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Research Article

Acute Toxicity of Clarias Gariepinus Fingerlings Exposed to 2,4-D Dimethylamine Salt

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Abstract

This study investigated the acute toxicity of *Clarias gariepinus* fingerlings. The fingerlings of *Clarias gariepinus* were acclimatized for 1 week before the range-finding test was carried out at varying concentrations. Sublethal concentrations (viz: 0.00ppm, 10.80 ppm, 18.00 ppm, 25.20 ppm, 32.40 ppm and 39.60 ppm of the 2,4-D Dimethylamine salt) were made in a rectangular aquarium. Each experimental concentration was carried out in triplicate with 10 fish each. The media were renewed at every 24 hours throughout the experimental duration viz: 96 hours. When the fish were introduced into the aquarium containing the toxicants, they exhibited some behavioural changes including opercular movement, air gulping and irregular swimming pattern. The mortality rate significantly increased as the concentration of the 2,4-D Dimethylamine salt increased for each of the exposure durations. The LC_{50} values at 24, 48, 72 and 96 were 86.15 ppm, 36.28 ppm, 18.72 ppm and 15.68 ppm, respectively. From the findings of this study, there is a need to exercise caution in the use of 2,4-D Dimethylamine salt based herbicides close to the aquatic ecosystem.

Keywords:
Clarias gariepinus; Contaminants; Fish; Environmental health; Herbicides

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Introduction

Pesticides have been found useful in the control of different kind of pest in both homes and agricultural fields [1]. Pesticides are often classified based on the target organisms. For instance, the group of pesticides used to control, mitigate or eradicate pests include insecticides, acaricides, herbicides, fungicides, rodenticides, fumigants. According to Inyang et al. [2], pesticides can also be grouped based on the formulation (soluble in solvent or dust-like), origin (synthetic or organic) and mode of action (contact or systemic).

In recent times, the use of herbicides in controlling weeds in agricultural and home fields has increased. This has raised the concern of many environmentalists, possibly due to the toxic nature of many herbicides to non-targeted organisms. Herbicides just like other pesticides (such as insecticides, acaricides, etc) tend to cause pollution in the environment depending on the concentrations and exposure duration. Over a prolonged period, herbicides could cause an alteration in the biochemical and physiological responses of exposed organisms especially fishes in the aquatic ecosystem and small mammals such as rabbits in the terrestrial environment.

Several herbicides have been studied in different parts of the world. But in Nigeria, some of the commonly used herbicides for controlling weeds in agricultural settings include paraquat dichloride [3], [4], glyphosate [5] and 2,4-D Dimethylamine salt [6]. The toxicity of some of these herbicides such as glyphosate and paraquat dichloride has been widely reported in the literature with respect to enzymatic, haematological, histopathological, biochemical, morphological/behavioural responses and mortality rate on fishes.

One of the herbicides that have not been commonly studied and are used for the control of weeds is 2,4-D Dimethylamine salt. Most herbicides that have 2,4-D Dimethylamine salt as the active ingredient are used as a selective, pre-emergent and post-emergent systemic herbicide which diminishes the number of broad leaves weeds and vegetation in agricultural fields [6] of both annual and perennial plants.

Most chemical toxicants have the tendency to persist for some time in the environment depending on their nature, concentration and prevailing climatic/ environmental condition. 2,4-D Dimethylamine salt tends to persist in the soil for a long period. According to Inyang et al. [6], 2,4-D Dimethylamine could bioaccumulate in animals (including rabbits, fishes etc) and vegetation which are a food source of humans.

Generally, herbicides may find their way into the aquatic ecosystem when they are applied close to surface water resources, and/ or when empty cans of herbicides are discharged into the water bodies directly. Runoff resulting from precipitation is the major method through which pesticides enter the aquatic ecosystem [3], where they impact on the water quality and some of the associated fauna and flora.

Fish have been widely used to assess the effect of herbicides such as 2,4-D Dimethylamine salt on surface water resources. Toxicants such as herbicides could cause dysfunction in the reproductive, food conversion efficiency, growth, and mortality rates in fishes [3]. Hence, this study assessed the mortality rate of *Clarias gariepinus* fingerlings exposed to 2,4-Dimethylamine salt.

Materials and Methods

Fish Sources and Acclimatization

A total of two hundred and ten *Clarias gariepinus* fingerlings (mean length of 5.6cm) were procured from a private fish farm in Yenagoa metropolis, Bayelsa state, Nigeria. The fish were acclimatized to an ambient environmental condition in a rectangular aquarium. A sub-lethal concentration of 2,4-D Dimethylamine salt was made and renewed daily. During the process, the fish were fed with their normal coupen fish diet (fish meal).

Range Finding Test (Trial Test)

A renewal bioassay was employed in this study, and the solution of the 2,4-D Dimethylamine salt was renewed daily. A range-finding test of the toxicants was carried out at 10ppm, 25ppm, 40ppm and 55ppm. This was done to define a safe sub-lethal concentration for the main experimental run. Each of the concentrations made for the trial test contains 4 fishes.

Main Experiment

About 0.00mls, 0.15mls, 0.25mls, mls, 0.35 mls, 0.45 mls and 0.55 mls of 2,4-D Dimethylamine salt (720g/L) (equivalent to 720000 mg/L) was pipetted into a rectangular aquarium containing 10 litres of water (equivalent to 10000 mg/L). This brings the concentration to 0.00ppm, 10.80ppm, 18.00ppm, 25.20 ppm, 32.40 ppm and 39.60 ppm. Each of the experimental groups contains 10 fish. Each group was carried out in triplicate. The toxicant was renewed after 24 hours until the experiment was terminated at 96 hours. These concentrations were made by using the formula previously described by Inyang *et al*. [7,8,9,10,11], Aghoghovwia and Izah [4], [5], Akinsorotan et al. [12]:

mls x stock solution (mg/L) = aquarium water (ml) x desired concentration (ppm or mg/l).

Mortality Determination

The fish were confirmed dead when they did not respond to repeated prodding [3], [4], and the percentage mortality was calculated as:

Percentage mortality =

$$\frac{\text{Number of dead fish}}{\text{Total number of fish exposed to the 2,4–D Dimethylamine salt}} \times 100$$

Water Quality Analysis

The in-situ water quality parameters analyzed include pH, temperature, conductivity, turbidity, dissolved oxygen and salinity and the results obtained ranged from 6.3 – 6.8, 24 - 26 ºC, 61.5 – 136.8 µS/cm, 3.6 – 7.0NTU, 5.2 – 6.5 mg/L and 0.02 – 0.05 ‰, respectively.

Statistical Analysis

Statistical package for social sciences version 20 and graph pad prism 5 were used for the statistical analysis. The data were expressed as mean ± standard error, and the charts were plotted using Graph pad prism 5 and Microsoft Excel. One way analysis of variance was used to show significant deviations at p=0.05, and Duncan statistics were used to determine the source of observed dissimilarity at p=0.05. The LC_{50} values were calculated using Finney Table – Microsoft Excel regression method as previously applied by Aghoghovwia and Izah [4], [5], Aghoghovwia et al. [3,15], Izah [16].

Results and Discussion

Figure 1 shows the percentage mortality of *Clarias gariepinus* fingerlings exposed to 2,4-D Dimethylamine salt. The percentage mortality at 0.00ppm, 10.80 ppm, 18.00 ppm, 25.20 ppm, 32.40 ppm and 39.60 ppm was 0.00±0.00 %, 13.33±3.33%, 23.33±3.33%, 26.67±3.33%, 30.00±0.00% and 33.33±3.33%, respectively at 24 hours, being significantly different at $p<0.05$. Duncan test statistics showed that there is no significant deviation ($p>0.05$) between 18.00 ppm, 25.20 ppm and 32.40 ppm concentrations of the toxicant. At 48 hours, the percentage mortality at 0.00ppm, 10.80 ppm, 18.00 ppm,

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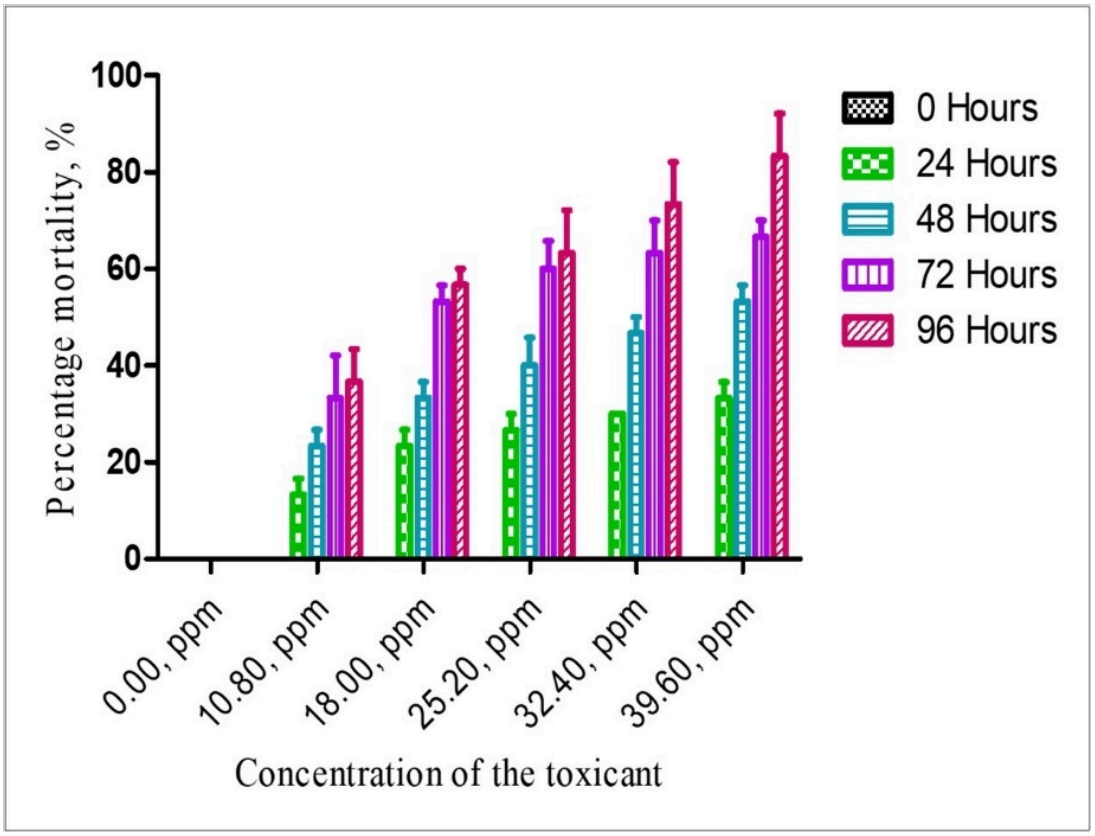

Figure 1. Mortality rate of Clarias gariepinus fingerlings exposed to 2,4-D Dimethylamine salt

25.20 ppm, 32.40 ppm and 39.60 ppm was 0.00±0.00 %, 23.33±3.33%, 33.33±3.33%, 40.00±5.77%, 46.67±3.33% and 53.33±3.33%, respectively. There were significant discrepancies at $p<0.05$ across the various concentrations of the toxicant. At 72 hours, the percentage mortality at 0.00ppm, 10.80 ppm, 18.00 ppm, 25.20 ppm, 32.40 ppm and 39.60 ppm was 0.00±0.00 %, 33.33±8.82%, 53.33±3.33%, 60.00±5.77%, 63.33±6.67% and 66.67±3.33%, respectively. Apart from 0.00 ppm and 10.80 ppm concentrations, there was no significant deviation at $p>0.05$ among the different concentrations. The percentage mortality at 0.00ppm, 10.80 ppm, 18.00 ppm, 25.20 ppm, 32.40 ppm and 39.60 ppm was 0.00±0.00 %, 36.67±6.67%, 56.67±3.33%, 63.33±8.82%, 73.33±8.82% and 83.33±8.82%, respectively at 96 hours. Statistically, there was dissimilarity ($p<0.05$) in the percentage mortality. The result showed that as the concentration of the toxicant increased the mortality rate of the fish also increased. This trend has been variously reported when fish are exposed to toxicants including the work of Aghoghovwia and Izah [4], [5] that exposed *Heterobranchus bidorsalis* fingerlings to paraquat dichloride and glyphosate; Seiyaboh and Izah [17] that exposed *Heterobranchus bidorsalis* fingerlings to cassava wastewater; Aghoghovwia et al. [3] that exposed *Clarias gariepinus* to paraquat dichloride; Aghoghovwia et al. [15] that exposed *Oreochromis niloticus* and *Clarias gariepinus* to palm oil mill effluents; Akinsorotan et al. [12], Ladipo et al. [18], Ariyo et al. [19] that exposed *Oreochromis niloticus, Clarias gariepinus* and *Labeo rohita* to paraquat dichloride; Ayoola [20], Nwani et al. [21] that exposed *Oreochromis niloticus* juvenile and *Tilapia zilli* to glyphosate; Nwani et al. [22] that exposed *Channa punctatus* to Carbosulfan, Glyphosate and Atrazine; Ojesanmi et al. [23] that exposed *Clarias gariepinus* fingerlings to 2, 3-dichlorovinyl dimethyl Phosphate; and Oyoroko and Ogamba [13] that exposed *Heterobranchus bidorsalis* and *Clarias gariepinus* fingerlings to detergent containing linear alkyl benzene sulphonate. The mortality is often proceeded by behavioural changes which are characterized by moderate swimming and opercular movement, body pigmentation, Intermittent swarming and Jerky movement, increased surfacing and air gulping [3,13,14,15].

Figure 2, Figure 3, Figure 4, Figure 5 show the LC_{50} values of *Clarias gariepinus* fingerlings exposed to 2,4-D Dimethylamine salt. The LC_{50} values at 24, 48, 72 and 96 were 86.15 ppm, 36.28 ppm, 18.72 ppm and 15.68 ppm, respectively. The LC_{50} values decreased as the concentration of the toxicant

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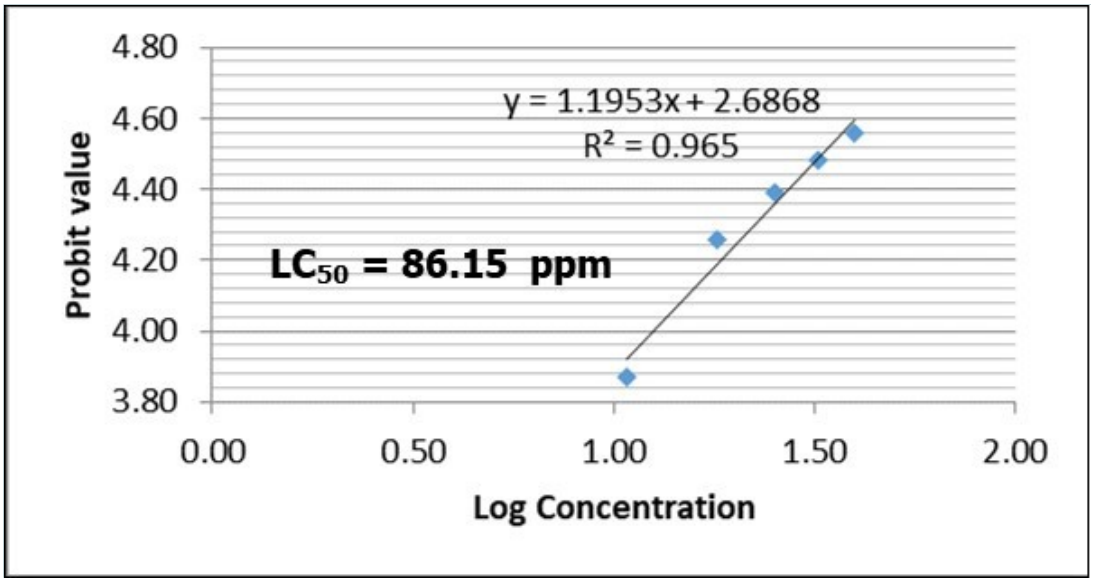

Figure 2. LC50 of Clarias gariepinus fingerlings exposed to varying concentration of 2,4-D Dimethylamine salt after 24 hours

increased. This indicates that as the concentration of 2,4-D Dimethylamine salt increased, its toxicity to fish increased. This trend is in line with the findings of authors when different fishes are exposed to different toxicants [3,4,5]. Though, slight variation exists in the LC_{50} values. Authors have attributed the variation to fish species, age, size and biochemical makeup of the fishes as well as the type of the toxicants [3,4,5]. As the acute toxicity duration increases, it could alter the cellular and biochemical processes in fish due to possible changes in enzymatic, haematological, histopathological, physiological and metabolic processes due to hassle and stress.

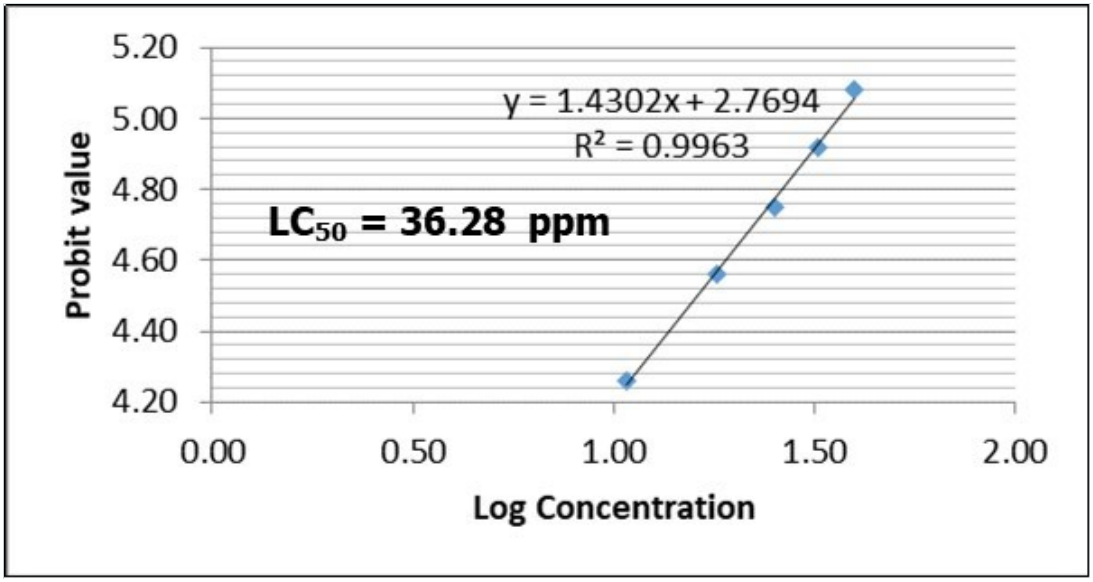

Figure 3. LC50 of Clarias gariepinus fingerlings exposed to varying concentration of 2,4-D Dimethylamine salt after 48 hours

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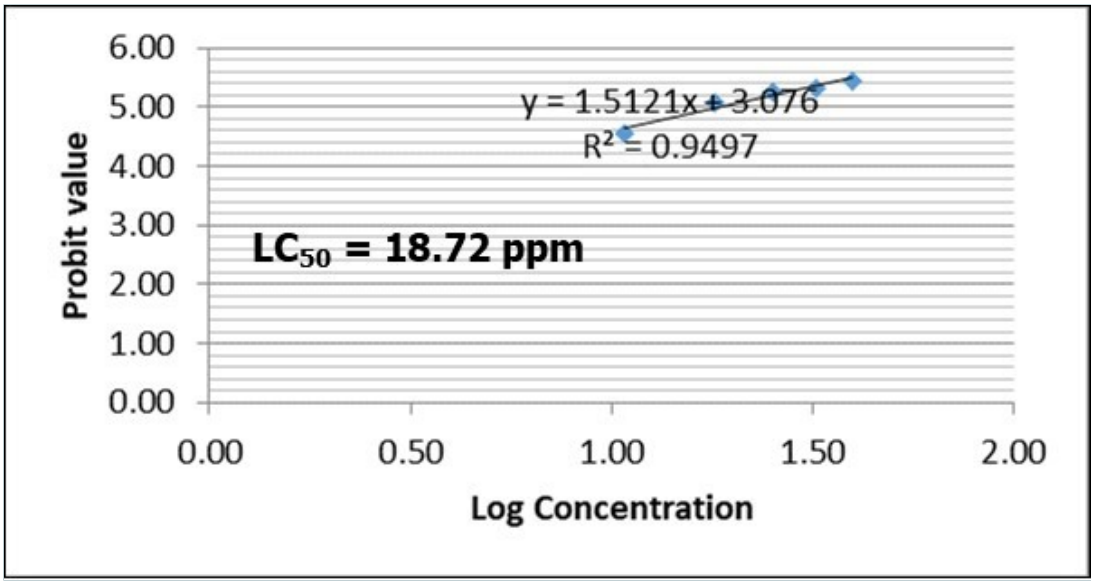

Figure 4. LC50 of Clarias gariepinus fingerlings exposed to varying concentration of 2,4-D Dimethylamine salt after 72 hours

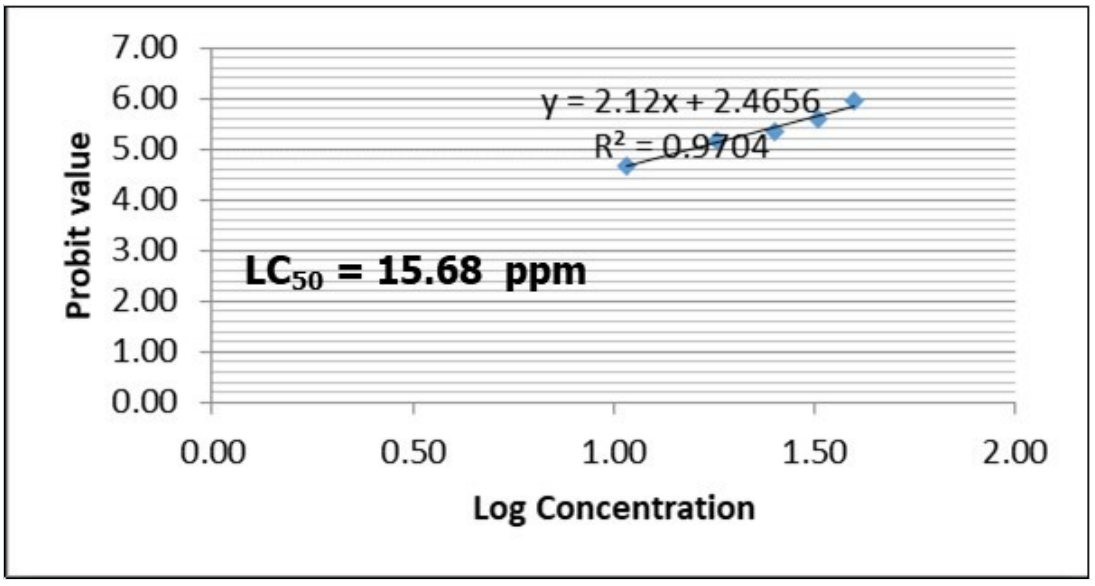

Figure 5. LC50 of Clarias gariepinus fingerlings exposed to varying concentration of 2,4-D Dimethylamine salt after 96 hours

Conclusion

In recent times, the rate of pesticide use especially herbicides has increased. Different brands of herbicides are marketed in Nigeria. This study evaluated the acute toxicity of 2,4-D Dimethylamine to *Clarias gariepinus* fingerlings. The study found that the mortality rate decreased as the concentration of the toxicant and exposure increased. LC_{50} values of 15.68 ppm after 96 hours is an indication that the 2,4-D Dimethylamine salt is toxic to fingerlings of *Clarias gariepinus*. As such, there is the need to exercise caution in the use of 2,4-D Dimethylamine salt based herbicides for the control of grasses especially in the coastal region where surface water resources abound.

Competing Interests

The authors have declared that no competing interests exist.

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