Journal of Environment & Biotechnology Research
VINANIE PUBLISHERS
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Studies on acute toxicity of lindane (gamalin 20) exposed to *Hetrobrancus bidorsalis* juveniles

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

The study was carried out to determine the acute toxicity of lindane (gammalin 20) exposed to *Heterobrancus bidorsalis* juveniles using static bioassays. The mean weight and total length of the fish samples were 2.5 g and 8.6 cm, respectively. Six groups of the experimental units were set up containing 10 fish individuals in each bowl with 20 L water capacity. Gammalin 20, organochlorine pesticide was distillated and the active ingredient lindane was condensed and collected. The stock solution was prepared with graded concentration of 0.02, 0.04, 0.06, 0.08 and 0.10 mL/L. The fish juveniles of 10 individuals were put in each of the bowls and exposed to the different concentration of the lindane with a control experiment where the toxicant was not introduced. The experimental set was replicated three times. The exposed fish were observed daily and dead fish were removed immediately and mortality was recorded for 24 and 48 h exposure period. The LC_{50} was determined to be 0.06 mL/L for the 24 and 48 h exposure period. The obtained result was transformed to Probit analysis which was plotted against the graded concentration of lindane for the 24 and 48 h exposure period. The R^2 values of 0.76 and 0.80 were obtained for the 24 and 48 h respectively indicating a strong relationship of lindane with mortality. The result of the water quality was, pH: 7.80 - 8.46, temperature: 28.39 - 28.42 ^oC, DO: 5.06 - 5.17 mg/L, conductivity: 462 - 482 µS/cm and TDS: 231 - 241 mg/L. The water quality parameters increase with increase in the lindane concentration; however they were within the maximum permissible level and did not have any effect on the fish and its death. The death of the fish may be due to the toxic potential of lindane. It was recommended through this study that gammalin 20 is very toxic and persistent in the aquatic environment and its use should be greatly discouraged.

KEYWORDS
bioassay; gammalin 20; *Heterobrancus bidorsalis*; lindane; organochlorine

1. INTRODUCTION

Gammalin 20 is an organochlorine pesticide that is widely used in veterinary and human medicine to treat ectoparsites and pediculosis. Gammalin 20 has a wide application in the elimination of phytophagous and soil inhibiting insects. It is also used to destroy ectoparasites of animals, crop pests, stored product pest, and in the treatment of seeds for the attack of pest. In fish farming, lindane is used to kill fish (Tomlin, 1997). The active ingredient contain in Gammalin 20 is lindane (Lawson et al., 2011). Lindane is widely used in the treatment of seeds. It is also used in lotions, creams and shampoos for the control of lice and mites in humans (Adedeji et al., 2008). Benzane hexachloride (BHC) is the 100% pure form of gammalin 20 while lindane is slightly less pure with 99% purity (Afful et al., 2010). Lindane is very toxic to fish, bees and aquatic invertebrates. Similarly lindane is very stable in both fresh and marine water environments (Afful et al., 2010).

A pesticide is any substance or a combination of substances used for the elimination and reducing the effect of any pest (UNEP, 2005). Broadly, pesticides are classified as insecticides, fungicides and herbicides (Afful et al., 2010). Insecticides are mainly organochlorine,

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Received: 05-10-2016
Revised: 22-11-2016
Accepted: 06-12-2016
Available online: 01-01-2017

organophosphus, carbamates and pyrethroids. As a result of the persistency of organochlorine insecticides, most of them have been banned by legislation in most countries as agrochemicals used for the elimination of pest in agriculture (Bouwan, 2004). The injurious effect of pesticide application to the control pest is the contamination of the environment (Lawson et al., 2011). Many studies have revealed that only about 1% of the pesticides applied get to the target organism. The remaining of the pesticide contaminates the soil and water environment (Lawson et al., 2011). Bioassay is the determination of the toxicity of chemical substances and to find out which of the organisms that is most sensitive to the chemical (Ndimele and Jenyo-Oni 2009). This study is to ascertain the toxicity of lindane to *Heterobranchus bidorsalis* in order to determine the level of tolerance and its suitability as bio indicator in the freshwater environment. This fish is common in the studied fresh water sources and is widely consumed and cultured.

2. MATERIALS AND METHODS

2.1. Test organisms collection

Hetrobrancus bidorsalis specimens numbering about 270 individuals were obtained from the fish farm of the Federal University of Agriculture Makurdi, Benue state Nigeria. The mean weight and total length of the body of the specimens were 2.5 g and 8.6 cm respectively. The fish samples were immediately taken to the fisheries Department of Federal University of Agriculture Makurdi in plastic containers filled with oxygenated and cool clean water. This was to reduce the stress on the fish samples before getting to the laboratory.

2.2. Acclimatization of the fish samples

The fish samples obtained from the fish farm were taken to the laboratory and kept in 27 plastic bowls of 20 L capacity containing dechlorinated water. The fish samples were acclimatized to the laboratory conditions for a period of 14 days. During the period of acclimatization, the water in each of the plastic bowls were stocked with ten (10) individuals of the fish juveniles each and the water was changed daily throughout the 14 days period. This was done to avoid the contamination of the water with metabolic wastes. The pH and DO of the water in the bowls were determined and recorded. The bowls in which the fish samples were aerated with an electric aerator and the fish samples were fed with commercial feed (40% cp) two times daily at 30% body weight (Reish and Oshida, 1987).

2.3. Source of the toxicant (gammalin 20)

Lindane (gammalin 20) used for the study was bought from a chemical shop in Makurdi, Benue state, Nigeria. The toxicant was kept in the laboratory under laboratory conditions prior to the commencement of the test.

2.4. Preparation of toxicant standard solution

The toxicant gammalin 20 was taken to chemistry laboratory, of the Federal University of Agriculture Makurdi. In the laboratory, the gammalin 20 was unsealed and the content was transferred into a boiling vessel or distillation column. Heat application was in the collection of the active ingredient lindane through the process of fractional distillation. This process help to precipitate or distillate lindane and the vapour was evaporated and condensed to colourless substance. However, the residue was dark in colour. The distillate was measured in concentrations of 0.02, 0.04, 0.06, 0.08 and 0.10 mL/L.

2.5. Bioassay procedure

Standard bioassay procedure was adopted during the course of this study (Reish and Oshida, 1987). The acute toxicity test commenced with a range finding test that was carried out for a 96 h period to ascertain the lethal concentration of lindane to *Heterobrancus bidorsalis*.

2.6. Exposure of fish to toxicant

The definitive test was carried out after the range finding test. The control and the treatments were carried out concurrently. Out of the 270 fish samples that were acclimatized 60 active individuals of the fish were selected and divided into a group of ten members separately for each of the concentrations. The concentration that the fish were exposed includes: 0.00 mL/L (control), 0.02, 0.04, 0.06, 0.08, and 0.10 mL/L. The test was triplicated with a total of 18 experimental set up carrying 180 juveniles of the fish specimens. The water in the control and treatments were changed daily and freshly prepared concentrations were added across the experimental set up. Mortality was observed at regular intervals of 4 h and subsequently about every

6 h up to 48 h. Fishes were considered dead when they showed no movement of the body upon gentle prodding and inability of the gills to move. Dead fishes were removed to avoid further contamination of the water. Behavioral changes were also observed. Dechlorinated water was used throughout the experiment (Davies et al., 2006).

2.7. Determination of physicochemical parameters of water

After introducing the chemical in the fishes in different concentrations, physicochemical parameters of the water were observed using multi-parameter checker HANNA model 2011. The temperature, DO, TDS, pH and EC of the water samples were determined by selecting the appropriate programed number and the readings were recorded.

2.8. Data analysis

The test concentrations were converted into logarithm and the corresponding mortality percentage into the probit value. The obtained probit values were plotted against the concentration of the lindane. The physico-chemical results were subjected to student t test analysis and descriptive statistics.

3. RESULTS AND DISCUSSION

3.1. Toxic effect of lindane

The results presented in Table 1 and 2 are the mortality record of *Heterobrancus bidorsalis* exposed to lindane (gammalin 20) for 24 and 48 h, respectively in the course of the study. The pesticide was more toxic at 48 h exposure time as compared to the 24 h exposure period. However, no death was observed in the control exposure after 24 and 48 h exposure. Across the graded concentrations of the toxicant, 0.10 mL/L produced highest mortality in both exposure times (24 and 48 h), while the same mortality of 20% was recorded in the 24 and 48 h exposure of 0.02mL/L concentration of the lindane (gammalin 20).

Similarly the results in Figures 1 and 2 are the Probit Transformed Response for mortality of *Heterobrancus bidorsalis* exposed to graded concentration of lindane (gammalin 20) for 24 and 48 h, respectively. The R^2 value for the 24 and 48 h exposure period of the pesticide to the fish was 0.76 and 0.81, respectively. This clearly indicates that the pesticide has a strong relationship with the death of the fish with graded concentration within the time of exposure. Thus, it was evident that the increase in concentration as well as increase in exposure time to

Table 1. Mortality record of *Heterobrancus bidorsalis* exposed to lindane (gammalin 20) for 24 h

S.No	Concentration (mL/L)	Log of concentration	Number of fish exposed	Number of fish died	%Mortality	Probit Value
1	0.00	0	10	0	0	0.00
2	0.02	-1.69	10	2	20	4.16
3	0.04	-1.39	10	3	30	4.48
4	0.06	-1.22	10	5	50	5.00
5	0.08	-1.09	10	6	60	5.26
6	0.10	-1.00	10	8	80	5.84

Table 2. Mortality record of *Heterobrancus bidorsalis* exposed to lindane (gammalin 20) for 48h

S.No	Concentration (mL/L)	Log of concentration	Number of fish exposed	Number of fish died	%Mortality	Probit Value
1	0.00	0	10	0	0	0.00
2	0.02	-1.69	10	2	20	4.16
3	0.04	-1.39	10	4	40	4.75
4	0.06	-1.22	10	5	50	5.00
5	0.08	-1.09	10	7	70	5.52
6	0.10	-1.00	10	9	90	7.33

lindane results in more death of the fish during the course of the study.

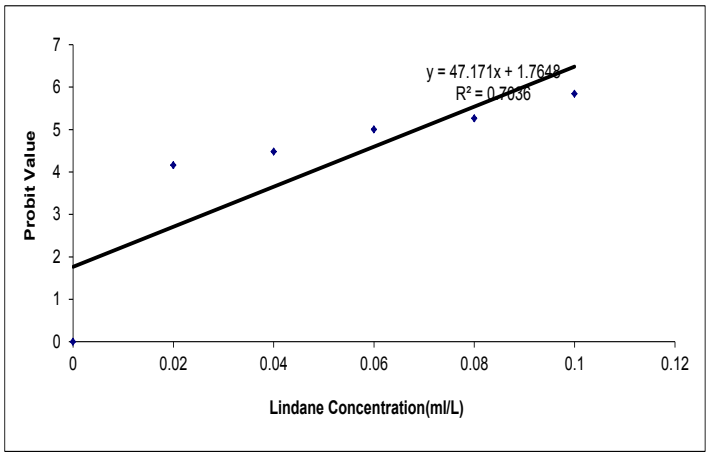

Figure 1. Probit transformed response for mortality of *Hetrobrancus bidorsalis* exposed to graded concentration of lindane (gammalin 20) for 24 h.

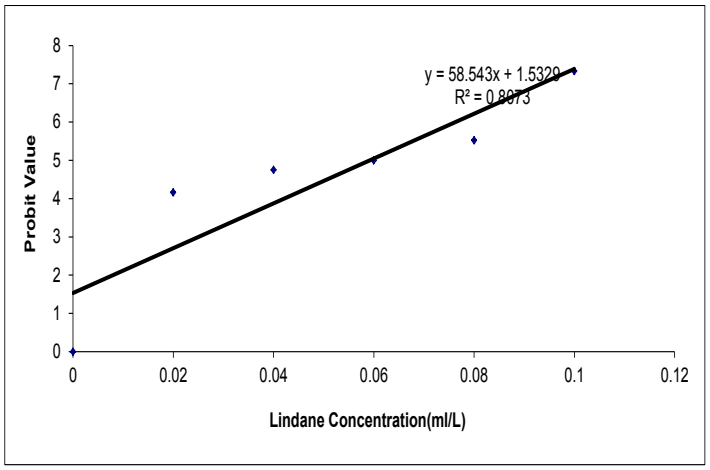

Figure 2. Probit transformed response for mortality of *Hetrobrancus bidorsalis* exposed to graded concentration of lindane (gammalin 20) for 48 h.

3.2. Physicochemical changes of water sample

The results presented in Table 3 were the physico-chemical characteristics of the water samples of the control and lindane treatments exposed to the fish during the period of the study. A perusal of the results indicated that among all the parameters examined their lowest concentration was obtained from the water samples of the control experiments where the pesticide was not introduced. The DO of the water samples exhibited the highest concentration of 5.17 mg/L in the control. In general, the values of all the parameters except for DO was obtained lowest for the lowest lindane concentration of 0.02mL/L; whereas the highest values of parameters were obtained for the highest lindane concentration of 0.10mL/L. In the case of DO, the value decreases as the concentration of lindane increases. This result clearly showed the effect on the pesticide on the DO of the water. The descriptive statics of the water quality parameters of the control and treatments of the experimental set up of the study are presented in Table 4. It should be noted that the mean values of all the water quality parameters were within the permissible level. The results of correlation analysis between the water quality parameters are presented in Table 5. The correlation was significant between DO and conductivity, as well as between DO and TDS at 0.01 level of significance. The student t test analysis was significant among all the water parameters examined ($P<0.05$).

The bioassay result of the lindane graded concentration exposed to juveniles of *Heterobranchus bidorsalis* indicated clearly that lindane is poisonous. The results of this study revealed that *Heterobranchus bidorsalis* exposed to lindane (gammalin 20) LC50 for 24 and 48 h was 0.06mL/L. The results showed that the higher the concentration of gammalin 20, it incurs more toxicity to the fish based on the findings of this present investigation. This finding was similar to that of earlier study of an organochlorine pesticide (Ezemonye and Ogomida, 2010). A perusal at the result of the present investigation revealed that the result of the probit transformed mortality of *Heterobranchus bidorsalis* juveniles exposed to graded concentration of lindane for 24 and 48 h have R^2 of 0.76 and 0.81, respectively. This results indicate clearly that lindane have a strong relationship with mortality and have high potential to kill. These findings conform to the result of an earlier study that reported that there is a positive relationship between concentration of pesticides to mortality of organisms (Ayuba et al., 2013; Reddy et al., 2016). Toxicants may affect aquatic organisms within a short period of time 24 or 48 h which may be acute as was observed in this present study. The fish in this study started showing the signs and symptoms of the toxic nature of lindane through unusual and erratic swimming behavior in the water and thus finally lead to the death of the fish. These observations were equally reported in earlier studies (Ezemonye and Ogbomomida, 2010; Ayuba et al., 2013). The result of this study presented in Figures 1 and 2 indicate conspicuously that the rate of mortality for a fixed time is proportional to the concentration of the lindane for 0.10miL concentration with increase in exposure time. This is also due to the regular mode of action of the toxicant as a result of the accumulation to the lethal level. These observations were consistent with Reddy

Table 3. Physico-chemical characteristics of the water samples of the control and lindane treatments exposed to the fish

S.No	Conc. (mL/L)	pH	Temperature (°C)	DO (mg/L)	EC (µS/cm)	TDS (mg/L)
1	0.00	7.80	28.40	5.17	462	231
2	0.02	8.34	28.42	5.15	468	234
3	0.04	8.39	28.44	5.11	470	235
4	0.06	8.43	28.39	5.13	475	237
5	0.08	8.44	28.40	5.09	480	240
6	0.10	8.46	28.42	5.06	482	241

Table 4. Descriptive statics of the water quality parameters of the control and treatments of the experimental set up

Parameter	Range	Minimum	Maximum	Mean	Std.Error	Std. Deviation	Variance
pH	0.66	7.80	8.46	8.31	0.10	0.25	0.06
Temp (°C)	0.05	28.39	28.44	28.41	0.01	0.02	0.00
DO (mg/L)	0.11	5.06	5.17	5.11	0.02	0.04	0.002
EC (µS/cm)	20.00	462.00	482.00	472.83	3.10	7.60	57.77
TDS (mg/L)	10.00	231.00	241.00	236.33	1.54	3.77	14.27

Table 5. Correlation analysis between the water quality parameters of control and treatments of the experimental set up

Parameter	pH	Temperature (°C)	DO (mg/L)	EC (µS/cm)	TDS (mg/L)
pH	1.0	0.241	-0.78	0.80	0.79
Temperature (°C)	0.241	1.00	-0.15	-0.07	-0.38
DO (mg/L)	-0.78	-0.15	1.00	-0.95*	-0.96*
EC (µS/cm)	0.80	-0.06	-0.95*	1.00	0.99*
TDS (mg/L)	0.79	-0.04	-0.96*	0.99*	1.00

*Correlation is significant at 0.01 level (2-tailed)

et al. (2016). The acute toxicity test was carried out to ascertain the susceptibility and survival potential of the test organisms to lindane (gammalin 20).

In this study the LC_{50} values increased with time of exposure of the fish to the lindane. The variation in the LC_{50} of this study as compared to other studies may be due to the difference in the test organisms and the graded concentration with the exposure time. These findings were consistent with (Omitoyin et al., 2006, Lawson et al., 2011). The LC_{50} concentration of 0.06 mL/L was reported in this study for 24 and 48 h exposure period. This result differs significantly from the findings of earlier study that reported LC_{50} of 1.29 mg/L of lindane exposed to *C. gariepinus* (Lawson et al., 2011). However Omitoyin et al.,(2006) reported LC_{50} of 0.38 mg/L for *C. gariepinus* during their study on lindane. The difference in the toxic effect of lindane of the fish species can be attributed to the differences in the susceptibility and the ability of tolerance of the fish exposed to the toxicant, biotransformation and the elimination of the toxicant within the body of the fish. This finding is consistent with Omitoyin et al., (2006). It was observed that a positive relationship existed between graded concentrations and the time of exposure of the fish to the toxicant. The result of the examined water quality parameters indicated that the toxicant gammalin 20 did not affect the quality of the water adversely. To be precise, the water quality parameters indicated the suitability of water for aquaculture. This result is similar to that reported by Akaahan et al. (2015). Pesticides induce different types of toxic effect in the fish that result to different changes of the fish was observed in this study (Ullah et al., 2014). The results of the water quality were within the acceptable limit for the survival fish. The death of the fish may be due to the toxic stress on the fish.

4. CONCLUSIONS

The result of this investigation reveals that lindane (gammalin 20), an organo chlorine pesticide exhibited high toxicity towards *Heterobrancus bidorsalis* juveniles and has the potential to persist in the aquatic ecosystems. The use of this pesticide as technique for killing fish for human consumption, as it is done by some fishermen, should be discouraged.

REFERENCES

Adedeji, B.A., Adedeji, A.O., Adeyemo, O.K. and Agbede, S.A. (2008) Acute toxicity of diazin the African catfish (*Clarias gariepinus*). African Journal of Biotechnology, 7, 651-654.

Afful, S., Anim, A.K. and Sarfa-Armah, Y. (2010) Spectrum of organochlorine pesticide residue in fish samples from Densu basin. Research Journal of Environment and Earth Science, 2, 133-138.

Akaahan, T.J., Eneji, I.S. and Azua, E.T. (2015) Evaluation of water quality suitability in river Benue for aquaculture production in Benue state Nigeria. International Scientific Research Journal, 1, 66-73.

Ayuba, V.O., Iyakwaji, S.P. and Oyeniyi. M.E. (2013) Acute toxicity of formalin on *Clarias gariepinus* juveniles. PAT, 9, 21-28.

Bouwman, H. (2004) South Africa and the Stockholm on persistent organic pollutants. African Journal of Science, 100, 323-328.

Davies, O.A., Inko-Tariah, M.B. and Amachree, D. (2006) Growth rate response and survival of *Hetrobrancus longifilis* fed at different feeding frequencies. African Journal of Biotechnology, 5, 778- 780.

Ezemonye, L. and Ogbomida, T.M. (2010) Histopathological effects of gammalin 20 on African Catfish(*Clariasgarie pinus*). Applied and Environmental Soil Science, Article ID 138019, 8 pages.

Lawson, E.O., Ndimele. P.E. Jimoh, A.A. and Whenu, O.O. (2011) Acute toxicity of lindane (gammahexachloro-cyclohexane) to African catfish (*Clarias gaeriepinus* Burchell 1822). International Journal of Animal and Veterinary Advances, 3, 63-68.

Ndimele, P.E. and Jenyo-Oni, A. (2009) Evaluation of toxicological impact of Nigerian crude oil (Bonny light) to *Tilapia guineensis*. Journal of Environmental Extension, 8, 76-80.

Omitoyin, B.O., Ajani, E.K., Adesina, B.T. and Okuagu, C.N.F. (2006) Toxicity of lindane (gammahexachloro-cyclohexane) to *Clarias gariepinus* (Burchell 1822). World Journal of Zoology, 1, 57-63.

Reddy, P.P., Jagadeshwarly, R. and Devi, G.S. (2016) Determination of lethal concentration of (LC_{50}) of copper to *Sarotherodon mossambica*. International Journal of Fisheries and Aquatic Science, 4, 172-175.

Reish, D.L. and Oshida, P.S. (1987) Manual of methods in aquatic environment research part 10 short term static bioassay. Food and Agricultural Organization Fish Technology, Technical Paper, 247, 62-68.

Tomlin, C.D.S. (1997) British crop protection council. The pesticide manual. Library Cataloguina Publication Data, pp. 1606.

Ullah, R., Zuberi, A., Ullah, S., Ullah, I. and Dawar, F.U. (2014) Cypermethrin induced behavioral and biochemical changes in mahseer, Tor puttilora. Journal of Toxicological Science, 39, 829-836.

United Nations Environmental Programme, UNEP. (2005) Ridding the world of persistent organic pollutants. United Nations Environmental Programme, 11-13, Chemin des Anemone, CH-129 Chatelain Geneva Switzerland, pp. 24.