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Acute Toxicity and Haemotological effect of Delmin Forte® on *Clarias gariepinus* Juveniles

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Abstract

A static bioassay method to determine the acute toxicity of the herbicide Delmine forte® to *Clarias gariepinus* juveniles was investigated under Laboratory condition for 96hours exposure period. Concentration of Delmine forte® used include 0.25, 0.50, 0.75, 1.00, 1.25µl/L respectively. The lethal concentration (LC_{50}) value of Delmin forte® on Clarias gariepinus was 0.06µl/L for 96hours of exposure. The Regression equation for probit from Delmine forte® was found to be Y= 2.40 + 6.44 x Log conc. (R^2 = 0.91, Y= probit kill). Fish exhibited various abnormal behaviors upon exposure to Delmine forte®. Immediate reaction was erratic swimming, loss of equilibrium, restlessness, and respiration distress, accumulation of mucus on the body surface and gill filament and death. Haemotological parameters such as PCV, RBC, MCV, Hb, MCH, MCHC, WBC and platelet investigated decreased with increase in concentration. This is an indication of the disruptive effect of Delmine forte® herbicide on the erythropoietic tissue as well as cell viability.

Keywords: Delmine forte®; Acute toxicity; Heamatological effect; *Clarias gariepinus*; Mubi

Introduction

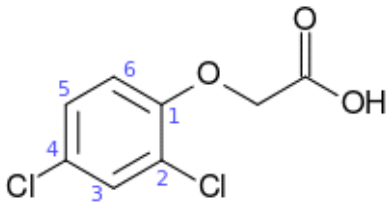
Delmine forte® is a trade name of 2, 4 -D amine salt with IUPAC name 2, 4-dichloropheoxyl acetic acid. This is a yellow brown and clear liquid in the physical state, it is a white powder with a melting point of 140.50^{0}C and soluble at 25^o C of 620mg/l water. This chemical is soluble in aqueous alkali, alcohol, and diethyl ether and insoluble in petroleum oil. Delmine forte® powder is a strong acid and corrosive (Akobundu,1981). Delmine forte® is one of the most widely used chemicals in Nigeria especially along Fadama areas for rice farmers. Farmers' use 2, 4- D amine salt in the control of broadleaved weeds in rice, wheat, corn, millet and sorghum farm. Below is the chemical and structural formula of Delmine Forte®; 2, 4-Dichloropheoxyl acetic acid.

Molecular Formula $C_8H_6Cl_2O_3$

Average mass 221.037 Da

Monoisotopic mass 219.969406 Da

ChemSpider ID1441



(Wikipedia, 2018)

Run-off from such treated field ends up in water bodies closed to those fields and accumulate in stagnate waters creating serious effect on the aquatic animals. In Nigeria, use of herbicide for weed control has been on increase. This has cause aquatic pollution as the poison are washed into water bodies through surface run off during rainy season (Akobundu, 1987) and (Svobodova *et al*; 2010). The advance effect of herbicide and their residue on non target organism have not been seriously considered in Nigeria (Ayoola, 2008). Modern agricultural activities have also introduced

several polluting substances such as herbicides and insecticide into the river and drainage systems causing a lot of havoc to aquatic life (Khallaf, 1998). The occurrence of herbicide in terrestrial and aquatic environment is due to their persistence toxicity and the fact that certain species have high bioaccumulation of these chemicals. In an attempt to address associated environmental problems, serial studies on the generation and discharged of herbicide from agricultural uses have been carried out in many countries of the world. The usual concentration at which most herbicide occurs in natural aquatic system is seldom high enough to bring about acute toxicity. However, sub lethal concentration that usually prevail in such water bodies are known to cause adverse biological effect manifesting at subtle physiological, biological, anatomical or behavioral changes in the exposed organism which can be employed as negative response of impacted species at concentration well below acute toxicity thresholds (poulsen *et al* 1982, Momoh,1995).

Material and Methods

Acclimatization: Juveniles of African catfish *Clarias gariepinus* with a mean weight 6.80g±0.25 and about 10.30cm±0.2 of length were collected from the Adamawa State University Mubi fish farm to its Fisheries laboratory in a de-chlorinated aerated tap water with the temperature of 21.5^0C±0.12. Juveniles were acclimatized for seven days prior to the commencement of the experiment in a plastic tanks measured 40cmx30cmx30cm dimension. Water was changed at 3 days interval to prevent build up of metabolic waste and aerated to increase oxygen supply. The fish were fed with vital feed twice daily (morning and evening) respectively at 5% body weight.

Definitive Test: Feeding was stopped 24 hours prior to the commencement of the experiment. Ten juveniles of *Clarias gariepinus*, were randomly selected and transferred from the holding tanks into the respective test tanks (with 20 liters each of water) within 30 minutes of preparing the toxicant mixture. The Delmine forte® concentration used was 0.25, 0.50, 0.75, 1.00, and 1.25µl/l respectively. There was a control in which 10 fish were exposed to Adamawa State University Mubi de-chlorinated tap water only. Haematological effect of the toxicant were determined using the method described by Patnaik *et al (*2006) and Svobodova *et al* (2010),).The haematological indices examined include, white blood cell (WBC), red blood cell (RBC), haemoglobin (HB), packed cell volume,(PCV), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and platelet. Result obtained was subjected to statistical analysis of variance (ANOVA) at 5% level of significance. Probit transformation of mortality was carried out to determine the 96hour LC_{50} of Delmine forte® on *Clarias gariepinus* juveniles. Regression analysis was done to determine the 96hour LC_{50} of Delmine forte® on the test fish.

Water quality parameters: Daily water quality parameters were also monitored during the course of the experiment. Daily water quality parameters values were obtained using the methods described by APHA (1998) and Apollos and Jamala (2011). Parameters recorded include Dissolved oxygen (DO), alkalinity, and hardness, free CO_2, temperature, and pH.

Results and Discussion

The physico-chemical parameters of the test solution in the experimental tanks as shown in **Table** 1 fluctuated slightly during bioassay, but were not enough to have affected mortality. There was no death nor abnormal behavior observed in the control group throughout the exposure period. The LC_{50} value derived from the toxicity test revealed that *Clarias gariepinus* juvenile is sensitive to the herbicide. The observed responses include: loss of balance, erratic swimming, restlessness, and respiratory distress, accumulation of mucus on the body surface and gill filament and death. At higher concentration, the percentage mortality increased as shown in table 2 and 3 respectively.

Table 1: Physico-chemical parameters of the test solution

Concentration(µl/l)	D.O(mg/l)	Alkalinity(mg/l)	Hardness(ppm)	Temperature(^{o}C)	pH
0.25	7.3±0.28^{a}	24.62±0.53^{a}	39.2±0.08^{a}	22.1±0.32^{a}	7.1±0.007^{a}
0.50	7.0±0.02^{a}	24.50±0.75^{a}	40.0±0.06^{a}	22.3±0.38^{a}	6.9±0.044^{b}
0.75	6.8±0.13^{b}	24.50±0.75^{a}	40.1±0.75^{a}	22.3±0.70^{a}	6.7±0.163^{b}
1.00	6.8±0.16^{b}	24.51±0.44^{a}	40.1±0.03^{a}	23.7±0.40^{a}	6.5±0.069^{b}
1.25	6.4±0.13^{b}	23.20±0.50^{a}	41.5±0.03^{a}	23.2±0.39^{a}	6.5±0.070^{b}
0.00 (control)	7.5±0.06^{a}	24.63±0.65^{a}	38.0±0.03^{a}	22.1±0.17^{a}	7.1±0.041^{a}

Means on the column with the same superscript are not statistically significant (p>0.05) Means on the same row with different superscript are statistically different (P< 0.05)

Table 2: Percentage Mortality rate of *Clarias gariepinus*

Concentration(µl/l)	Number of Test fish	Number of death in in Test After 96hr	Number death in Replicate After 96hr	Percentage Mortality (%)
0.25	10	6	5	55
0.50	10	7	7	70
0.75	10	9	8	85
1.00	10	9	10	95
1.25	10	10	10	100
0.00 (control)	10	0	0	0

Table 3: Log Concentration and the probit value of the mortality

Concentration(µl/l)	Log concentration	Percentage Mortality (%)	Probit Value
0.00 (control)	-	0	-
0.25	-0.6021	55	5.13
0.50	-0.3010	70	5.52
0.75	-0.1249	85	6.04
1.00	0	95	6.64
1.25	0.0969	100	-

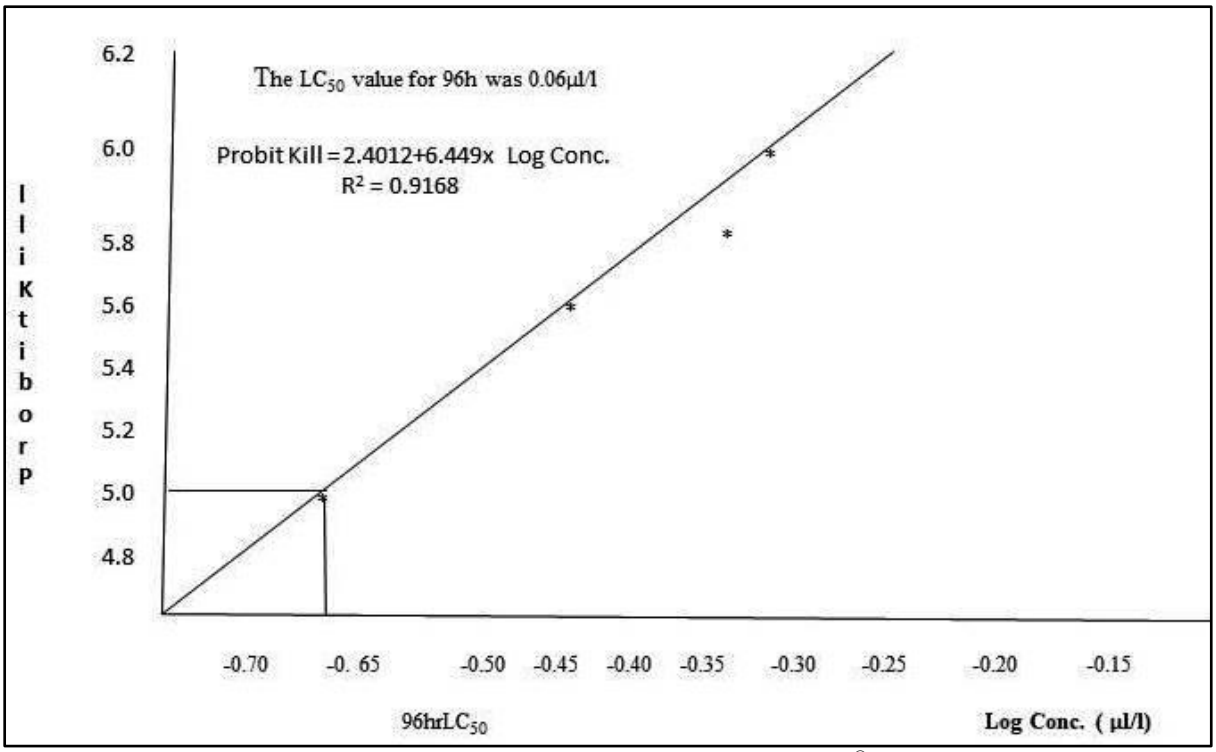


Figure 1.Regression curve of probit kill and Log concentration of Delmin force® on *Clarias gariepinus*

The computed regression equation was found to be Y= 2.40+ 6.44 x Log conc. (R^2= 0.91, Y= probit kill). The R^2 value of 0.91 obtained in the regression equation shows that there is strong correlation between probit kill and the toxicant concentration. This implies that the higher the concentration of the herbicide the higher the mortality. The LC_{50} value in the present study for *Clarias gariepinus* juveniles is similar to the findings of Annune *et al* (1994), Annune and Ejike (1999), Anadu and Ajana(1988), Avoaja and Oti (1997), Lovely (1998) and Oti (2000) who observed that at higher concentration of herbicide (toxicant) exposed at 96hr to fish showed several fish abnormal behavior of restlessness, loss of equilibrium, eractic swimming, respiratory distress, air gulping and death. The mortality increased with increasing concentration following the exposure of fish to varied concentration of Delmine forte®. The haemotological effect of Delmine forte® on *Clarias gariepinus* juvenile is shown in **Table** 4.

Table 4: Haematological parameters of *Clarias gariepinus* exposed to Delmine Forte®

	Treatment (µl/L)						
parameters	00 (control)	0.25	0.50	0.75	1.00	1.25	LSD
PCV	24.40±0. 5^a	23.40±0.1^a	21.50±0.30^b	20.50±0.2^c	18.50±0.2^d	17.51±0.2^d	1.6
HB	8.05±0.04^a	7.85±0.10^a	7.14±0.05^b	6.65±0.02^b	5.50±0.2^c	4.9±0.0^e	0.17
WBC	10.05±0.0^a	5.85±0.02^b	5.45±0.06^b	4.35±0.03^c	3.95±0.05^c	2.45±0.05^d	0.12
RBC	3.3±0.01^a	3.01±0.06^a	2.55±0.05^b	2.25±0.07^c	2.15±0.04^d	2.00±0.10^d	0,21
MCV	74.50±0.01^a	72.50±0.3^a	60.50±0.02^b	59.40±0.5^b	55.50±0.2^c	49.50±0.0^d	1.60
MCH	24.15±0.01^a	18.65±0.0^a	18.55±0.25^a	18.45±0.0^a	17.35±0.0^b	16.70±0.3^c	0.41
Platelete	142.50±0.60^a	123.50±0.50^b	121.50±0.3^b	121.40±0.20^b	120.50±0.50^c	116.50±05^d	2,62
MCHC	36.75±0.0^a	34.10±0.1^b	33.85±0.05^b	32.70±0.1^b	32.20±0.1^c	31.45±0.5^d	0.18

Means on the row with the same superscript are not statistically significant (p>0.05) Means on the same row with different superscript are statistically different (P< 0.05) where; WBC = White Blood Cell, RBC = Red Blood Cell, PCV = Packed Cell Volume, MCV = Mean Corpuscular Volume, MCH

= Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration.

The PCV, RBC, MCV, HB, MCHC, WBC and platelet decreased with increase in concentration of the toxicant. All the mentioned alterations indicated that exposed fish to toxicant suffered from anemia induced by the herbicide. This is an indication of the disruptive effect of Delmine forte® on erythropoietic tissue as well as cell viability. This corroborated with the report of Sultz (1971), Patnaik *et al (*2006), Kori- siakpere *et al* (2007) and Svobodova (2010), who reported of several cell damage, anemia, loss of immunity and death.

Conclusion

Delmine forte® was toxic to *Clarias gariepinus*, and effect increased with increasing concentration. Environmental Authorities need to set quality standard on the use of Delmin forte® in aquatic ecosystem. This will reduce the deleterious effect on the environment, other living aquatic organism and man.

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