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Growth response and nutrient utilization of *Clarias gariepinus* fingerlings exposed to Dichlorvos

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Abstract

Organophosphate pesticides exert neurotoxicity by inhibiting acetylcholinesterase (AChE) in animals. Early-life exposure to agrochemicals affects growth performance and survival of non-target aquatic resources. In this study, lethal and growth responses of *Clarias gariepinus* fingerlings exposed to Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate or DDVP), commonly known as 'Sniper' or 'Ota-piapia' in Nigeria were evaluated. The acute toxicity test shows that the mortality recorded after every 24 hours was concentration-dependent. The median lethal concentration (LC50) of DDVP on *C. gariepinus* fingerlings at 24, 48, 72 and 96 hours post-exposure (hpe) were 3.30 mg/L (3.197 to 3.441; R2 = 0.964), 2.30 mg/L (1.844 to 2.518; R2 = 0.866), 2.27 mg/L (2.117 to 2.373; R2 = 0.959) and 1.88 mg/L (0.7704 to 2.211; R2 = 0.74), respectively. After a feeding trial experiment on post-exposed fingerlings, there was no significant difference ($p > 0.05$) between the feed intake of the control and DDVP exposed groups. Except for protein intake, significant differences ($p < 0.05$) were found in the growth and nutrient utilization parameters. The findings of this study reveal that *C. gariepinus* fingerlings could recover after acute exposure to Dichlorvos at different concentrations.

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Recovery, post-exposure, organophosphate pesticide, acute toxicity

Introduction

The Globally, the use of chemicals for the control of pests has continued to increase (Sharma *et al*., 2019; Al-Kawaz, 2019). These chemicals are also utilized in agriculture due to losses of around 30% initiated by insect attacks and other pest organisms (Das, 2013; Kumar *et al*., 2016). 2, 2-dichlorovinyl dimethyl phosphate (DDVP) is a commonly used organophosphate pesticide (OP) in controlling insect pests in residential and agricultural areas; however, its toxicity has gone beyond the target organism, and this chemical is frequently detected in water bodies (Ezike, 2017).

Compared to other chemicals, organophosphate pesticides (OPs) have gained universal popularity especially because they degrade rapidly (Das, 2013). Regrettably, OPs

can negatively affect both terrestrial and aquatic animals due to their non-target specificity (Das, 2013; Sturve *et al*., 2016). Dichlorvos, or 2, 2-dichiorovinyl dimethyl phosphate (DDVP) is one of the most commonly applied OPs especially in developing countries (Das, 2013). It has a wide application including its use in agriculture for crop protection and the protection of animals from parasitic attack. It is also used in aquaculture to eradicate ectoparasites (Das, 2013).

In Nigeria, DDVP is also used for the protection of crops and animals (Omoniyi *et al*., 2013). The aquatic environment receives toxic contaminants that get to the water bodies through agricultural, domestic, and industrial activities (Ololade and Oginni, 2010, Ö Fırat *et al*., 2011). When this chemical gets into the aquatic environment, it may be highly toxic to the aquatic organisms, is capable of inhibiting the health of fish by impairing metabolism, and could lead to death. Fishes are practically the most vital aquatic organisms that are frequently exposed and, therefore, are susceptible to the effects of these toxic pesticides (Rao *et al*., 2017). The acute toxicity of dichlorvos to freshwater fish has been studied in many freshwater fish species including Mossambique tilapia (*Tilapia mossambica*) (Rath and Misra, 1981), African air-breathing catfish (*Heterobranchus longifilis*) (Oribhabor and Ikeogu, 2016), Philippine catfish (*Clarias batrachus*) (Narra, 2016), and African mud catfish (*Clarias gariepinus*) (Omoniyi *et al*., 2013, Ashade *et al*., 2011; Egesi, 2017).

The African mud catfish (*C. gariepinus*) is a freshwater fish species that is of high commercial importance in Africa (Dadebo *et al*., 2014). Due to their hardy nature, they are known to survive in harsh environmental conditions (Ibrahim *et al*., 2016; Basharat *et al*., 2020). Previous studies have sought to estimate the potential hazards of these chemicals as a form of risk assessment through toxicity testing (Omoniyi *et al*., 2013). Some of these studies have assessed the toxicity of DDVP on various life stages of *C. gariepinus* including Omoniyi *et al*. (2013) who studied the lethal toxicity of DDVP on *C. gariepinus* fingerlings and juveniles. Ojesanmi *et al*. (Ojesanmi *et al*., 2017) studied the mortality rate of *C. gariepinus* fingerlings exposed to this chemical, while Ndimele *et al*. (2013) studied the effect of dichlorvos-induced stress on the growth of the fingerlings.

Apart from Ndimele *et al*. (2013), other available studies had only considered the toxicity of the chemical on *C. gariepinus* and not the recovery of the survived fish post-exposure. This study, therefore, adds to the knowledge regarding the post-exposure influence of DDVP on the growth of *C. gariepinus* fingerlings. In addition to the growth parameters reported by Ndimele *et al*. (2013), this study presents information on the nutrient utilization of the exposed fishes. The objectives of this study are, therefore, to assess the acute toxicity of DDVP on fingerlings of *C. gariepinus* and the effects of DDVP on the growth and nutrient utilization of surviving individuals, post-exposure.

Materials and Methods

Brood stocks of *C. gariepinus* were sourced from Lagos State University hatchery. Thereafter, artificial reproduction was carried out following De Graaf *et al*. (1995). After the first eight weeks, 1000 fingerlings were transferred to the laboratory of the Department of Fisheries, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria. The experimental fish were fed 2.0 mm size commercial pelleted feed (42% crude protein) at 3% body weight (Viveen *et al*., 1985), and this was administered twice daily: morning (09.00 h) and evening (16.00 h). Removal of waste food and water exchange was also done daily.

Dicholorvos, 2, 2-dichlorovinyl dimethyl phosphate (DDVP) was obtained from the stocks available at Lagos State Agricultural Inputs Supply Authority, Ojo, Lagos, Nigeria. Concentrations were prepared from stock (10 mg/L). A 96-hour range-finding test was conducted before the definitive test on the selected fingerlings of *C. gariepinus* (Odiete, 1999).

For the toxicity test, the experimental set-up consisted of 21 transparent rectangular (61.5 cm × 33.2 cm × 20 cm) plastic tanks with various concentrations of pesticides: 0, 2.4, 2.6, 2.8, 3.0, 3.2, and 3.4 mg/L (Control, T1, T2, T3, T4, T5, and T6, respectively) with three replicates each. The control, T7 had no toxicant. Each aquarium (containing 10 litres of dechlorinated water) was randomly stocked with 10 *C. gariepinus* fingerlings (average body weight 3.3g). Before the recovery phase, a new set of fingerlings with an average weight of 3.8 g were exposed for 24 hours, which is the time with the least mortality from the previous toxicity experiment.

Feeding was done twice a day at 3% body weight between 09:00-16:00 h for 56 days. The fishes were fed through an even distribution of the feed on the water surface of each holding tank. This was to give an even feeding opportunity to all the individual fishes. Feeding in all tanks was generally completed in about 10-15 min. The mean size of the fish {weight (g) and total length (cm)} for each treatment and its replicates were measured every 2 weeks (Ndimele *et al*., 2012). Feed intake (FI): quantity of feed fed per day. This was enumerated as follows; = 3% body weight of fish/day

The growth and nutrient utilization parameters were calculated as follows:

Growth parameters:

i. Weight Gain (WG) = W2 — W1 (g)
ii. Percentage Weight Gain % (PWG) = 100 (W2 — W1)
iii. Specific Growth Rate (SGR) % per day = (Loge W2– Loge W1) × 100/T
iv. Average Daily Gain (ADG) = (W2 - W1)/ T

Where:
W2= Final mean weight (g),
W1 = Initial mean weight (g),
T2 = Final time (days)
T1 = Initial time (days),
Loge = Natural logarithm,
T = period of experiment (days)

Nutrient Utilization:

i. Protein Intake (PI): Feed intake (g) × % protein in the diet
ii. Feed Conversion Ratio (FCR) = Weight of dry feed fed (g)/ Live weight gained (g)
iii. Protein Efficiency Ratio (PER) = Gain in weight of test fish (g)/ Protein consumed (g)

Statistical Analysis

The concentrations and mortality were transformed to Log10 and percentage, respectively based on the probit model (Sprague, 1969). The median lethal concentration (LC50) and median lethal time (LT50), were determined (Finney, 1971) using GraphPad prism ver. 7.0. Analysis of variance (ANOVA) was used to test for significant differences in growth performance and feed utilization. This was after exploring the data to confirm that they satisfied the conditions for a parametric test. The ANOVA was carried out with

Statistical Package for Social Science (SPSS) for Windows (v 15.0). A pairwise comparison was carried out using Fisher's Least Significant Difference (LSD) at $p < 0.05$.

Results and Discussion

This study assessed the toxicity stress of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) on the growth performance and nutrient utilization of *C. gariepinus* fingerlings. Early-life exposure to environmental stressors (either chemical or physical factors) might be reversible or irreversible depending on the physical and chemical properties of the xenobiotic, the duration of exposure, age, and health condition of an organism (Rodriguez-Dominguez *et al*., 2018).

The mortality recorded after every 24 hours was substantially concentration-dependent. However, no mortality was recorded in the control group (Figure 1). The median lethal concentration of DDVP on *C. gariepinus* fingerlings at 24, 48,72, and 96 hours post-exposure (hpe) were 3.30 mg/L (3.197 to 3.441; R2 = 0.964), 2.30 mg/L (1.844 to 2.518; R2 = 0.866), 2.27 mg/L (2.117 to 2.373; R2 = 0.959) and 1.88 mg/L (0.7704 to 2.211; R2 = 0.74), respectively.

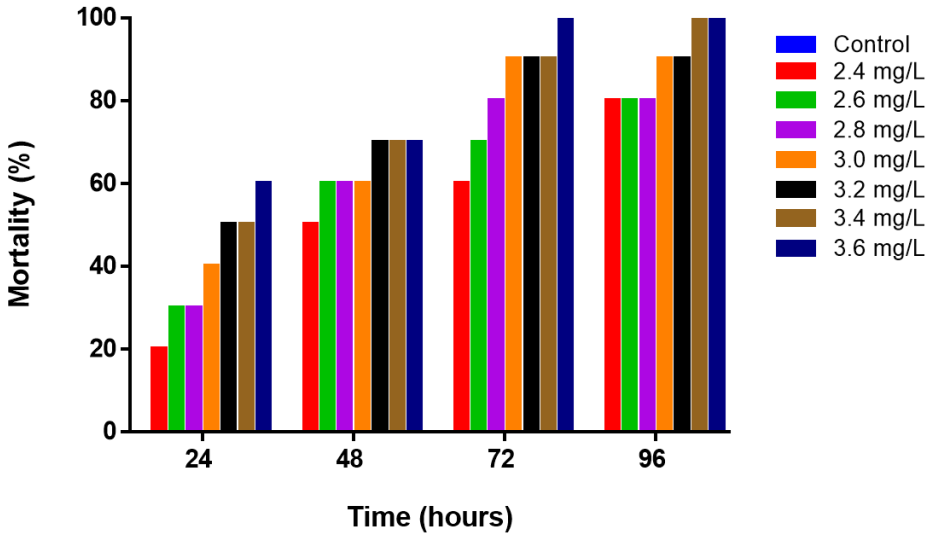

Figure 1. Time-mortality relationship of Dichlorvos to fingerlings of *C. gariepinus*

Figure 2 presents the concentration-mortality at different times of exposure. Figure 3 shows the median lethal-time (LT_{50}) of the cumulative mortality at 96 hours post-exposure of *C. gariepinus* fingerlings. The LT_{50} was 27.23 hours (6.185 to 41.21; R2 = 0.946).

Fish under the controlled group expectedly did not die, and they survived better than those exposed to DDVP. This is in agreement with the findings of earlier researchers who also reported that the effects of the pesticides could be regarded as the possible cause of death of the test fish (Ugwu *et al*., 2006; Khan *et al*., 2018). Ezike (2017) reported that the 96 hour LC_{50} of DDVP in freshwater and brackish water fish is 0.2 - 12 mg/L with > 4 mg/L in marine fish, this is similar to the result in this study. This is contrary to the 96-hour LC_{50} of DDVP *C. gariepinus* juveniles reported as 0.93 mg/L by Ezike (2017). The mortality recorded in this study is in agreement with the 24, 48, 72, and 96 hours mortality observed in Tilapia mossambica exposed to DDVT with the LC_{50} values ranging between 1.42 – 2.53 mg/L. The highest concentration of the pesticide led to the highest mortality and this suggests a

concentration influenced lethality and dose-dependent survival (Khan *et al*., 2018, Ezemonye and Tongo, 2009). Higher mortality values recorded may be mainly due to the metabolites of dichlorvos that were formed in animals. Dichlorvos is a potent enzyme inhibitor capable of killing organisms directly by inhibiting acetyl cholinesterase (Kumar *et al*., 2016).

There was no significant difference ($p > 0.05$) in the feed intake of the control and other Dichlorvos concentration groups. Except for protein intake, significant differences ($p < 0.05$) were found in the growth and nutrient utilization parameters. Mean + SD values for growth and nutrient utilization indices can be found in Table 1. The highest value for weight gain was 2.45 ± 2.30 g. The percentage weight gain (57.84 ± 43.39 %), specific growth rate (0.96 ± 0.84) and average daily growth (0.18 ± 0.17) were recorded in treatment with concentration of 3.2 mg/L while their lowest values (0.07 ± 0.33), (2.32 ± 1.02), SGR (0.03 ± 0.47) and ADG (0.00 ± 0.03) were respectively recorded in group exposed to a concentration of 2.8 mg/L.

Except for the 2.6 mg/L group, the weight gain of the control was significantly different ($p < 0.05$) from the other groups. The percentage weight gain of the control was only significantly similar to those of 3.0 mg/L and 3.4 mg/L. The specific growth rate of the control was only significantly different from those of 2.4 mg/L and 2.8 mg/L groups, while the average daily gain of the control was only significantly different from the 2.48 mg/L, 2.8 mg/L, and 3.4 mg/L groups. The feed conversion ratio, of control, was only significantly different from that of the 2.8 mg/L group, while the protein efficiency ratio was significantly different from those of 2.4 mg/L, 2.8 mg/L, and 3.2 mg/L.

Apart from the control group, slow response to feeding by fish exposed to different concentrations of dichlorvos was observed in the first 14 days of the recovery phase, which improved in the second fortnight. This is an indication that feed intake was likely affected by the presence of the toxicant. The feed intake (FI) increased irrespective of dichlorvos concentration (2.8 - 3.4 mg/L) between days 28 and 56. Better or faster response to feeding in the 28-56 days also implied that the effects of dichlorvos fractions in water were reducing and the fish response to feeding has improved. This was also reported by Ugwu *et al*. (2006).

Table 1. Growth and nutrient utilization of *Clarias gariepinus* fingerlings exposed to Dichlorvos at 24hp

Treatment (mg/L)	WG (g)	PWG (%)	SGR (%/day)	ADG (g/day)	PI (%/g)	FCR	PER
Control	1.24±0.41^{b}	22.36±7.19^{c}	0.71±0.27^{b}	0.09±0.29^{a}	6.65±1.09^{a}	0.02±0.01^{a}	2.52±0.8^{b}
2.4	0.22±0.37^{a}	2.58±9.34^{a}	0.04±0.37^{a}	0.0±0.26^{a}	4.43±0.34^{a}	0.01±0.02^{a}	0.29±1.01^{a}
2.6	1.73±0.90^{b}	33±14.15^{b}	0.83±0.32^{b}	0.12±0.06^{b}	5.37±0.91^{a}	0.07±0.06^{a}	3.95±1.6^{b}
2.8	0.07±0.33^{c}	2.32±1.02^{a}	0.03±0.47^{a}	0±0.03^{a}	4.01±0.02^{a}	0.19±0.12^{b}	0.17±0.10^{a}
3.0	0.84±0.25^{d}	20.34±5.28^{c}	0.6±0.14^{b}	0.06±0.18^{b}	4.98±0.72^{a}	0.02±0.00^{a}	2.27±0.5^{b}
3.2	2.45±2.03^{e}	57.84±3.39^{d}	0.96±0.84^{b}	0.18±0.17^{b}	6.29±1.12^{a}	0.01±0.01^{a}	8.73±0.28^{c}
3.4	0.86±0.39^{d}	18.36±9.06^{c}	0.6±0.20^{b}	0.06±0.28^{a}	4.67±0.46^{a}	0.02±0.02^{a}	2.45±0.8^{b}

WG;Weight gain, PWG; Percentage weight gain, SGR; Specific growth rate; ADG; Average daily gain; PI; Protein intake FCR; Feed conversion ratio, PER; Protein efficiency ratio. Means followed by the same letter in the same column are not significantly different ($p < 0.05$) using LSD. Values present mean ± standard deviation.

In this study, the growth and nutrient utilization parameters of *C. gariepinus* fingerlings were generally not dependent on the concentration group to which they belong. This is different from the finding of Ndimele *et al*. (2012) who reported a concentration-dependent growth for *C. gariepinus* fingerling exposed to varying concentrations of dichlorvos. This may be due to different conditions to which the fishes were subjected in their study. For example, the time for which the fishes were exposed before the growth trial was not clearly stated in their study as opposed to this study where the time with the lowest record of mortality was selected. As opposed to their report, this study showed that the fishes belonging to the control group did not necessarily record the best growth or nutrient utilization parameters. This finding points to the possible recovery of the fishes that survived exposure to varying levels of the pesticide for 24 hours.

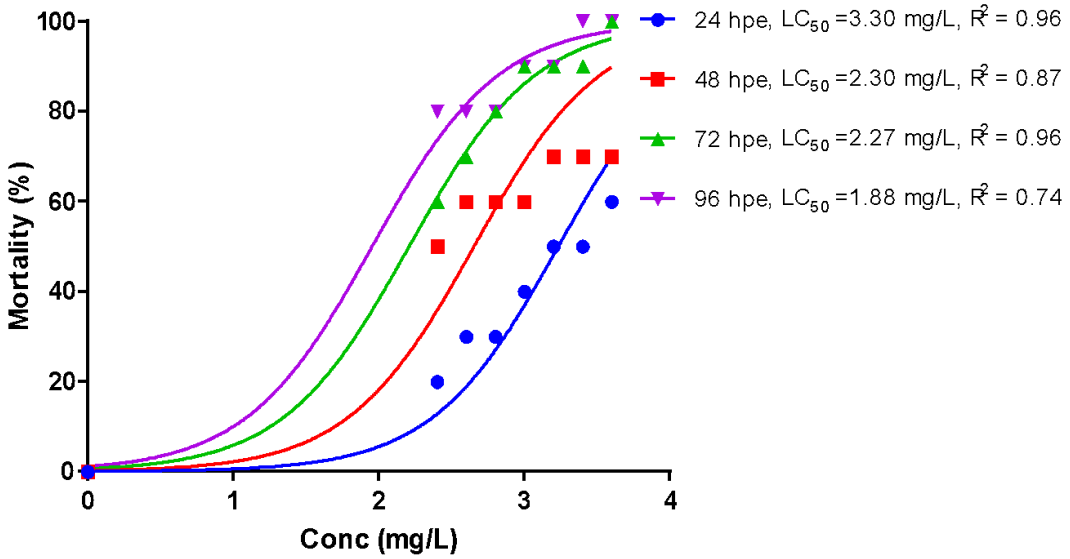

Figure 2. Median Lethal Concentrations (LC50) of Dichlorvos to fingerlings of *Clarias gariepinus*

Although it is expected that the residue of the pesticide in the tissues of the fish will fade out within 56 days for which the growth trial lasted, this is not to suggest the possible absence of residual effects on the biochemical parameters of this fish. Some studies that reported this had ignored the possible impacts of pesticides on the biochemical parameters after a substantial period (post-exposure) as was the case in this study. For example, based on analyses carried out immediately after the exposure period, Ndimele *et al*. (2015) concluded that another organochlorine pesticide known as endosulfan alters the hematology of *C. gariepinus* fingerlings. Likewise, Harabawy *et al*. (2014) only considered the immediate post-exposure hematological, biochemical, and cytogenetic response of *C. gariepinus* to carbofuran pesticide. Although this study adds to what has been known, there is the need for further research into the post-exposure recovery and the assessment of growth, nutrient utilization, and biochemical and hematological parameters for a while during recovery of exposed fishes to either lethal or sublethal concentrations of chemicals to which they are exposed.

Conclusions

This study found a dose-dependent survival and concentration graded lethality of dichlorvos based on the 96-hour acute toxicity test. Besides, the growth and nutrient utilization parameters of *C. gariepinus* fingerlings post-exposure were generally not concentration-dependent. This finding reveals that *C. gariepinus* fingerlings could recover after acute exposure to 2, 2-dichlorovinyl dimethyl phosphate (Dichlorvos) at different concentrations.

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