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Original Article

Effects of dimethyl 2, 2-dichlorovinyl phosphate on the sodium, potassium and calcium content in the kidney and liver of *Clarias gariepinus*

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Abstract

Dimethyl 2, 2-dichlorovinyl phosphate is an organophosphorus insecticide used in the control of several plant and animal pests. This study evaluated the effects of dimethyl 2, 2-dichlorovinyl phosphate on the electrolytes (calcium, sodium and potassium) in the kidney and liver of *Clarias gariepinus.* The fish samples were purchased from private farm in Aluu Port Harcourt, Nigeria. The fishes were carefully housed to prevent contamination and three different concentrations of the toxicant were prepared i.e. 0.00ppm, 0.10ppm and 0.20ppm. At the end of the experiment (four days), the fishes were dissected and the internal organs i.e. liver and kidney were collected and 0.5g of each macerated, and a few drop of physiological saline added before centrifuging at 3000 rpm for 10 minutes. The calcium, sodium and potassium content were analyzed using standard methods. Results showed that calcium, potassium and sodium ranged from 0.80 – 1.40mg/l, 3.80 – 7.10mg/l and 10.20 – 16.40mg/l respectively (Kidney) and 1.00 – 1.13mg/l, 10.60 – 17.73mg/l and 6.80 – 12.80 mg/l respectively (liver). With the exception of calcium there was significant variations ($P<0.05$) among the various concentrations of sodium and potassium. The study confirms that dimethyl 2, 2-dichlorovinyl phosphate can alter the electrolytes balance of *Clarias gariepinus.*

1. Introduction

Xenobiotics are substances that are recalcitrant to degradation. A typical example includes plastic, pesticides, health care wastes. Pesticides are substances used to prevent and or control pests on crops and animals [1 – 3]. These pesticides could have adverse effects on the environment biota and components including crops, animals, soil and water on application [1, 2], and humans through food contaminated by pesticides [3]. Pesticides are vital chemical that is often encountered by human nearly on daily basis [4]. Wilson and Tisdell [5] and Ezemonye and Ogbomida [4] stated that the use of chemical pesticides increases agricultural productivity, but its application have negative effects including damage to the environment, agricultural land, fisheries, fauna and flora. Some indirect method through which pesticides enter the environment include water runoff and or as aerosols carried by winds [1, 6], abandonment of emptied cans of pesticides in the environment without proper disposal approach. In the world today, different pesticides manufactured contain different chemicals including organochlorines, halogenates, hydrocarbons, carbamates, heterocyclic compounds, organophosphates, chlorinated phenoxy substances, amines and ureas, phenolic compounds and pyrethroids [2]. A typical example of organophosphorus pesticide is dimethyl 2,2-dichlorovinyl phosphate (DDVP) [7] and Nuvan [8]. Pesticides can be classified by on biological perspectives and these include viruses, bacteria, fungi, and plants, nematodes, insects and other parasites or predators. Some of the biological pesticides include insecticides, rodenticides, fungicides, herbicides and fumigants [3, 9].

Dichlorvos, is the common name of dimethyl 2,2-dichlorovinyl phosphate, some authors have reported it as 2,2-dichlorovinyl dimethyl phosphate, whose properties include; 120°C/14 mm (boiling point), <-18 °C (Freezing point), 0.142 kg per litre at 20°C (density), about 1% solubility in water, heat stable, corrosive to black iron and mild steel, noncorrosive to aluminium, nickel and stainless steel in the absence of moisture, nonreactive to teflon and polyethylene, stable in hydrocarbon solvents [10]. The toxicity of dichlorvos to mammals is moderately high by ingestion, inhalation and absorption through the skin [10]. Dichlorvos is used as insecticides for the control of mushroom flies, aphids, spider mites, caterpillars, thrips and whiteflies in vegetable crops and fruits and for the treatment of parasitic worm infections in dogs, livestock, and humans [11].

The aquatic ecosystems are known to receive a wide range of pollutants which are directly or indirectly introduced into such water bodies [12]. These toxicants may enter the food chain of the receiving water body that inhabits several edible zooplankton, fishes (shell and fin) and affects the fatty part of the organisms [3]. According to Dahunsi et al. [13], toxic materials in the aquatic ecosystem alter the water quality parameters, which could affect aquatic life in such water. When the concentration of such toxicant is higher than the homeoistasis of the aquatic organisms can tolerate, it could result to organ damage and even death. Most of the notable organs that could be impaired by toxicants include the opercula, skin, liver and gill [14] cited in [13], kidney and blood vessel [15]. Pesticides in fisheries could lead to acute toxicity, behavioral and histopathological changes [16]. Liver is the metabolic site for detoxification of chemicals [17]. Other histopathological effects associated with pesticides in fisheries include partial loss of liver plate, radial orientation, shrinkage of some liver cell mass and exhibition of cytoplasmic granularity by the liver [15]. Murthy et al. [15] further reported that the glomeruli in the posterior kidney could show pycnotic changes of cell nuclei, vocalization of cytoplasm, and atrophy of some cells, while the gill filaments and lamellae could also show precipitated masses that could plug the central capillaries.

Several fin and shelled fish species are major sources of animal protein and nutrients in human diets [18-22]. In the natural habitats of fisheries, pesticides could be intentionally added to the aquatic ecosystem for the purpose of killing, injuring, or occasionally enhancing the development of some forms of life [4]. Contamination of aquatic ecosystem with pesticides could lead to bioaccumulation in fisheries. These could impair the electrolytes including sodium, potassium and calcium concentration. Akinsorotan [23] and Ogamba et al. [6] reported that pollutants in aquatic ecosystem could adversely affect the ability of fish to detect and respond to chemical stimuli, feeding, growth and reproductive performances, which could lead to inability to bread in polluted environment or even death. Napit [24] also noted that presence of pesticides in aquatic ecosystem could alter the behavior, biochemical processes in fisheries as well as bio-accumulation of the pesticides in the fish body tissues. Typically, it is difficult to evaluate the toxicity of pesticides in natural aquatic ecosystem probably due to their complex nature [25].

Clarias gariepinus an African catfish, which belong to sub-order Siluridae and Clariidae family [26] is one of the commonest freshwater mud fish that are normally reared in fish ponds and they also occur freely in their natural stock (Rivers) [27]. *Clarias gariepinus* have accessory air breathing organs, which enable them to withstand harsh aquatic conditions which other fish species cannot withstand [28] cited in [27]. Therefore, this study assessed the toxicity of dimethyl 2,2-dichlorovinyl phosphate on the kidney and liver of *Clarias gariepinus* with respect to the electrolytes (calcium, potassium and sodium) balance.

2. Materials and methods

2.1 Animals procurement

Forty-five adult *Clarias gariepinus* (mean weight 166.70 ±3.3g, mean length 22.43±2.42cm) was purchased from a private fish farm in Aluu, Port Harcourt, Rivers State, Nigeria. The fish samples purchased were transported to Biological sciences Department, Niger Delta University, Nigeria were the experiment was conducted.

2.2 Pre-experimental fish management

On fishes arrival, they were acclimated individually in rectangular aquaria with sealed top using mesh (i.e net) for 7 days. A slit was made at the middle of the net to ease the aquaria maintenance i.e cleaning [25]. The aquarium was washed with piece of foam and the water was changed at 24 hours interval [6]. Pipette and suckers was used for the introduction of the toxicant after the water is changed on daily basis during the trail experiment. The fishes were fed once a day with 1% biomass containing 35% crude protein [1, 6] between 10.00 and 11.00 hours.

2.3 Source of Dimethyl 2, 2-dichlorovinyl phosphate and Concentration prepared

The toxicant i.e. dimethyl 2,2-dichlorovinyl phosphate was obtained from private chemical dealer in Port Harcourt, Nigeria. A two concentration range finding text (trial test) was carried out on the toxicant (i.e. dimethyl 2,2-dichlorovinyl phosphate) based on the original concentration using the formula;

$N1\ V2 = N2V2$ [29] cited in [1, 25]

N1 = Manufacturer concentrated

N2 = Concentration of test solution desired.

V1 = Volume of the original solution added.

V2 = Volume of the test solution (30 litre).

The test trial concentrations were 0.10ppm and 0.20ppm.

2.4 Experimental design

The completely Randomized Design (CRD) experimental method was used in this study. The use of the CRD experimental design was due to the fact that the experimental fisheries are homogenous. The *Clarias griepinus* used for this study were randomly divided into three groups of five fishes per group representing one control group and two treatment i.e. A (0.00ppm), B (0.10ppm) and C (0.20). Group A served as the control group. The fishes in the different group were fed with the same feed during this study. The duration of the experiment was four days.

2.5 Inoculation of the toxicants dimethyl 2, 2-dichlorovinyl phosphate on the definitive test

The definitive test was done based on the range finding test which was prepared by pipetting 0.00ml/l, 0.10ml/l and 0.20ml/l respectively of the original concentration of the dimethyl 2, 2-dichlorovinyl phosphate and making it up to 30 litre with tap water in the aquaria to make 0.00ppm, 0.10ppm and 0.20ppm. The three treatments were carried out in triplicate levels.

2.6 Sample collection and laboratory analysis

At the end of the experiment, the fishes were dissected and the organs such as liver and kidney were collected. About 0.5 g each of the organ was grounded using mortar and pestle. About 5ml drops of physiological saline were added to the sample before centrifuging at 3000 rpm for 10 minutes. The supernatants were collected using plain bottle and stored at -20ºC prior to analysis.

2.7Physico-Chemical Parameters of Water

Triplicate samples of the aquarium water were collect after the introduction of the toxicants and the physico-chemical parameters including conductivity, temperature, pH, Turbidity and Dissolved oxygen were analyzed in-situ using probes.

2.8 Statistical Analysis

SPSS software used to carry out the statistical analysis. The data were expressed as Mean ± standard error and one-way analysis of variance was carried out at α = 0.05, and Tukey HSD Test was used for mean separations.

3. Results and Discussion

Table 1 presents the physiochemical properties of the aquarium water. The concentration ranged from 6.20 – 6.80mg/l (dissolved oxygen) and 6.28 – 6.37 (pH) with significant difference ($P<0.05$) among the different concentrations. Similarly, there was no significant variation ($P>0.05$) in the concentration of conductivity, temperature and turbidity which ranged from 136.08 – 136.12 µS/cm, 26.00 – 26.07 ºC and 5.00 NTU respectively. The absence of variation in the conductivity, temperature and turbidity is an indication that the toxicant may not have impaired these parameters. Also the slight variation that occur with regard to the pH showed that the toxicant increased the pH of the water toward alkalinity slightly as observed in concentrations 0.10 and 0.20ppm, while decreasing the dissolved oxygen concentration. Similar physico-chemical parameters concentration including temperature (26ºC), pH (6.20-6.37), dissolved oxygen (5.38-7.21) mg/l, conductivity (99.50-136.12µs/cm) and turbidity (0.42-0.50NTU) have been reported aquarium water (*Clarias gariepinus)* exposed to diazinon [30]. Seiyaboh et al. [1] reported the physicochemical parameters of aquarium water exposed to Paraquat dichloride whose concentration ranged from 0.00 – 0.20ppm in the range of 99.5 – 108.75 µS/cm (conductivity), 6.27 – 6.37 (pH), 0.20 -0.50 NTU (turbidity) and 6.61 – 7.21mg/l (dissolved oxygen). The high similarity with regard to the physico-chemical parameters could be attributed to the fact that these previous study and current study were carried out in the same location, whose water source is the same with the only difference being the time of the study.

Table 1: Physico-chemical properties of the aquarium water exposed to dimethyl 2, 2-dichlorovinyl phosphate

Treatments/ Concentration, ppm	pH	Conductivity, µS/cm	Temperature, ºC	Turbidity, NTU	DO, mg/l
0.00	6.28±0.01a	136.10±0.06a	26.00±0.21a	5.00±0.00a	6.80±0.06c
0.10	6.37±0.01b	136.08±0.58a	26.00±0.15a	5.00±0.00a	6.60±0.00b
0.20	6.37±0.01b	136.12±0.58a	26.07±0.24a	5.00±0.00a	6.20±0.00a

The same letters along the column is not significantly different ($P>0.05$) according to Tukey HSD statistics; Mean ± Standard error; n=3

The concentration of the electrolytes in the kidney of *Clarias gariepinus* exposed to dimethyl 2, 2-dichlorovinyl phosphate is presented in Table 2. The concentration of calcium ranged from 0.80 – 1.40 mg/lbeing significantly the same (P>0.05) in all the treatment. However, there was decreased in the calcium level when they were exposed to dimethyl 2, 2-dichlorovinyl phosphate. Similarly, the concentration of potassium in the kidney ranged from 3.80 – 7.10 mg/l being significantly different (P<0.05) in all the concentrations. Basically, the concentration increased with the addition of the toxicants. Like potassium, the sodium level increased with the addition of toxicant (dimethyl 2, 2-dichlorovinyl phosphate), being significantly different (P<0.05) in all the concentration. However, the concentration ranged from 10.20 – 16.40 mg/l.

Table 2: Concentration of the different electrolytes in the kidney of *Clarias gariepinus* exposed to dimethyl 2, 2-dichlorovinyl phosphate

Treatment/ Concentration, ppm	Sodium, mg/l	Potassium, mg/l	Calcium, mg/l
0.00	10.20±0.20a	3.80±0.06a	1.40±0.23a
0.10	16.40±0.17c	7.10±0.00c	0.80±0.06a
0.20	11.33±0.20b	4.40±0.21b	0.80±0.06a

The same letters along the column is not significantly different (P>0.05) according to Tukey HSD statistics; Mean ± Standard error; n=3

Table 3 presents the concentration of the electrolytes in the liver of *Clarias gariepinus* exposed to dimethyl 2, 2-dichlorovinyl phosphate. The concentration of sodium and potassium were typically lower in the control samples. However, the sodium and potassium concentration ranged from 10.60 – 17.73 mg/l and 6.80 – 12.80 mg/l respectively. Basically significant variation (P<0.05) exist between the various concentrations. The concentration of calcium was highest 1.13 mg/l (0.20ppm) and least 1.00 mg/l (0.00 and 0.10ppm). However no significant difference (P>0.05) exist among the concentrations.

Table 3: Concentration of the different electrolytes in the liver of *Clarias gariepinus* exposed to dimethyl 2, 2-dichlorovinyl phosphate

Treatment/ Concentration, ppm	Sodium, mg/l	Potassium, mg/l	Calcium, mg/l
0.00	10.60±0.06a	6.80±0.06a	1.00±0.12a
0.10	17.73±0.17c	12.80±0.20c	1.00±0.15a
0.20	14.93±0.15b	9.40±0.21b	1.13±0.28a

The same letters along the column is not significantly different (P>0.05) according to Tukey HSD statistics; Mean ± Standard error; n=3

The concentration of the toxicant in the liver and kidney of *Clarias gariepinus* were lower in the control (0.00ppm) than other treatments. This is an indication that the toxicant used in this study has altered the electrolyte composition of the fish under study. Similarly, the concentration of the toxicant at 0.10ppm was higher than when it was 0.20ppm. The reason for this variation remains unknown. A decline in the calcium content in the kidney when exposed to toxicant could be due to increased osmoregulatory activity in the kidney. The trend of results reported in this study is comparable to previous report by Ogamba et al. [25] that reported a fluctuation pattern in the different concentrations of paraquat dichloride on gills, liver, muscle and plasma metabolites (total protein, albumin, urea, creatinine and total bilirubin) of *Clarias gariepinus* and the study of Seiyaboh et al. [1] on the haematological response of *Clarias gariepinus* when also exposed to paraquat dichloride*.* Generally the physicochemical parameters were within the permissible limits, hence the variation in the electrolytes could not be attributed to water quality [25]

4. Conclusion

Pesticides are substances that control, prevent and mitigate any pest. Pesticides are classified according to their role including herbicides, insecticides, fungicides etc. these pesticides have beneficial role in agriculture including crop protection, food and material preservation and disease control. On the adverse effects, they may pose threat to human and impact on the ecosystem (i.e terrestrial and aquatic). This study evaluated the effects of dimethyl 2, 2-dichlorovinyl phosphate on the kidney and liver of *Clarias gariepinus* – a freshwater fish. The study found that with the introduction of the toxicant (dimethyl 2, 2-dichlorovinyl phosphate), the concentration of sodium and potassium increased in both organs. While the concentration of calcium declined in the kidney and increased in the liver. Hence, this study showed that dimethyl 2, 2-dichlorovinyl phosphate has the potential to alter the electrolytes of *Clarias gariepinus.*

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