

## Toxic Effect of Ladosulfan-Pesticides on Biochemical Indices of *Clarias Gariepinus* (Burchell 1822) Juveniles

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### Abstract

*ladosulfan* is an insecticide extensively used in agriculture for pest control. The 96h LC<sub>50</sub> estimated from probeit analysis was 1.89mg/L. Sub lethal concentrations of 1/10th (0.19 mg/L), 1/5th (0.49mg/L) and 1/2th (0.95mg/L) of LC<sub>50</sub> were used to determine the effects of the *ladosulfan* -pesticide on biochemical parameters at day 1, 7, 14 and 21 exposure periods. Water quality test was determined on pH, temperature, dissolved oxygen, hardness and alkalinity. The results show that biochemical parameters viz; Total Protein, Alkaline Phosphatase ALP, Alanine Transferase ALT, Aspartate Amino Transferase (AST) concentrations increased significantly ( $P \leq 0.05$ ) in time and concentration dependent manner. The biochemical parameters concentration had higher values in treated samples than control. The total protein increased from (8.80±0.002b -9.20±0.103b) at 0.19 mg/L, (9.20±0.102a -9.70±0.103c) at 0.48 mg/L and (9.27±0.152a -9.80±0.103c) at 0.95 mg/L of *ladosulfan* when compared with the control (7.67±0.931c -9.06±0.763c); Alkaline Phosphatase increased from (60.00±0.002a -86.00±1.001b) at 0.19 mg/L, (62.00±1.002b -88.00±1.001c) at 0.48 mg/L and (65.00±1.001a -92.00±1.002d) at 0.95 mg/L of *ladosulfan* when compared with the control (40.00±1.002a -53.67±5.511a); Alanine Transferase also increased from (30.00±1.002a -40.00±1.001c) at 0.19 mg/L, (35.00±1.002a -54.00±1.001a) at 0.48 mg/L and (38.00±1.002b -89.00±1.001d) at 0.95 mg/L of *ladosulfan* when compared with the control (22.33±2.083c -27.33±2.882b); Aspartate Amino Transferase increased from (64.00±1.002b -87.00±1.001c) at 0.19 mg/L, (66.00±1.002c -88.00±2.001d) at 0.48 mg/L and (68.33±0.582c -90.00±1.001d) at 0.95 mg/L of *ladosulfan* when compared with the control (63.00±2.002b -68.00±1.001b); respectively. The results indicate that commercial formulation of *ladosulfan* is toxic to *C. gariepinus*, the insecticide should be prudently used in both terrestrial and aquatic eco-systems to avoid eco-toxicological hazards.

**Keywords:** *Clarias gariepinus*, Ladosulfan, Effects, Biochemical Parameters

### Introduction

Ladosulfan is abroad-spectrum insecticide which is one of the most frequently applied pesticides in agriculture for the control of a great variety of annual, biennial and perennial terrestrial aquatic weeds and woody shrubs [1]. Ladosulfan is used widely in the rural communities, highly toxic to aquatic animals, miscible with water at pH 4.5 for 1% solution, boils at 113°C, having vapor pressure 1.9. 10<sup>-3</sup> Pa at 25°C indicating increased loss to the atmosphere from treated surfaces [2-4]. It is washed from nearby farm lands and accidental discharges into aquatic systems and may contribute to long term eco-toxicological effects to non-target aquatic organisms [5]. Due to affordability, availability and solubility of *ladosulfan* herbicide, its utilization has increased in

recent years in Africa. It then became necessary to study the lethal toxicity and biochemical stress of the herbicide on local species like catfish which will help in formulating the strategies for safeguarding aquatic organisms.

Catfish (order siluriformes) are a very diverse group of bony fish. They are named for the barbells (whiskers) around their mouths like cats. They have scale less skin, fleshy ray less posterior fins and sharp defensive spines in the shoulder and dorsal fins. Catfishes are highly esteemed group of fishes, they have high growth rate, commands high market value as they form part of food chain, hardy in nature as they possesses air breathing organs that enables them tolerate difficult aquatic conditions. The present study thus aims at the determination of the biochemical response of *ladosulfan* on juveniles of *Clarias gariepinus*.

## Materials and Methods

450 specimens of *Clarias gariepinus* (Family: Clariidae) juveniles were bought from Sacem fish farm and transported to Applied Biology Special Laboratory Agbani, ESUT, Enugu State, for the investigation. The fish were acclimatized for fourteen days in plastic tanks.

During the acclimation period, juveniles were fed twice daily with Coupons feed (2mm) at 3% body weight. Feeding was terminated 48hrs before the experiment to empty their stomach and avoid pollution of the water with their faeces. The test was conducted using a semi-static bioassay in 40L glass aquaria (60x30x30cm). A range finding test was carried out prior to determine the concentrations of the test solution for the definitive test. This was determined by subjecting juveniles of *Clarias gariepinus* to different concentrations of ladosulfan. Six different ladosulfan concentrations (1.63, 1.73, 1.83, 2.00, 2.20, and 2.40) mg/L were selected for definitive exposure after a series of range findings. A set of 10 specimens of *C. gariepinus* juvenile were randomly exposed to each of the selected concentrations and set in triplicates. Simultaneously, another set of 10 fish specimens was maintained as control in triplicate under the same conditions but contained only tap water. The test solution was renewed on alternate days to maintain the concentration of the chemicals.

The experiment lasted for 4 days and involved 2 phases: Monitoring and recording of water quality parameters and also monitoring and recording the cumulative mortalities of the juveniles under different concentrations of ladosulfan. Mortalities and survival were determined at 24, 48, 72 and 96 hours of exposure period. Dead fishes were removed to avoid pollution. The median lethal concentration ( $LC_{50}$ ) value was determined following the probit analysis method described by Finney (1971) [27]. Sub lethal concentrations of  $1/10^{th}$  (0.19 mg/L),  $1/5^{th}$  (0.49mg/L) and  $1/2^{th}$  (0.95mg/L) of  $LC_{50}$  were used to determine the effects of the ladosulfan -pesticide on biochemical parameters at day 1, 7, 14 and 21 exposure periods in triplicates.

Biochemical assay serum was used throughout the biochemical assay to determine total Protein by folinphenol reaction method as described by Lowry et al., [25], Alanine Transferase (ALT) was determined following the methods described by Reitman and Frankel (1957), Alkaline Phosphatase (ALP) was determined using Continuous Assay Method as described by Gouri, et al., Aspartate Amino Transferase (AST). The determination of AST was done according to the method of calorimetric assay as described by Reitman and Frank using Randox kit [26].

The data obtained were statistically analyzed by statistical package SPSS (Version 17). The data were subjected to one-way Analysis of Variance (ANOVA) and Duncan's multiple range test to determine the significance difference at 5% probability level.

## Results

The water temperature ranged from 26.90 to 27.6°C during the experimentation. The pH of the water ranged from 7.00 to 7.29 which was slightly higher than neutral. Dissolved oxygen varied

from 6.00 to 6.08mgI-1. The conductivity value ranged from 10-20ppm during the experimental period. The  $LC_{50}$  values (with 95% confidence limits) of 1.891 mgI-1. The results of effects of ladosulfan on biochemical parameters are shown on Table 1. The total protein increased from (8.80±0.002b -9.20±0.103b) at 0.19 mg/L, (9.20±0.102a -9.70±0.103c) at 0.48 mg/L and (9.27±0.152a -9.80±0.103c) at 0.95 mg/L of ladosulfan when compared with the control (7.67±0.931c -9.06±0.763c); Alkaline Phosphatase increased from (60.00±0.002a -86.00±1.001b) at 0.19 mg/L, (62.00±1.002b -88.00±1.001c) at 0.48 mg/L and (65.00±1.001a -92.00±1.002d) at 0.95 mg/L of endosulfan when compared with the control (40.00±1.002a -53.67±5.511a); Alanine Transferase also increased from (30.00±1.002a -40.00±1.001c) at 0.19 mg/L, (35.00±1.002a -54.00±1.001a) at 0.48 mg/L and (38.00±1.002b -89.00±1.001d) at 0.95 mg/L of ladosulfan when compared with the control (22.33±2.083c -27.33±2.882b); Aspartate Amino Transferase increased from (64.00±1.002b -87.00±1.001c) at 0.19 mg/L, (66.00±1.002c -88.00±2.001d) at 0.48 mg/L and (68.33±0.582c -90.00±1.001d) at 0.95 mg/L of ladosulfan when compared with the control (63.00±2.002b -68.00±1.001b); respectively. There were concentration and duration dependent significant ( $P \leq 0.05$ ) increase in total protein, alkaline phosphatase, alanine transferase, aspartate amino transferase at day 1, 7, 14 and 21 at 0.19, 0.48 and 0.95mg/L of ladosulfan.

## Discussion

Alteration in the concentrations of the biochemical parameters is certain in this research. These alterations have detrimental effects on vital organs where the biochemical parameters perform physiological functions. Alanine transaminase, alkaline phosphatase, are indicators of liver function, creatinine activity portrays kidney health, bilirubin indicates free radical levels in the body of the fish, glucose concentration indicates pancreatic condition of the fish. All these parameters play vital role in metabolic activities of the fish. The results from this research therefore show that ladosulfan can affect many functional organs of fish and possibly affect other organisms that consume fish in the food chain. These reports are in agreement with the following reports; biochemical responses can be applied to determine water quality requirements and safe lethal concentrations for fish life. Physiological changes are the most sensitive indicators of potential toxic effects in fishes [6-9]. The observed physiological changes in the studied formulation of ladosulfan are consistent with previous reports on some herbicides and also pesticides like malathion, chlorpyrifos; profenofos, Nwamba and Ajima [15] *endosulfan* and *endosulfan*, Pragatheeswaram et al., [18], posited that such abnormal and altered physiological characteristics are considered to be the result of excessive elimination of skeletal minerals while Pandey et al., [14], shifted the secretion of mucus over the body and dispigmentation to the function of the endocrine gland under toxic stress resulting to aberrations in the number and area of mucus gland and chromatophores [10-20]. It is however important to note that physico-chemical parameters (temperature, pH, dissolved oxygen), size and age, type of species, water quality, concentration and formulation of test chemicals can affect the toxicity of chemicals to aquatic organisms [21-23].

## Conclusion

It was concluded from the present study that the commercial formulation of lodosulfan is toxic to *C. gariepinus*. Most of the physiological abnormalities were recorded mainly at the higher concentrations. The herbicide should be prudently used in both terrestrial and aquatic eco-systems to avoid eco-toxicological hazard. More studies related to toxicity in *C.gariepinus* exposed to

lodosulfan and its formulations are necessary to understand the oxidative stress and hematological effects on this fish [24-26].

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**Table 1:** Biochemical parameters of clarias gariepinus exposed to different concentraions of lodosulfan-pesticides

Parameter	Concentration (ug/l)	Exposure Duration (Days)			
		DAY 1	DAY7	DAY14	DAY21
AST	Control	63.00±2.002b	65.00±2.002b	67.67±3.051a	68.00±1.001b
	0.19	64.00±1.002b	80.00±1.002b	84.38±2.082b	87.00±1.001c
	0.48	66.00±1.002c	86.00±1.002d	87.00±2.643c	88.00±2.001d
	0.95	68.33±0.582c	88.33±0.582d	89.33±1.533c	90.00±1.001d
ALT	Control	22.33±2.083c	25.33±2.083a	26.33±3.051a	27.33±2.882b
	0.19	30.00±1.002a	38.00±1.002b	39.00±2.642b	40.00±1.001c
	0.48	35.00±1.002a	43.00±1.002b	45.33±3.213c	54.00±1.001a
	0.95	38.00±1.002b	68.00±1.002c	81.33±1.523d	89.00±1.001d
ALP	Control	40.00±1.002a	59.00±1.002a	60.00±2.002a	53.67±5.511a
	0.19	60.00±0.002a	60.00±0.002a	81.33±1.533b	86.00±1.001b
	0.48	62.00±1.002b	62.00±1.002b	83.00±3.003c	88.00±1.001c
	0.95	65.00±1.001a	68.00±1.001a	90.67±0.583d	92.00±1.002d
TOTAL PROTEIN	Control	7.67±0.931c	7.68±0.931c	7.70±1.132d	9.06±0.763c
	0.19	8.80±0.002b	8.88±0.002b	9.13±0.151c	9.20±0.103b
	0.48	9.20±0.102a	9.30±0.102a	9.63±0.251b	9.70±0.103c
	0.95	9.27±0.152a	9.47±0.152b	9.66±0.151b	9.80±0.103c

**Note:** Values with different alphabetic superscripts differ significantly ( $p < 0.05$ ) between exposure durations. Values with different numeric superscripts differ significantly ( $p < 0.05$ ) between concentrations within exposure duration.

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