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Bioassay of Lindane (Gamalin 20) to *Hetrobrancus bidorsalis* Juveniles

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Abstract

The study was carried out to determine the acute toxicity of lindane (Gammalin 20) to *Hetrobranchus 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 litres water capacity. The Gammalin 20, organo chlorine pesticide was distillated and the active ingredient lindane was condensed and collected and the stock solution was prepared. Graded concentration of 0.02, 0.04, 0.06, 0.08 and 0.10 ml/L were prepared and the fish of 10 individuals in each of the bowls were exposed to the different concentration with a control experiment where the toxicant was not introduced. The experimental set was replicated three times. The exposed fish were observed daily and death ones were removed immediately and mortality was recorded for 24 and 48 hours exposure period. The LC_{50} was determined to be 0.06 ml/L for the 24 and 48 hours exposure period. The obtained result was transformed to probit analysis which was plotted against the graded concentration of lindane for the 24 and 48 hours exposure period. The R^2 values of 0.76 and 0.80 were obtained for the 24 and 48 hours respectively indicating a strong relationship of lindane with mortality. The result of the water quality varied from pH: 7, 80-8.46, temperature: 28.39-28.42°C DO: 5.06-5.17 mg/L, Conductivity: 462.00-482.00 µS/cm and TDS: 231.00-241.00 mg/L. Although the water quality parameters increase with increase in the lindane concentration yet it was within the maximum permissible level and did not have any effect on the fish and the death of the fish was due to the toxic potential of lindane. It was recommended that Gammalin 20 is very toxic and persistent in the aquatic environment and it use should be greatly discouraged.

Keywords: Bioassay; *Hetrobrancus bidorsalis*; Lindane; Organochlorine gammalin 20

Introduction

Gamalin 20 is organochlorine pesticide that is used widely in veterinary and human medicine to treat ectoparsites and pediculosis. It also has application as a broad spectrum in the elimination of phytophagous and soil inhibiting insects, public health pest, ectoparasites of animals, used on crops to control their pest and stored product pests and seed treatment. In the fishing industry it is used for killing of the fish as a fishing technology [1]. The active ingredient contain in Gamalin 20 is lindane [2]. Lindane has application in the treatment of seeds. It is also used in lotions, creams and shampoos for the control of lice and mites in humans [3]. Benzane hexachloride (BHC) is the 100% pure form of Gamalin 20 while lindane is slightly less pure with 99% purity. Lindane is highly toxic to fish, bees and aquatic invertebrates and it is very stable in both fresh and salt water environments.

A pesticide is any substance or a combination of substances used for the elimination and stopping the effect of any pest [4]. Broadly, pesticides are classified as insecticides, fungicides and herbicides [5]. Insecticides are mainly organochlorine, organophosphus, carbamates and pyrethroids. As a result of the persistency of organochlorine insecticides, most have been banned by legislation in most countries as agrochemicals used for the elimination of pest in agriculture [6]. The ill effect of pesticide application to control pest is the contamination of the environment [2]. More so studies have revealed that only about 1% of the pesticides applied that get to the target organism, the remaining contaminate the soil and water environment [2]. 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 [7]. 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.

Materials and Methods

Test organisms collection

Hetrobrancus bidorsalis life juveniles specimens numbering about 270 members were obtained from the fish farm Tekeze dam tigray Ethiopia. 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 mekelle university 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.

Acclimatization of the fish samples

The fish samples obtained from the fish farm and taken to the laboratory were kept in 27 plastic bowls of 20 liters 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 decchlrinated water in each of the plastic bowls was 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 put into were equally aerated with

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an electric aerator and the fish samples were fed with commercial feed (40% cp) two times daily at 30% body weight.

Source of the toxicant (Gammalin 20)

Lindane (Gammalin 20) used for the study was bought from a chemical shop in mekelle tigray Ethiopia. The toxicant was kept in the laboratory under laboratory conditions prior to the commencement of the test.

Preparation of toxicant standard solution

The toxicant Gammalin 20 was taken to chemistry laboratory, of the Mekelle University. In the chemistry Laboratory the Gammalin 20 was unsealed and the content was transferred into a boiling vessel or distillation column, by application of heat the active ingredient lindane was collected through the process of fractional distillation, which serves as the precipitate or distillate and the vapour was evaporated and condensed to colourless substance in appearance. However the residue was dark in colour. The distillate was measured in concentrations of 0.02 ml/l, 0.04 ml/l, 0.06 ml/l, 0.08 ml/l and 0.10 ml/l.

Bioassay procedure

Standard bioassay procedure was adopted during the course of this study. The acute toxicity test commenced with a range finding test was carried out for a 96 hour period to ascertain the lethal concentration of lindane to *Heterobranchus bidorsalis*.

Exposure of fish to toxicant: The definitive test was carried out after the range finding test was conducted. 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 (10) members separately for each of the concentrations. The bioassay concentration were 0.00 ml/L (control), 0.02, 0.04, 0.06, 0.08, and 0.10 ml/L. The test was replicated in three replicates 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 hours and subsequently about every 6 hours up to 96 hours. 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 were used throughout the experiment [8].

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.

Temperature: The temperature of the water in each bowl used for the experiment was measured by gently inserting the temperature sensor of the multi-parameter water checker in each bowl. Through this process various temperatures were measured and noted.

Dissolved oxygen (DO): The dissolved oxygen in the water in each bowl was measured using a dissolved oxygen sensor of the multi-parameter water checker gently inserted or dipped in each bowl of water and the readings appeared on the screen.

Total dissolved solid (TDS): The total dissolved solid was measured by dipping the total dissolved solid sensor of the multi-parameter water checker into the water contained in each bowl, the value for total dissolved solid appeared on the screen.

pH: The PH of each water sample contained in the bowls was examined by dipping the PH sensor of the multi-parameter water checker into each of the water sample, after some seconds, the PH value appeared on the screen and was noted.

Electrical conductivity (EC): This was measured by inserting or dipping the conductivity sensor of the multi-parameter water checker into each water sample in the experimental bowls, the value for electrical conductivity appeared on the screen and was noted.

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.

Results

Toxic effect of lindane

The results presented in Tables 1 and 2 is the Mortality record of *Hetrobranchus bidorsalis* Exposed to Lindane (Gammalin 20) for 24 and 48 hours respectively in the course of the study. The pesticide was more toxic within the 48 hours exposure time as compared to the 24 hours exposure period. However no death was recoded in both and 24 and 48 hours exposure of the control of experimental set up of the study. Across the graded concentrations of the toxicant the 0.10 ml/L was recoded with the highest mortality in both exposure times(24 and 48 hours), while the same mortality of 20% was recorded in the 24 and 48 hours exposure for 0.02 ml/L concentration of the lindane (Gammalin20).

Similarly the results in Figures 1 and 2 is the Probit Transformed Response for mortality of *Hetrobranchus bidorsalis* exposed to graded concentration of Lindane(Gammalin 20) for 24 and 48 hours respectively. The R^2 value for the 24 and 48 hours exposure period of the pesticide to the fish was 0.76 and 0.81 respectively. This clearly indicate that the pesticide has a strong relationship with the death of the fish with graded concentration within the time of exposure. This is evident in the fact the more the concentration with mores exposed time to lindane the more the death of the fish that will be recorded.

Physicochemical changes of water sample

The results presented in Table 3 is the Phyico-chemical Characteristics of the water samples of the control and Lindane treatments exposed to the Fish during the period of the study. A perusal at the results indicate that among all the parameters examined their lowest concentration was obtained in the waters of the control experiments where the pesticide was not introduced except for the DO that it was the highest (5.17 mg/L). All the same among all the parameters except for DO the lowest concentration was measured in the lowest concentration of 0.02 ml/L and the highest in the highest concentration of 0.10 ml/L. In the case of DO it was decreasing in its value from the control to the highest concentration of lindane during the study. This result clearly showed the effect on the pesticide on the DO of the water. This similar trend is observed in Table 4 which is the descriptive statics of the water quality parameters of the control and treatments of the experimental set up of the study. All the same the mean values of all the water quality parameters were within the permissible level acceptable. The correlation analysis results between the water quality parameters is presented in Table 5. The correlation was significant between DO and conductivity, and between DO and

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 1: Mortality record of *Hetrobranchus bidorsalis* Exposed to Lindane (Gammalin 20) for 24 hours.

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

Table 2: Mortality record of *Hetrobranchus bidorsalis* Exposed to Lindane (Gammalin 20) for 48 hours.

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	462.00	231.00
2	0.02	8.34	28.42	5.15	468.00	234.00
3	0.04	8.39	28.44	5.11	470.00	235.00
4	0.06	8.43	28.39	5.13	475.00	237.00
5	0.08	8.44	28.40	5.09	480.00	240.00
6	0.10	8.46	28.42	5.06	482.00	241.00

Table 3: Phyico-chemical Characteristics of the water samples of the Control and Lindane Treatments Exposed to the Fish.

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 4: Descriptive Statics of the Water quality Parameters of the Control and Treatments of the Experimental set up.

Parameter	pH	Temperature (°C)	DO (mg/L)	EC (µS/cm)	TDS (mg/L)
pH	1	0.241	-0.78	0.8	0.79
Temperature (°C)	0.241	1	-0.15	-0.07	-0.38
DO (mg/L)	-0.78	-0.15	1	-0.95*	-0.96*
EC (µS/cm)	0.8	-0.06	-0.95*	1	0.99*
TDS (mg/L)	0.79	-0.04	-0.96*	0.99*	1

*Correlation is significant at 0.01 level (2 tailed)

Table 5: Correlation Analysis between the Water Quality parameters of control and Treatments of the experimental set up.

TDS only at 0.01 level of significance. The student t test was analysis was significant among all the water parameters examined ($P<0.05$).

Discussion

The bioassay result of the lindane graded concentration exposed to juveniles of *Heterobranchus bidorsalis* indicate clearly that lindane is poisonous. The results of this study revealed that *Heterobranchus bidorsalis* exposed to lindane (Gammalin 20) LC_{50} for 24 and 48 hours was 0.06 ml/L. The results showed that the higher the concentration of Gammalin 20 the more toxic it becomes to the fish. This finding is similar to that of earlier study of an organochlorine pesticide [9]. A perusal at the result of the present investigation revealed that the probit transformed response to mortality of *Heterobranchus bidorsalis* juveniles exposed to graded concentration of lindane for 24 and 48 hours 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. This 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 [10,11]. Toxicants may affect aquatic organisms within a short period of time 24 or 48 hours 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 [9,10]. 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

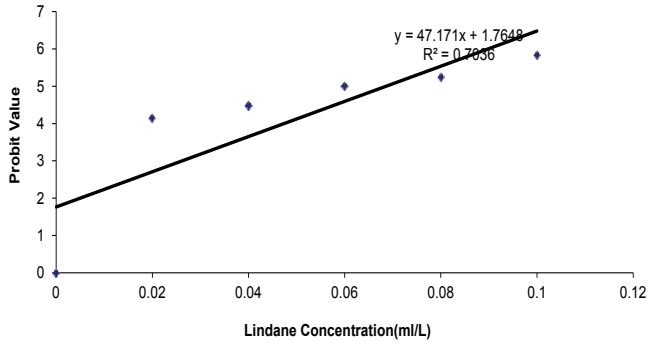

Figure 1: Probit Transformed Response for mortality of *Hetrobranchus bidorsalis* Exposed to Graded Concentration of Lindane (Gammalin 20) for 24 hours.

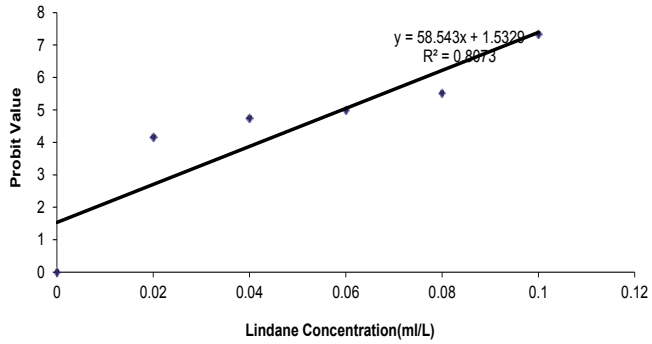

Figure 2: Probit Transformed Response for mortality of *Hetrobranchus bidorsalis* Exposed to Graded concentration of Lindane (Gammalin 20) for 48 hours.

lindane for a particular 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 observation are consistent with Reddy [11]. 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. This findings are consistent with [2,12] The LC_{50} concentration of 0.06 ml/L was reported in this study for 24 and 48 hours exposure period. This result differs significantly from the findings of earlier study that reported LC_{50} of 1.29 ppm of lindane exposed to *C. gariepinus* [12]. However [2] reported LC_{50} of 0.38 ppm 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. [2]. 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 water quality parameters examined indicate that the toxicant Gammalin 20 did not affect the quality of the water adversely. All the same all the water quality parameters tested were suitable for aquaculture. This result is similar to that reported by [13]. Pesticides induce different types of toxic effect in the fish that result to different changes of the fish was observed in this study [14]. Generally the water quality results were suitable for the survival of the fish and may not have pronounced effect on the mortality of the fish but the lindane has established by this present investigation.

Conclusion

The result of this investigation reveals that lindane (Gammalin 20) an organo chlorine pesticide is very toxic to *Heterobranchus bidorsalis* juveniles and has the potential of persisting in the aquatic ecosystems. The use of this pesticide as technique for killing fish for human consumption as is done my some fisher men should be discouraged greatly. This is because the pesticide has the ability of biomanification along the food chain may be toxic to man when he consumes the fish.

References

1. Tomlin CDS (1997) British Crop Protection Council. The pesticide Manual. Library Cataloguina Publication Data, p: 1606.
2. Lawson EO, Ndimele PE, Jimoh AA, Whenu OO (2011) Acute toxicity of lindane (gamma hexachloro-cyclohexane) to African catfish (Clarias gaeriepinus Burchell 1822). Inter J of Ani and Veter Advan 3: 63-68.
3. Adedeji BA, Adedeji AO, Adeyemo OK, Agbede SA (2008) Acute toxicity of diazin the African catfish (Clarias gariepinus). Afr J Biotechnol 7: 651-654.
4. United Nations Environmental Programme UNEP (2005) Ridding the World of persistent organic Pollutants. United Nations Environmental Programme, Chemin des Anemone, CH -129 Chatelain Geneva Switzerland, p: 24.
5. Afful S, Anim AK, Sarfa-Armah Y (2010) Spectrum of organochlorine pesticide residue in fish samples from Densu Basin. Resear J of Environ and Ear Scei 2: 133-138.
6. Bouwman H (2004) South Africa and the Stockholm on persistent organic pollutants. Afr J Sci 100: 323-328.
7. Ndimele PE, Jenyo-Oni A (2009) Evaluation of toxicological impact of Nigerian crude oil(Bonny light) to Tilapia guineensis. J Environ Extens 8: 76-80.
8. Davies OA, Inko-Tariah MB, Amachree D (2006) Growth rate response and survival of Hetrobranchus longifilis fed at different feeding frequencies. Afri J Biotechnol 5: 778-780.
9. Ezemonye L, Ogbomida TM (2010) Histopathological effects of Gammalin 20 on African Catfish (Clarias gariepinus). Appli and Environ Soi Sci, pp: 1-8.
10. Ayuba VO, Iyakwaji SP, Oyeniyi ME (2013) Acute toxicity of formalin on Clarias gariepinus juveniles. PAT 9: 21-28.
11. Reddy PP, Jagadeshwarly R, Devi GS (2016) Determination of lethal concentration of (LC50) of copper to Sarotherodon nossambiea. Inter J of Fisher and Aqua Sci 4: 172-175.
12. Omitoyin BO, Ajani EK, Adesina BT, Okuagu CNF (2006) Toxicity of lindane (gamma hexa chloro-cyclohexane) to Clarias gariepinus (Burchell 1822). World J Zool 1: 57-63.
13. Akaahan TJ, Eneji IS, Azua ET (2015) Evaluation of water quality suitability in River Benue for aquaculture production in Benue state Nigeria. Inter Scienti Resaera J 1: 66-73.
14. Ullah R, Zuberi A, Ullah S, Ullah I, Ullah Dawar F (2014) Cypermethrin induced behavioral and biochemical changes in mahseer, Tor putitora. J Toxicol Sci 39: 829-836.