 h, 48 h, 72 h, 96 h and above 96 h (Tripathi, Mishra and Girdoniya, 2011; Kovendan *et al.*, 2013).

Acute Exposure Studies Experimental Design: Based on the results of the range-finding test according to APHA (1998) and Abolagba *et al.*, (2011), four (4) concentrations (2%, 5%, 10% and 20%) of the dichlorvos and four (4) control (standard pond water without DDVP pesticide) were prepared in duplicate. Ten (10) fish samples were introduced randomly into each of the 16 aquaria (of 30 litres capacity) and the tanks were filled up to 20 litres mark with standard pond water. The post-fingerlings were left to acclimatize to nursery temperature condition before the commencement of inoculation. Fish samples in four aquaria were inoculated in duplicate with 5 mL of 2%, 5%, 10% and 20% of the DDVP test solution respectively. Specimens selected as control were kept intact (without inoculation with pesticides) in each of the 8 aquaria containing standard pond water. The set up was aerated and pond water (in the control) was renewed daily. Fish samples showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately for storage at -20 °C until used for further analysis. Parameters such as weight loss, mortality and survival changes of each post-fingerling were measured *in situ* at 6 h intervals (Lawrence and Temiotan, 2010; Abolagba *et al.*, 2011; Tripathi *et al.*, 2011; Hassan *et al.*, 2013; Kovendan *et al.*, 2013).

Chronic Exposure Studies Experimental Design: Two (2) sub-lethal dichlorvos concentrations (2% and 5%) and two (2) control (0.0%) were selected in duplicate for chronic exposure studies (APHA, 1998; Abolagba *et al.*, 2011). Ten (10) healthy specimens were selected and kept in each of the aquaria containing 20 litres of standard pond water. The fish samples in each pond were fed with multi-feed (inoculated with 2% DDVP and 5% DDVP) at 5% of body mass once a day, whereas specimens selected as control were fed with only multi-feed (without inoculation with 2% and 5% of DDVP) at 5% of body mass once a day in each of the 8 aquaria containing standard pond water. The set up was aerated and pond water (in the control) was renewed daily. Fish samples showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately for storage at -20 °C until used for further analysis. Parameters such as weight loss, mortality and survival changes of each post-fingerling were measured *in situ* at 6 h intervals (Lawrence and Temiotan, 2010; Abolagba *et al.*, 2011; Tripathi *et al.*, 2011; Hassan *et al.*, 2013; Kovendan *et al.*, 2013).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SPSS Version 16 to determine the differences in the concentration of each of the dichlorvos residue in each sample analyzed (Kovendan *et al.*, 2013; Shinggu *et al.*, 2015). Four concentrations for acute test and two concentrations for the chronic test of the dichlorvos were

derived based on the results and used as the experimental concentrations (Kovendan *et al.*, 2013). Variations were considered statistically significant at $p<0.05$ (Shinggu *et al.*, 2015).

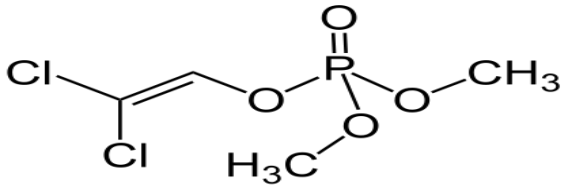

Figure 1:2, 2-Dichlorovinyl dimethyl phosphate (DDVP

RESULTS

Table 1 Post-inoculation variation in dissolved oxygen (acute studies

Concentration of DDVP Solution (mg/L)	Dissolved Oxygen of Test (mg/mL)	Dissolved Oxygen of Control (mg/mL)
4.420 ± 0.000	6.090± 0.000	7.000± 0.020
11.049 ± 0.000	5.000± 0.005	7.000± 0.020
22.098 ± 0.000	4.670± 0.007	7.000± 0.020
44.196 ± 0.000	3.990± 0.007	7.000± 0.020

Dissolved oxygen in pond water is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean dissolved oxygen levels of fish pond water.

Table 2 Post-inoculation variation in dissolved oxygen (chronic studies)

Concentration of DDVP Solution (mg/L)	Dissolved Oxygen of Test (mg/mL)	Dissolved Oxygen of Control (mg/mL)
44.200 ± 0.000	2.040 ± 0.007	7.000 ± 0.020
55.300 ± 0.000	2.010 ± 0.000	7.000 ± 0.020

Dissolved oxygen in pond water is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean dissolved oxygen levels of fish pond water.

Table 3 Post-inoculation variation in pH (acute studies)

Concentration of DDVP Solution (mg/L)	pH of Test	pH of Control
4.420 ± 0.000	7.200 ± 0.000	7.000 ± 0.200
11.049 ± 0.000	7.200 ± 0.000	7.000 ± 0.200
22.098 ± 0.000	7.400 ± 0.000	7.000 ± 0.200
44.196 ± 0.000	7.400 ± 0.000	7.000 ± 0.200

pH of pond water is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean pH levels of fish pond water.

Table 4 Post-inoculation variation in pH (chronic studies)

Concentration of DDVP Solution (mg/L)	pH of Test	pH of Control
44.200 ± 0.000	7.100 ± 0.000	7.000 ± 0.200
55.300 ± 0.000	7.100 ± 0.000	7.000 ± 0.200

pH of pond water is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean pH levels of fish pond water.

Table 5 Post-inoculation variation in temperature (acute studies)

Concentration of DDVP Solution (mg/L)	Temp. of Test (0C)	Temp. of Control (0C)
4.420 ± 0.000	27.100 ± 0.071	26.000 ± 1.000
11.049 ± 0.000	27.100 ± 0.071	26.000 ± 1.000
22.098 ± 0.000	27.200 ± 0.000	26.000 ± 1.000
44.196 ± 0.000	27.100 ± 0.071	26.000 ± 1.000

Temperature of pond water is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean temperature levels of fish pond water.

Table 6 Post-inoculation variation in temperature (chronic studies)

Concentration of DDVP Solution (mg/L)	Temp. of Test (^{0}C)	Temp. of Control (^{0}C)
44.200 ± 0.000	27.100 ± 0.071	26.000 ± 1.000
55.300 ± 0.000	27.100 ± 0.071	26.000 ± 1.000

Temperature of pond water is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean temperature levels of fish pond water.

Table 7 Post-inoculation variation in fish weight

Concentration of DDVP Solution (mg/L)	Weight of Control (fish) Sample (g)	Weight of Test (fish) Sample (g)	Variation in Weight (loss of weight (g))	(%)
(a) Acute Studies				
4.420 ± 0.000	26.540 ± 0.007	25.490 ± 0.007	1.050 ± 0.000	3.956
11.049 ± 0.000	27.750 ± 0.014	26.650 ± 0.014	1.100 ± 0.000	3.964
22.098 ± 0.000	27.220 ± 0.007	25.970 ± 0.007	1.250 ± 0.007	4.592
44.196 ± 0.000	27.180 ± 0.000	25.830 ± 0.000	1.340 ± 0.000	4.930
(b) Chronic Studies				
44.200 ± 0.000	26.860 ± 0.050	25.930 ± 0.014	0.780 ± 0.036	2.904
55.300 ± 0.000	26.710 ± 0.042	25.890 ± 0.007	0.850 ± 0.035	3.182

Weight of fish sample is presented as mean ± SE (standard error). Comparison of values shows statistically different ($p<0.05$) mean weight of the fish sample.

Table 8 Post-inoculation behavioural variation (chronic studies)

Concentration of DDVP Solution (mg/L)	Behavioural Changes of Test	Behavioural Changes of Control
44.200 ± 0.000	Rapid suffocation was observed after 1 h. Loss of equilibrium was observed. 10% Mortality was registered after 6 h.	No suffocation, no loss of equilibrium and no mortality were observed.
55.300 ± 0.000	More rapid suffocation was observed after 1h. Loss of equilibrium was observed. 10% Mortality was registered after 4 h.	No suffocation, no loss of equilibrium and no mortality were observed.

Table 9 Post-inoculation behavioural variation (acute studies)

Concentration of DDVP Solution (mg/L)	Behavioural Changes of Test	Behavioural Changes of Control
4.420 ± 0.000	Suffocation was observed after 1h and Loss of equilibrium and was observed. 10% Mortality was registered after 3 h	No suffocation, no loss of equilibrium and no mortality were observed.
11.049 ± 0.000	Severe suffocation was observed after 1h. Loss of equilibrium and was observed. 10% Mortality was registered after 2 h.	No suffocation, no loss of equilibrium and no mortality were observed.
22.098 ± 0.000	More severe suffocation was observed after 1 h. Loss of equilibrium was observed. 10% Mortality was registered after $1^{1/}_{2}$ h.	No suffocation, no loss of equilibrium and no mortality were observed.
44.196 ± 0.000	Most severe suffocation was observed after 1 h. Loss of equilibrium was observed. 10% Mortality was registered after 45 minutes.	No suffocation, no loss of equilibrium and no mortality were observed.

DISCUSSION

The result of the acute and chronic exposure studies revealed that *Clarias gariepinus* post-fingerlings treated with different concentrations of dichlorvos pesticide depict significant different variations ($p<0.05$) in behaviour (Table 8 and 9), weight loss (Table 7) as well as dissolved oxygen or water quality parameter (Table 1 and 2). However, there were no significant post-inoculation variations ($p>0.05$) in skin and eye morphology and other water quality parameters such as pH (Table 3 and 4) and temperature (Table 5 and 6), whereas the periods of exposure (24, 48, 72, 96 h and above) between test and control cultured post-fingerlings under acute and chronic exposure studies of dichlorvos toxicity showed significant inverse relationships (inverse post-inoculation changes). Table 1 represents significant difference ($p<0.05$) in the mean levels of dissolved oxygen (6.090 ± 0.000 mg/mL; 5.000 ± 0.005 mg/mL; 4.670 ± 0.007 mg/mL; 3.990 ± 0.007 mg/mL) of pond water at acute levels of dichlorvos test solution as compared to the dissolved oxygen of control or standard aquarium water (7.000 ± 0.020 mg/mL) at the same level of exposure of the post-fingerlings. Similarly, there was a significant variation ($p<0.05$) in the mean levels of dissolved oxygen (Table 2) of pond water at chronic levels of concentration (2.040 ± 0.007 mg/mL; 2.010 ± 0.000 mg/mL) of DDVP. These variations are statistically significant, and they indicate inverse relationships (a decrease in the mean level of dissolved oxygen of aquarium water with a corresponding increase in the concentration of DDVP). The results imply that high concentration of acute DDVP test solution may have triggered changes in the physicochemical property (Gupta *et al.*, 2008). The absence of post-inoculation variation ($p>0.05$) in skin and eye morphology, pH (Table 3 and 4) and temperature (Table 5 and 6) among four different acute concentrations of DDVP test solution (4.420 ± 0.000 mg/L; 11.049 ± 0.000 mg/L; 22.098 ± 0.000 mg/L; 44.196 ± 0.000 mg/L) and two different chronic concentrations of dichlorvos test solution (44.200 ± 0.000 mg/L; 55.300 ± 0.000 mg/L) as well as their corresponding skin and eye morphology of the control is an indication that increase in the level of acute and chronic concentrations of DDVP may not have impaired the physicochemical properties and the normal skin and eye colouration of the post-fingerlings. This also shows that DDVP is short lived and non-persistent or non-bio accumulative in fish (Das, 2013). The cultured post-fingerlings exposed to acute dichlorvos toxicity exhibited significant behavioural manifestations like erratic swimming, copious mucus secretion, loss of equilibrium and hitting to the walls of test tank prior to mortality in acute dichlorvos test solutions. Similarly, these variations indicate that increase in the level of acute concentrations of DDVP may have caused changes in behavioural patterns of cultured *Clarias gariepinus* post-fingerlings exposed to four different acute concentrations of dichlorvos test solutions. The post inoculation behavioural variations cultured post-fingerlings exposed to chronic dichlorvos toxicity in fish were characterized by weak morphological manifestations which are indicated by diagnosed diverse effects including oxidative damage, inhibition of AchE activity, histopathological changes as well as developmental changes, mutagenesis and carcinogenicity (Das, 2013). Since dichlorvos is present in the environment with other similar organophosphate compounds, additive responses to organophosphate compounds may induce lethal or sub-lethal effects in fish (Das, 2013). Moreover, there is a correlation between this research result and the studies carried out by Das (2013), Ogamba, *et al.* (2015.) and Abolagba *et al.*, (2011) which reported some health implications of pesticide residues in fresh and smoked catfish (Clarias gariepinus) in Nigeria

CONCLUSION

From the result of the analysis, it could be concluded that variations and positive correlations in behaviour, mortality, weight loss and hydrobiological parameter (dissolved oxygen) were statistically significant ($p<0.05$). smoked catfish (*Clarias gariepinus*) in Nigeria

The organophosphate pesticide sampled as dichlorvos was present in the aquatic environment. Dichlorvos constituted a major constraint to hydrobiological and behavioural cycles of fresh catfish under study.

Acute and chronic dichlorvos concentrations constituted a potential health risk to fish and could as well serve as potential diagnostic tools for comparative purposes in assessing the lethal and sub-lethal effects of dichlorvos on fish. Hydrobiological and behavioural indices could serve as useful biomarkers in environmental bio-monitoring of pesticides contamination.

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