Toxicity of Dakshtm Insecticide to Clarias Gariepinus Post Fingerlings

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Abstract:
The study was carried out to investigate the toxicity of daksh insecticide on the African catfish *C. gariepinus* post fingerlings using a 96 hour static bioassay. The experiment was carried out under laboratory conditions with 360 post fingerlings of the test fish species distributed randomly in triplicate concentrations. The test fishes were treated with concentrations of 0.003, 0.004, 0.005, 0.006 and 0.007mg/l of daksh insecticide. The 96 hour LC_{50} was determined for Log toxicant concentration after 24, 48, 72 and 96 hour exposure. The 96 hour LC_{50} was 0.0028mg/l with a 95% confidence limits of between 3.4 – 7.69. The maximum safe concentrations were 2.8^{-04} – 2.8^{-05} for *C. gariepinus* exposed to daksh insecticide. The result also showed that there were significant differences ($P < 0.05$) in the water quality parameters as the concentration of the toxicant increased, also, variations in the 24 and 96 h-LC_{50} showed a significant difference ($p < 0.05$), while variations between the 48 and 72 h-LC_{50} was not significant ($p > 0.05$). Water quality parameters tested for this experiment were temperature, Dissolved Oxygen and pH. Temperature was observed to be near normal while pH and D.O reduced drastically as concentration of toxicant increased.

Keywords:
Toxicity, Daksh, Insecticide, Clarias Gariepinus, Post Fingerlings

1. Introduction

The loss of biodiversity owing to anthropogenic activities during the past 50 years is unprecedented in human history (Millennium Ecosystem Assessment, 2005). Despite general concern and several international initiatives (United Nations, 2008; Secretariat of the Convention on Biological Diversity, 2003 and SCBD, 2010), the current rate of biodiversity loss appears to be accelerating rather than slowing (Walpole, 2009 and Burtchart, *et al*., 2010). The future consequences of this crisis may be dramatic, as the latest analyses show that a planetary scale ecosystem shift to

an unknown and irreversible state may occur (Barnoski, 2012). To date, no unequivocal link has been established between the measured exposure (i.e., the concentration of toxicants in the environment) and quantitative measures of biodiversity whether globally or regionally (i.e., the taxonomic richness pool) (SCBD, 2010). The only exceptions are two studies that addressed effects of salinity which are studies carried out by (Kefford, *et al*., 2006; 2011), respectively. Hence, although chemical contaminants are well known as an important driver for biodiversity loss, there is scarce empirical evidence to support such opinion for the large-scale taxonomic pools.

This problem holds true even for agricultural pesticides, which are among the best eco-toxicologically characterized and regulated groups of contaminants (Fischer *et al*., 2010). Essentially, it remains unknown whether to what degree, and at what concentrations pesticides cause species loss. However, there are many investigations showing the effects on the local biodiversity related parameters in both freshwater and terrestrial systems as reported by (Geiger, *et al*., 2010; Gibbs, *et al*., 2009; Colignon, *et al*., 2001; Liess and Von Der Ohe, 2005 and Schäfer van den Brink and Liess, 2011).

Pesticides are chemicals or biological agents (such as a virus, bacterium, antimicrobial, or disinfectant) that deters, incapacitates, kills, or otherwise discourages pests. They can also be described as substances meant for seducing, attracting and then destroying, or mitigating any pest, (United States Environmental Protection Agency, 2007). They are a class of biocide. Target pests can include insects, plant pathogens, weeds, mollusks, birds, mammals, fish, nematodes (roundworms), and microbes that destroy property, cause nuisance, or spread disease, or are disease vectors. The most common use of pesticides is as plant protection products (also known as crop protection products), which in general protect plants from damaging influences such as weeds, plant diseases or insects. This use of pesticides is so common that the term pesticide is often treated as synonymous with the protection of plant product. In a broader term, pesticides are also used for non-agricultural purposes. The term pesticide includes all of the following: herbicide, insecticide, molluscicide, piscicide, avicide, insect growth regulator, nematicide, termiticide, rodenticide, predacide, bactericide, insect repellent, animal repellent, antimicrobial, fungicide, disinfectant and sanitizer (Carolyn, 2013). Although pesticides have benefits, some also have drawbacks, such as potential toxicity to humans and other desired species. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are organochlorine pesticides (Gilden *et al*., 2010). These substances when applied whether on land and on air or even in water, find their ways into the aquatic systems, thereby causing aquatic pollution.

The toxicant DAKSH is made of 100% Dichlorvos which is a synthetic organic chemical used as an insecticide. It does not occur naturally in the environment, but is manufactured by industries. Daksh can also be used for fumigation and pest control in vegetable farms. Dichlorvos is sold under many trade names including: Vapona, Atgard, Nuvan, Nogas 50 and Task, (Mir *et al*., 2012). Dichlorvos may also be called Dimethyl Dichlorovinyl Phosphate (DDVP).

2. Materials and Methods

Three hundred and sixty (360) Healthy post fingerlings of *Clarias gariepinus* with mean weight of 13.57g ± 5.6g and length of 12.5 ±8.4cm were obtained from Department of Fisheries and Aquatic Environmental Mgt. Fish Farm, University of Uyo, Uyo, Akwa Ibom State. Fish were acclimatized for 2 weeks in the laboratory, in eighteen (18) 30 L plastic tanks, with a minimum of 10 individuals per tank. The fish were fed *ad libitum* with pelleted fish diet containing 35% crude protein during the acclimatization period. Daksh insecticide was bought from a chemical store in Ikot Ansa Calabar.

2.1. Preparation of Stock Solution

The method employed for test solution preparation was the serial dilution method (Shai-Shafir et al., 2014). A stock solution of daksh insecticide was prepared by adding 1ml in 1 litre of distilled water, according to (Shai-Shafir et al., 2014). The stock solution was preserved at standard temperature (STP) 18oC to avoid loss of potency prior to use.

2.2. Acute Toxicity Test

A Preliminary test was conducted to determine the toxicity level of daksh insecticide following OECD (2001) Direction No. 203 and Methodical Manual ISO 7346/2. Triplicate of six (6) test concentrations were used for this investigation: one control and five tests solutions of daksh insecticide. *C. gariepinus* post fingerlings were batch-weighed with a top-loading balance (Mettler Toledo (K), and distributed randomly in triplicate per treatment.

The glass tanks were covered with mosquito net to prevent fish from jumping out; there was no aeration, no water change nor feeding throughout the test. The toxicant was introduced at concentrations; 0.002, 0.004, 0.006, 0.008 and 0.010 mg/l with a control of 0 g/l. The behavior and mortality of the test fishes in each tank were monitored for 24 hours and recorded every 15 min for the first hour, once every hour for the next three hours and every four hours for the rest 24 hours period.

Based on the result from the range finding test, a 96-hour definitive test was carried out using *Clarias gariepinus* post fingerlings. Test solution was introduced at 0.003, 0.004, 0.005, 0.006 and 0.007mg/l into five treatments with triplicates respectively. Fish mortality was monitored and recorded hourly for the first four hours, every 4 hours for the next 24 hours, and subsequently every 24 h, for the next 96 hours. Apart from monitoring and recording fish mortality, behavioral changes such as: erratic swimming, air gulping, loss of reflex, discoloration and molting was monitored. 96-LC50 (concentration of Daksh insecticide, estimated to be lethal to 50% of test organisms after exposure time of 96 h) was determined graphically using Probit transformation (Herwig, 1979; USEPA, 2000).

2.3.Water Qulity Analysis

Water quality was checked for before, during and after the experiment using the required test kits. The water quality parameters considered include; temperature which was tested with a mercury glass thermometer, the apparatus was placed in the medium inside the test container until reading was taken, dissolved oxygen was tested with a Dissolved Oxygen meter once daily at 8.00 a.m. and pH was tested with a pH meter (Mettle Toledo 320). The electrode was inserted into the test chambers containing the

water samples after standardization in different buffer, after which the readings were taken.

2.4.Statistical Analysis

Toxicological dose responses (fish mortality) were determined graphically using Microsoft excel (Herwig, 1979; USEPA, 2000; Newman, 1995). The median lethal concentration LC50 at selected period of exposures, and an associated 95% confidence interval for each replicate toxicity test were subjected to probit analysis (Finney, 1971) using Statistical Package for Social Sciences (SPSS) 20.0 for Windows. Data were analyzed using descriptive statistics (mean, standard deviation, frequencies and percentages). Comparison of data and physico-chemical properties between the control and other treatments in the definitive tests were carried out using analysis of variance (ANOVA) (Steal and Torrie, (1980).

3. Results and Discussion

3.1. Result

Acute Toxicity Test

The mortality pattern of *Clarias gariepinus* to Daksh is shown in figure 1 and table 1 and 2. Mortality commenced in the 8th hour of exposure. Mortality was observed to increase with increase in concentration and time of exposure. There was no 100 percent mortality in the experiment; the highest mortality recorded in the experiment was 98% which was observed in the highest concentration of 0.007mg/l. At concentration 0.003mg/l LC50 was achieved at 96hrs which was the highest mortality recorded for that concentration. The highest concentration of 0.007mg/l recorded a highest mortality level of 96.6 percent while the LC50 was achieved at 24 hours of the experiment. Increase in mortality did not follow any pattern as it was observed that there were no steady percentage increases. Nonetheless, percentage mortality maintained a rise in all concentrations. Variations in the response of catfish fingerlings during the 48 and 72 h-LC50 values were not significant ($p > 0.05$), but variations in the 24 and 96 h-LC50 showed a significant difference ($p < 0.05$). During the course of conducting the acute toxicity assays a series of symptoms were observed in the test organisms. Symptom include; erratic swimming movements, air gulping, restlessness, loss of reflex, discoloration and death. This is in line with toxicologically related symptoms reported by (Oladimeji and Offem, 1989, Ayotunde and Offem, 2005, Ayotunde and Offem, 2008, Ayotunde et al., 2010). Acute toxicity occurred at concentrations higher than those of diazinon (Olufemi et al., 2008), phenol (Cowgill and Milazzo, 1991) and tetrachloromethane (LeBlanc, 1980), benzene (Canton and Adema, 1978), methanol (Tong et al., 1996) and acetonitrile (Guilhermino et al., 2000). According to Oh et al. (1991), three main factors are responsible for the selective toxicity of toxicants for various fish species, which are: different inhibition of acetylcholinesterase, different detoxification and absorption. The aforementioned factors were probably responsible for the different toxic reactions showed in this experiment by catfish fingerlings to varying concentration of Daksh insecticide. The intensity of these symptoms was directly related to the concentration of toxicant in water and duration of exposure, which is in line with the submission of Oh et al (1991). We observed that Catfish *C. gariepinus* post fingerlings showed variations in their tolerance to Daksh. We also observed that the reaction of the test fish to the toxicant were more pronounced at higher concentrations of daksh insecticide due to

increased inhibition of acetylcholinesterase which eventually led to the death of the test fish (Olufemi et al., 2008). The results from this experiment disagree with the size-specific sensitivity to acute chemical toxicity observed in some aquatic animals with the smallest individuals showing the highest sensitivity (Goyer and Clarkson, 2001; Bianchini et al., 2002; Bossuyt and Janssen, 2005).

3.2. Discussion

It was observed that in higher concentrations of daksh, the behavioral responses of the test organisms greatly increased and the organisms later became inactive. This pattern is described as a normal situation in acute and sub-acute toxicity tests (Kulakkattolickal and Kramer, 1997).

Erratic swimming observed in this study can be attributed to the disturbances in the metabolic state resulting in the depletion of energy. This report agrees with the work of Shai-shafir *et al*, 2014 who examined the toxicity of household detergent on reef corals. Same has also reported same on the effect of DDVT on carp (Mir *et al* 2012). It is possible that animals which have higher metabolic activities could require higher level of oxygen and thus would embark on higher respiratory activities (Canli and Kargin, 1995). Loss of reflex observed in this study has been attributed to depletion of energy. Also, loss of reflex as recorded in this work has been reported to be an indication of impairment of abnormal carbohydrate metabolism and those organisms that could not tolerate the toxicants, entered into a state of coma and later died as observed by Anderson, *et al*., (1988*)*.

In this present study, the 95% confidence limits were found to be between 3.4 – 7.69 for daksh insecticide. The maximum safe concentrations were 2.8^{-04} – 2.8^{-05} exposed to daksh insecticide for a 96-hour period. Koesomadinata (1980), stated that the safe level of a compound is derived by multiplying the 96hour LC_{50} with an application factor of 0.1 – 0.01, such application factors are applied to acute toxicity test data to estimate the concentration that is safe for chronic exposures. These results are far lower than that of Ayotunde *et al*., (2010) who reported MATC for pawpaw seed on *C. gariepinus* at 0.1 to 1.29 mg/L and confidence level of 22% in the highest concentration to 78.8% in the lowest concentration. This implies that daksh insecticide is highly toxic to fish.

According to Oh *et al*. (1991) there exist three factors for classifying toxicity of toxicants for various fish species as: different detoxification, different inhibition of acetylcholinesterase and absorption. The above factors were probably responsible for the different toxic reactions shown in this experiment by catfish post fingerlings to varying concentration of daksh insecticide. The reactions were more pronounced at higher concentration due to increased inhibition of acetylcholinesterase which eventually resulted in the death of the fish (Olufemi *et al*., 2007).

In toxicological experiments, the time of exposure has large effect on biological response. The general rule of thumb is that the longer the exposure time, the lesser the LC_{50} value and the greater the toxicity. Results of this study showed similar pattern having lesser 96 h-LC_{50} than 48 h-LC_{50} and so on. The water quality variables in the experimental tanks were within the range acceptable for optimum performance of fish in culture medium (Boyd, 1998). There was concentration dependent reduction in the values of dissolved oxygen levels in the exposure tanks from 4.7575 ± 0.07099^{a} toas low as 0.6 ± 0.107^{c}. This is an indication that daksh insecticide affected the dissolved oxygen concentration during the experimental procedure resulting in asphyxiation.

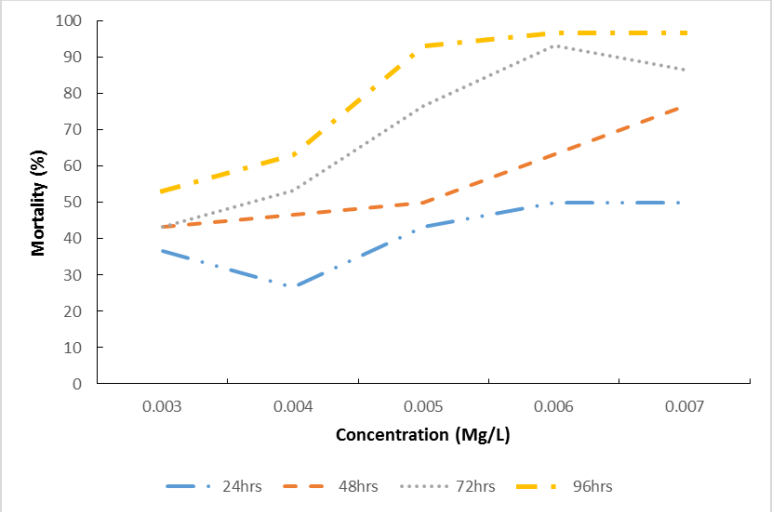

Figure 1. *Percentage cumulative mortality of C. gariepinus exposed to daksh insecticide.*

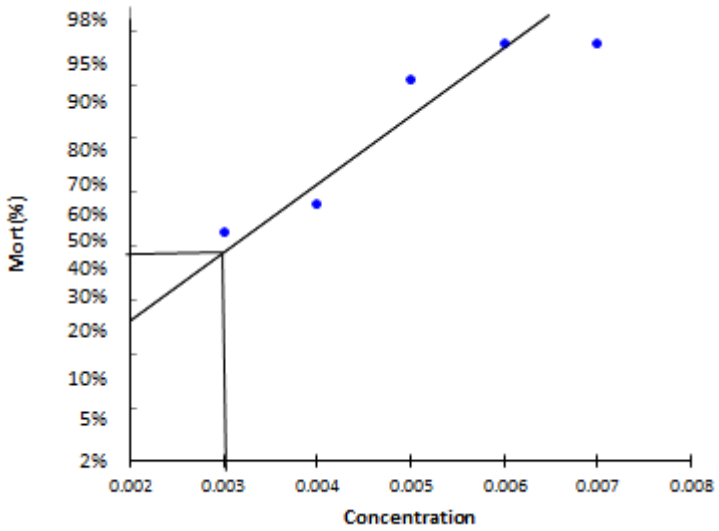

Figure 2. *LC_{50} of Clarias gariepinus Exposed to Daksh Insecticide.*

Table 1. *Percentage Cumulative Mortality of C. gariepinus Exposed to Daksh Insecticide.*

Conc. Mg/L	MINUTES			HOURS								
	15	30	45	1	2	3	4	8	12	16	20	24
0.00	-	-	-	-	-	-	-	-	-	-	-	-
0.002	-	-	-	-	-	-	-	-	13.3	23.3	26.7	26.7
0.004	-	-	-	-	-	-	-	13.3	26.7	33.3	33.3	40
0.006	-	-	-	-	-	6.7	16.7	36.7	36.7	40.0	40.0	53.3
0.008	-	-	-	20.0	23.3	23.3	36.7	60.0	73.3	100	100	100
0.010	-	-	-	16.7	43.3	63.3	73.3	90.0	93.3	100	100	100

Table 2. *Summary of physico-chemical parameters of test media.*

Daksh (*Clarias gariepinus*)								
Par.	Control	1	2	3	4	5	P-Value	Sig.
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE		
Temp	28.875 ± 0.15478[a]	29.55 ±0.25331[ab]	29.7 ±0.4916[ab]	29.925 ± 0.11087[ab]	30.45 ±0.32275[bc]	30.45 ±0.32275[c]	0.84	0.23
D.O	4.7575 ± 0.07099[a]	3.0825 ± 0.22462[b]	2.8975 ± 0.07028[b]	2.8225 ± 0.10523[b]	0.6475 ±0.13431[c]	0.6 ±0.107[c]	0.922	0.11
pH	7.2775 ±0.04973[a]	7.3075 ± 0.0841[a]	6.8025 ± 0.23286[b]	6.005 ±0.07511[c]	5.8725 ±0.03838[cd]	5.5275 ± 0.14453[d]	0.824	0.32

Table 3. *Behavioural response of C. gariepinus exposed to daksh insecticide.*

	24hrs						48hrs					
Conc. (mg/l)	0.00	0.003	0.004	0.005	0.006	0.007	0.00	0.003	0.004	0.005	0.006	0.007
Air gulping	-	-	-	+	+	+	-	+	+	+	+	+
Discoloration	-	-	-	-	+	+	-	-	-	+	+	+
Erratic Swimming	-	-	+	+	+	+	-	-	-	-	-	-
Molting	-	-	-	-	-	-	-	-	-	-	-	-
Loss of reflex	-	-	+	+	+	+	-	-	+	+	+	+

Table 3. Cont.

	72hrs						96hrs					
Conc. (mg/l)	0.00	0.003	0.004	0.005	0.006	0.007	0.00	0.003	0.004	0.005	0.006	0.007
Air gulping	-	+	+	+	+	+	-	-	+	+	+	+
Discoloration	-	-	-	+	+	+	-	-	-	+	+	+
Erratic Swimming	-	-	-	-	-	-	-	-	-	-	-	-
Molting	-	-	-	-	-	-	-	-	-	-	-	-
Loss of reflex	-	-	+	+	+	+	-	-	+	+	+	+

Key: - = Not present
+ = Present

4. Conclusions

The toxicity effect of daksh insecticide had a positive correlation with exposure time from 24 to 96 h, for the catfish *C. gariepinus*. From the toxicity tests, daksh

insecticide concentration as low as 0.003 mgL-1 in the medium is potentially hazardous to all fish species and other aquatic biota in freshwater. Therefore, acute toxicity data of the present study provides baseline information needed to develop models of the effect of daksh insecticide use in riverbank farming and its effects on ecological systems. We recommend a Chronic test in order to provide more information is needed to assess the potential long-term impact of Daksh insecticide on the aquatic environment.

5. Conflicts of Interest

We Dodeye Eno Omini and Ekpo Imaobong Emmanuel, Obot Ofumbuk and Ogar Patrick Ogar hereby state that there is no conflict of interest regarding the publication of this article.

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