

## Toxicity of Gramoxone (paraquat) to Juvenile African Catfish, *Clarias gariepinus* (Burchell, 1822)

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**Abstract:** African catfish, *Clarias gariepinus* (Burchell 1822) juveniles were exposed to 0, 1.6, 3.6, 8, 18 and 40 mg L<sup>-1</sup> of gramoxone (paraquat). The median lethal concentration (LC<sub>50</sub>) value of gramoxone was 18 mg L<sup>-1</sup> for 96 h of exposure. The fish specie showed several abnormal stress behaviours which include excessive gulping of air, erratic swimming, restlessness, loss of movement, loss of equilibrium, increased opercular beats and paralysis during the exposure. The means mortality percentage was 0, 0, 0, 0, 50 and 100 in order of concentration of 0, 1.6, 3.6, 8, 18 and 40 mg L<sup>-1</sup> (weight/volume), respectively. Toxicity of gramoxone reduced with time, *Clarias gariepinus* juveniles succumbed only to higher concentration of gramoxone within 9-72 h of exposure. Congestion, necrosis and pyknosis of hepatocytes, cellular infiltration, hypertrophy, neuronal degeneration, lesion, pyknosis and degenerative changes such as necrosis of tubular and haematopoietic cells of kidney and liver were the major histopathological changes that occurred during the exposure to gramoxone. There were no lesions on the organs of the control experimental fish as signs of histopathological changes. The results showed that 'a safe level' of gramoxone was obtained at 18 mg L<sup>-1</sup> where there was 50% survival while 100% mortality was obtained at 40 mg L<sup>-1</sup>.

**Key words:** *Clarias gariepinus* • gramoxone • toxicity

### INTRODUCTION

Widespread use of herbicides and pesticides in agriculture and forestry is now a world wide phenomenon [1, 2]. The rate of increase of herbicide and pesticide use is relatively high in Africa, Central and South America, Far East Asia and the Middle East [3]. In recent years, awareness about the potential harmful effects of the residues on human health has been growing [2, 4] and its contribution to the gradual degradation of the aquatic ecosystem cannot be ignored [1, 5-7].

Pesticides and herbicides at high concentrations are known to reduce the survival, growth and reproduction of fish [1, 8] and produce many visible effects on fish [1, 9]. Moreover, it is known that diseases, pollution and the presence of agricultural chemicals in water cause alterations in blood cells of fish resulting in losses in aquaculture [10, 11]. Due to the residual effects of pesticides and herbicides important organs like the kidney, liver, gills, stomach, brain, muscles and organs are damaged [1, 12].

Gramoxone is a common herbicide use by farmers to control weeds in Nigeria. Its use around aquatic habitat

poses a serious threat on aquatic organism particularly fish. There is therefore the need to determine the tolerance limit of fish to gramoxone.

The aim of the present study was to determine the median lethal concentration (Lc<sub>50</sub>) value of gramoxone (paraquat) to *Clarias gariepinus*, the behavioural and histopathological effects of gramoxone on organs (brain, gill, liver, kidney and intestine) of *Clarias gariepinus* juveniles.

### MATERIALS AND METHODS

*Clarias gariepinus* juveniles with average length of 15±1.5 cm and weight: 20±0.5 g used in the study was bought from a reputable fish farm in Abeokuta, Ogun State, Nigeria. The fish were disinfected with 0.1% KMnO<sub>4</sub> and acclimatized for fourteen days in glass aquaria. The fish were fed with 47% commercial catfish feed during the period of acclimatization. After acclimatization, the fishes were transferred into eighteen 60 cm x 30 cm x 30 cm glass aquaria for the experiment. A range finding test was first determined as described by Fafioye [13], Omitoyin *et al.*, [12] and Solbe [14], respectively to

determine the concentration of the toxicant to use in the definite test. The following concentrations in weight per volume of gramoxone were used: 10 mg L<sup>-1</sup>, 20 mg L<sup>-1</sup>, 40 mg L<sup>-1</sup>, 60 mg L<sup>-1</sup>, 80 mg L<sup>-1</sup> and 100 mg L<sup>-1</sup>. Three *Clarias gariepinus* juveniles per concentration of toxicant were used with 3 replicates each for 24 h.

Prior to range finding test, the experimental fish were starved for 48 h. Mortality was recorded at 20 mg L<sup>-1</sup>, 40 mg L<sup>-1</sup>, 60 mg L<sup>-1</sup> and 100 mg L<sup>-1</sup>. At 40-100 mg L<sup>-1</sup> 100% mortality was recorded. Based on this, five concentrations of the herbicide were prepared and tested on the *C. gariepinus* juveniles. The different concentrations of gramoxone prepared for the definite test were: 1.6, 3.6, 8, 18 and 40 mg L<sup>-1</sup>, with three replications including a control (0 mg L<sup>-1</sup>). Ten acclimated fish were used in each aquarium containing different concentrations of herbicide as well as in the control as described by Solbe [14] and Rahman *et al.*, [1], respectively. The experiment was carried out in the laboratory at room temperature of 28.1°C.

The behavioural pattern of the fish and other external changes in the body of the fish were observed accordingly. Dead fishes were promptly removed and preserved in 10% formaldehyde.

Histopathological examination was performed on various organs such as liver, kidney, brain, gills and intestine by following standard methods of Luna [15] and Rahman *et al.*, [1]. At the end of the experiment, the critical organs were collected and preserved in small plastic vials with ten times 10% neutral buffer formalin fixatives. The numbers at section of sampled organs was prepared using a microtome, stained and studied under a Microscope as described by Luna [15], Fafioye [13] and Rahman *et al.*, [1]. The Lc<sub>50</sub> value volume of the fish species was calculated for 96 h exposure time by prohibit analysis. Water quality parameters such as Temperature, Dissolved oxygen and PH were measured using standard method of APHA [16].

## RESULTS

**General effects:** The effects of different concentrations and exposure time of gramoxone on *Clarias gariepinus*

juveniles are presented in Table 1. The Lc<sub>50</sub> value based on probit analysis was found to be 18 mg L<sup>-1</sup> for 96 h of exposure to the herbicide. Results obtained shows that there was no mortality of fish in the control experiment and those exposed to 1.8, 3.6 and 8 mg L<sup>-1</sup> gramoxone throughout the 96 h. However, at 18 mg L<sup>-1</sup> there was 50% mortality while at 40 mg L<sup>-1</sup>, 100% mortality were observed (Table 1).

The behaviour of the control fish was normal immediately the herbicide was introduced into the test fish in different aquaria at different concentration level, abnormal behaviour such as erratic swimming, restlessness, sudden quick movement, excessive secretion of mucus, rolling movements, swimming on the back (at higher concentrations) were observed. The fish became very weak, settled at the bottom and died in increasing number at 40 mg L<sup>-1</sup> (Table 1). There was discolouration on the body of the fish at high concentrations. Normal colour and behavioural responses were observed in the control experiment. However, the colour became pale progressively at higher concentrations at the end of 96 h of exposure time to the herbicide.

### Histopathological effect:

**Brain:** The histopathological observation on the brain showed that there was discolouration on the brain of fish exposed to different concentration of gramoxone, while there was none in the brain of fish in the control experiment. Fish exposed to concentration 1.6-8 mg L<sup>-1</sup> of gramoxone showed mild congestion, mononuclear cellular infiltration of the cerebral meninger, neuronal degeneration and moderate spongiosis in the white matter, whereas fish exposed to concentrations of 18 and 40 mg L<sup>-1</sup> showed severe brain damage as shown in Table 2.

**Gill:** The gill of the control experimental fish was normal while at different concentrations of gramoxone, there was swollen tip of the gill filament, infiltration of subamucosa, congestion necrosis in the lamellae, submucosa haemorrhage, stunting and matting of the gill filament. However, at 40 mg L<sup>-1</sup>, the gill were severely

Table 1: Mean cumulative mortality percentage of *Clarias gariepinus* Juveniles subjected to different concentrations of gramoxone (Paraquat) for 96 h

Treatment (Concentration mg L <sup>-1</sup> )	Mean cumulative mortality (%)											Total mortality
	3 h	6 h	9 h	12 h	24 h	33 h	36 h	45 h	54 h	72 h	96 h	
0 (control)	00	00	00	00	00	00	00	00	00	00	00	00
1.6	00	00	00	00	00	00	00	00	00	00	00	00
3.6	00	00	00	00	00	00	00	00	00	00	00	00
8	00	00	00	00	00	00	00	00	00	00	00	00
18	00	00	7.14	00	7.14	7.14	7.14	7.14	14.3	7.14	00	50%
40	00	00	7.14	14.29	00	00	00	00	00	00	00	100%

Table 2: Summary of histopathological changes observed in the Brian, gill, liver kidney and intestine of *Clarias gariepinus* juveniles subjected to different concentrations of gramoxone (paraquat) for 96 h

Treatment (Concentration mg L <sup>-1</sup> )	Hours of merit	Organs	Congestion	Necrosis	Cellular infiltration	Lesions	Pyknosis	Haemorrhage
0 (Control)	96	B	-	-	-	-	-	-
		G	-	-	-	-	-	-
		L	-	-	-	-	-	-
		K	-	-	-	-	-	-
		I	-	-	-	-	-	-
1.6	96	B	+	-	-	-	-	-
		G	-	+	+	-	-	-
		L	+	½	-	-	-	+
		K	+	½	+	+	+	+
		I	-	-	½	++	-	-
3.6	96	B	-	-	-	-	-	-
		G	-	+	+	+	-	-
		L	++	-	-	-	+	+
		K	++	½	-	++	+	+
		I	+-	-	-	+	-	-
8	96	B	+	-	-	-	-	++
		G	-	½	-	+	-	-
		L	+	+	+	+	+	+
		K	++	½	+	-	+	+
		I	+-	-	-	-	-	-
18	72	B	++	-	-	-	-	++
		G	-	++	+	++	-	-
		L	++	++	-	+	++	++
		K	++	++	½	++	++	++
		I	½	-	-	-	-	+
40	6	B	++	-	+	-	++	++
		G	-	++	+	-	-	++
		L	++	++	½	+	++	++
		K	++	++	½	++	++	++
		I	½	-	-	-	-	+

Legend: B = brain, G = Gill, L = Liver, K = kidney, I = Intestine, - = Completely absent, + = present, ½ = mild, ++ = severe

N.B: Treatments with negative signs indicated no histopathological changes were observed

damaged and congestion was severe at 3.6, 8 and 18 mg L<sup>-1</sup> while submucosa and heterophilic infiltration was observed at 1.6-8 mg L<sup>-1</sup>, respectively (Table 2).

**Liver:** The liver of fish in the control 0 mg L<sup>-1</sup> was normal without necrosis, hepatocytes and other cells were normal and systematically arranged. There was mild necrosis and minor vacuolation at the concentration of 1.6-8 mg L<sup>-1</sup>, hypertrophy of hepatocytes was also observed. At 18 and 40 mg L<sup>-1</sup>, severe necrotic hepatocytes, pyknosis, hypertrophy, haemorrhage and vacuolation were observed accordingly as described in (Table 2).

**Kidney:** Kidney tubules and haematopoietic cells were normal and well arranged in the control treatment for all the tested *C. gariepinus* juveniles. Whereas, kidney tissue from *C. gariepinus* juveniles exposed to 1.6-8 mg L<sup>-1</sup> showed necrosis, degenerated kidney

tubules and pyknosis having haemorrhagic areas. Degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage were more severe in *C. gariepinus* juveniles exposed to 18 and 40 mg L<sup>-1</sup> of the herbicides.

**Intestine:** The intestine of fish exposed to 0 mg L<sup>-1</sup> was normal, but in 3.6 mg L<sup>-1</sup> there was more severe lesion than at 1.6 mg L<sup>-1</sup>. There was moderate vascular congestion in the submucosal lamina propriae at 1.6 mg L<sup>-1</sup> while at 18 mg L<sup>-1</sup> and 40 mg L<sup>-1</sup> showed diffuse infiltration of villi lamina propriae by mononuclear cells as indicated in (Table 2).

**Water quality:** The average water temperature recorded during the experiment was 28.0±0.1°C, pH 6.0-7.0 and dissolved oxygen 7.3±1.0 mg L<sup>-1</sup>. There was little variation in the water quality parameters of the test media.

## DISCUSSION

The  $LC_{50}$  value for 96 h of gramazone was  $18 \text{ mg L}^{-1}$  for *C. gariepinus* juveniles. The  $LC_{50}$  value in the present study for *C. gariepinus* juveniles is similar to the findings of Haider and Inbaraj [17] that used formulations of melathion. The gramoxone exerted toxic effect on the fish in the present study. However the toxicity of pesticides on different species of fishes have been reported, the present findings agreed with Edwards *et al.*, [18], Haider and Inbaraj [17], Eells *et al.*, [19], Singh and Agarwal [20], Hoque *et al.*, [21], Dorucu and Girgin [10] and Cakmak and Girgin [11].

Accumulation of mucus in the gills of fish exposed to the different concentrations of gramoxone in this study might be responsible for the mortality recorded. Konar [5] reported that accumulation of mucus on the gills reduces respiratory activity in fish. The inability of the gill surface to actively carryout gaseous exchange might be responsible for the observed mortalities [12, 13, 22].

Several abnormal behaviour such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercular activities, surface to bottom movement sudden quick movement, resting at the bottom were similar to the observations of Hoque *et al.*, [21], Lovely [23], Omitoyin *et al.*, [12], Fafioye [13], Omoniyi *et al.*, [22] and Rahman *et al.*, [1], respectively. Swelling in the abdominal region and gas filled stomach were not observed, which is contrary to the findings of Kabir and Begun [24] and Lovely [23] and this is an indication that the effect of Pesticides and herbicides are species-specific [1]. Water quality parameters had little variation, physico chemical parameter measured seems to be within optimum range for fish culture as reported by Boyd [25], Viveen *et al.*, [26], Olaifa *et al.*, [27] and Fafioye *et al.*, [28].

Temperature, pH, sex, age and other physiological status of the test animals have profound effects on the toxicity of agro-chemicals [1, 25, 29]. Meletev *et al.*, [30] reported that pesticides affect the gas exchange of fish and other aquatic organisms.

At the agricultural dose of 1.13 ppm of diazinon 60EC, mild pyknotic and necrotic hepatocytes, hypertrophy and in a few cases vacuolation were observed in all the fish species as observed by Rahman *et al.*, [1]. However, at the highest concentration of  $40 \text{ mg L}^{-1}$  of gramoxone degenerative changes of hepatocytes and in some sample of the fish exposed to the herbicide; severe degenerative changes such as, necrosis, pyknosis, vacuolation, rupture of blood vessel causing hemorrhage were observed and this agreed with findings of Omitoyin *et al.*, [12] and Rahman *et al.*, [1]. Degeneration of kidney tubules and

haematopoietic cells, necrosis, pyknosis and hemorrhage observed in this study were similar to those reported by Rahman *et al.*, [1]. Severe brain damaged was observed in fish exposed to 18 to  $40 \text{ mg L}^{-1}$  of the herbicide and there was mononuclear cellular infiltration of the cerebral meninges and neuronal degeneration. Moderate spongiosis in the white matter was observed at  $1.6\text{-}8 \text{ mg L}^{-1}$  of gramoxone on *C. gariepinus* juveniles. This also agreed with the findings of Omitoyin *et al.*, [12]. Rand and Petrocelli [29] reported necrosis of tubular and surrounding haematopoietic cells, nuclear changes of pyknosis and karyorrheis of kidney tissue when Coho salmon was exposed to 100 ppm amitrole for 144 h.

At  $3.6 \text{ mg L}^{-1}$  of gramoxone exposure to *C. gariepinus* there was more severe lesion than at  $1.6 \text{ mg L}^{-1}$ . There was moderate vascular congestion in the submucosa lamina propriae at  $1.6 \text{ mg L}^{-1}$ ; while at  $18 \text{ mg L}^{-1}$  and  $40 \text{ mg L}^{-1}$  showed diffuse infiltration of villi lamina propriae by monuclear cells as observed by Omitoyin *et al.*, [12] in *S. galilaeus* fingerlings exposed to *Tetrapleura tetraoptera*. The results of the present study revealed that gramoxone is toxic to fish and causes histopathological changes in fish organs. Therefore indiscriminate use of gramoxone by farmers should be discouraged particular in a area close to aquatic environment.

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