THE EFFECTS OF IMIDACLOPRID EXPOSURE ON THE LIVER AND MUSCLE ALBUMIN OF CLARIAS GARIEPINUS

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DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

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FEBRUARY,2025.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN ANIMAL AND ENVIRONMENTAL BIOLOGY

FEBRUARY,2025.

CERTIFICATION

This is to certify that this project work was successfully carried out by **CERENA OGHENEDORO OGHORO LSC2002976** in the Department of Animal and Environmental Biology,Faculty of Life Sciences,University of Benin.

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PROF. M.O OMOIGBERALE DATE

Head of Department

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EXTERNAL SUPERVISOR DATE

DEDICATION

I dedicate this work to God Almighty for His endless blessings in my life.

ACKNOWLEDGEMENTS

I want to thank my parents Mr. Rex and [Barr.]Mrs Oghoro for their constant support in my life and all the encouragements. I also want to thank my project supervisor Dr. Opute for his guidance.

I also want to thank my siblings ,friends and Cece’s Hair for believing in me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This study was conducted to investigate the physico-chemical effect of imidacloprid (Imidacloprid 200G /L SL exposure on *Clariasgariepinus* juveniles.

Four (4) weeks old C. gariepinus juveniles werepurchased at the Department of Fisheries, Faculty of Agriculture, University of Benin. The experimental fishes were kept and used for twenty-eight (28) days in a static renewal bioassay. At the end of the 28 days exposure, juvenile samples were prepared for physico-chemical parameters using standard procedures. This study investigated the effects of imidacloprid on *Clariasgariepinus* and examined Albumin function in the liver and muscle. Albumin constitutes a major part of the proteins in the body. Functions of albumin includes distribution of extra cellular fluid, regulation of osmotic pressure ,acts as transport agent for hormones,lipids and vitamins. Increased levels are found in dehydration and decreased levels are found in liver disease, malnutrition and kidney disorders. Standard physicochemical analyses of the experimental water yielded the following parameters: temperature 28 °C, pH 5.74, electrical conductivity 32 µS/cm, total dissolved solids 16 mg/L, turbidity 2 NTU, dissolved oxygen 6.6 mg/L, biological oxygen demand 2.7 mg/L, hardness 18 mg/L, alkalinity 8 mg/L, and chloride 14.12 mg/L.

Fish were exposed to imidacloprid at three concentrations—5 µg/L (Concentration A), 10 µg/L (Concentration B), and 15 µg/L (Concentration C)—with an unexposed group serving as the control (Concentration D). These results suggest that imidacloprid exposure led to changes in albumin levels. At lower concentrations imidacloprid may affect albumin synthesis and at higher concentrations there was a significant increase in albumin levels. Increase in *Clariasgariepinus*, potentially serving as a protective mechanism against pesticide-induced oxidative damage. The research work shows the importance of antioxidant responses in aquatic environments on their exposure to neonicotinoid pesticides and teaches the management of contaminated aquatic systems

CHAPTER ONE

INTRODUCTION

Background to the study

Fishes are aquatic vertebrates with gills and limbs in the shape of fins(Peter Nelson 1994),

Fishes are poikilothermic ,aquatic chordates with appendages (when present)developed as fins ,whose chief respiratory organs are gills and whose body is usually covered with scales(Tim Berra 1981).

The African catfish ,Clarias gariepinus is a freshwater fish species in the family Claridae and belongs to the order Siluriformes (Nnatuanya *et al* .,2023).

Central and Western African countries began farming Clarias gariepinus in early 1970s(Temitope,2017).

The African sharptooth catfish ,Clarias gariepinus ,is in many senses a remarkable beast considering its phenomenal natural distribution ,Clarias gariepinus is an extremely hardy and adaptable animal efficiently able to exploit a variety of both animal and protein under diverse conditions. It is one of the most promising fish species for developing an aquaculture industry in Africa (Ali *et al* ., 2005).

There is a massive aggregation of catfish before spawning amd courtship is preceeded by aggressive encounters between males.

Mating takes places between isolated pairs in shallow water among indundated or semiterrestrial aquatic grasses and sedges.

There is no parental protection of the young as found in many other catfish families.

Egg and larval development is rapid and the larvae is capable of swimming strongly within 48hours of fertilization. The larvae are secretive and feed on small invertebrates which are abundant in the shallow inshore areas which they inhabit.

1.2 Justification of Study

The neonicotinoid imidacloprid is under revaluation by regulatory agencies because of the poor current information available regarding its potential effects.One of the goals was to determine imidacloprid uptake and distribution in the Clarias gariepinus fish.

The pollution of water by pesticides directly or indirectly can lead to the death of fish and an increase in undesired substances in healthy fishes.

This can negatively impact human health through consumption(Olatoyeb*et al* .,2021).

1.3 Aims and Objectives

The aim of this study was to determine the biochemical reaction of *Clarias gariepinus* after exposure to imidacloprid.

The specific objectives were to;

1. Evaluate the Albumin level in the muscle of *C.gariepinus* juveniles after exposure to imidacloprid.
2. Evaluate the Albumin level in the liver of *C.gariepinus* juveniles after exposure to imidacloprid.

CHAPTER 2

LITERATURE REVIEW

2.1 The role of Clarias gariepinus in the Ecosystem

The morphological and behavioural adaptations for feeding in Clarias gariepinus shows how it is well equipped to feed on a wide variety of food items.

An important aspect of their success as predators is their ability to switch from one prey to another.

They are need to dampen fluctuation in prey abundance and to restrict the distribution of prey under certain conditions.

2.2 The Effect of Water Pollution on Aquatic organisms

Imidacloprid is a systemic insecticide that belongs to the neoncotinoid class of chemicals that act on the central nervous system of insects .It is used to prevent and control sucking and chewing insect and as a result end up in aquatic environments via runoffs and discharges.Although its effects have been known to have serious implications ,they deserve more attention.

(Nosakhare *et al.,2023)*

2.2 The Effect of Water Pollution on Aquatic organisms

The neonicotinoids are one of the most widely used insecticides in the world .DNA damage is considered an early biological effect which could lead to reproductive and carcinogenic effects .These results show that short term exposures to environmentally relevant concentrations of IMI could affect the integrity of fishes through oxidative damage.[Castillo *et al* 1977]

To avoid the deleterious toxic impacts of IMI this pesticide use should be stopped

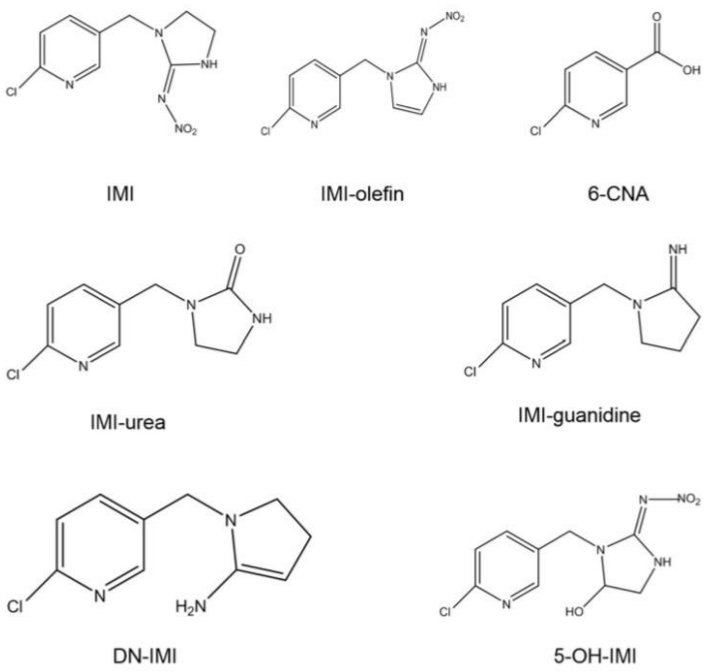

Figure 2.3.1 Chemical structures of IMI and its metabolites (Chen et al., 2023)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Test Chemicals

Liquid Imidacloprid ,Imiforce (manufactured by Jiangsu Noon Crop Science Co.LTD ,North of North Xuija fast track Xuzhou industrial park ,Jiangsu ,China) with Imidacloprid 200G/L SL as the active ingredient ,was purchased from the market place.

3.2 Experimental design and treatment

A batch of 400 eight week old Clarias gariepinus juveniles were purchased from Otaniyen farm,Ikpoba hill and taken care of at the Department of Animal and Environmental Biology . After being acclimatized fifteen juvenile fish were distributed to each tank and half filled with 15L of water, the fishes had an average weight of 5.29g and average standard length of 8.24cm. They were fed 2mm of commercial fish feed from Blue crown (Olam Group) twice a day at an eight hour interval and their water was changed every two days.

The fishes were exposed to three concentrations (5,10 and 15g/L) of Imidacloprid the tanks were labeled IMI1(A,B,C), IMI2(A,B,C), IMI3(A,B,C) respectively and control(0.0g/L) in a bioassay procedure for a period of twenty eight days /four weeks. At the end of the twenty eight day period the brain,muscle and tissues were harvested from all tanks including control and taken to the laboratory for analysis,

3.3 Extraction of tissue samples

At the end of the twenty eight days period fish specimens from the treatments and control tanks were dissected using a dissecting kit and then the brain, liver and muscle were deposited in an ice chest after collection. After which they were taken to the laboratory for analysis.

3.4 Biochemical analysis

For this analysis samples were collected from each of the tanks from treatment and control tanks alike at the end of the exposure period.

3.4.1 Albumin

Albumin which creates the colloid osmotic pressure blood plasma and is involved in lipid transport and other functions.

It is a functional protein that is highly beneficial for human health and vertebrates health especially in healing as it accelerates recovery .

Albumin the major serum protein binds a wide variety of lipophilic hormones and several xenobiotics that bind to receptors for steroids and other hormones.

It protects animals from endocrine inhibition caused by xenobiotics

CHAPTER FOUR

RESULT

Table 4.1: Physico-chemical parameters

PARAMETER	UNITS	BOREHOLE
Ph		5.74
Conductivity	µS/cm	32
TDS	mg/*l*	16
Turbidity	NTU	2
Dissolved Oxygen	mg/L	6.6
B.O.D	mg/L	2.7
Hardness	mg/L	18
Alkalinity	mg/L	8e
Chloride	mg/L	14.12
Temperature	^{o}C	28

Table 4.2: Result of Albumin

So/No	Name on Bottle Liver Sample	Albumin Result (g/l)	Muscle Sample	Albumin Results (g/l)
1	**IA1**	**13.97**	**IA1**	**4.65**
2	**IA2**	**5.10**	**IA2**	**6.92**
3	**IB1**	**4.84**	**IB1**	**7.10**
4	**IB2**	**11.0**	**IB3**	**5.6**
5	**IC1**	**3.9**	**IC1**	**5.12**
6	**IC3**	**3.1**	**IC2**	**4.07**
7	**D2**	**5.47**	**D1**	**3.29**
8	**D3**	**6.4**	**D3**	**4.13**

4.1Imidacloprid concentrations on exposure to prolactin (Table 4.1)

The mean Prolactin concentration was **2.63 ± 0.315 ng/ml**, with values ranging from **2.22 to 3.171 ng/ml**. The coefficient of variation (**CV% = 11.98%**) suggests a moderate level of variation in Prolactin levels across samples. The p-value (**p < 0.05**) indicates that the differences in Prolactin levels between groups are statistically significant, suggesting that the experimental conditions had an impact on Prolactin concentration.

The mean FSH concentration was **3.872 ± 0.152 mIU/ml**, with values ranging from **3.67 to 4.086 mIU/ml**. The coefficient of variation (**CV% = 3.93%**) is relatively low, indicating that FSH levels were more consistent across the samples. The p-value (**p < 0.05**) confirms that the observed differences in FSH levels are statistically significant, implying that the experimental treatment influenced FSH concentration.

Liver Albumin had a mean concentration of **6.723 ± 3.775 g/L**, with a wide range from **3.10 to 13.97 g/L**. The coefficient of variation (**CV% = 56.15%**) is quite high, indicating substantial variability in Liver Albumin levels among the samples. The significant p-value (**p < 0.05**) suggests that the differences observed are statistically meaningful, showing that the treatment had a significant effect on Liver Albumin concentration.

Muscle Albumin had a mean concentration of **5.110 ± 1.365 g/L**, with values ranging from **3.29**

to 7.10 g/L. The coefficient of variation (**CV% = 26.71%**) is moderate, showing some variability in Muscle Albumin levels across the samples. The p-value (**p < 0.05**) indicates that the differences

in Muscle Albumin levels are statistically significant, suggesting that the treatment influenced the protein content in muscle tissue.

Table 4.3: Imidacloprid concentrations on exposure to prolactin

Parameter	Mean +SD (Min-Max)	Cv%	P-value
Prolactin (ng/ml)	**2.63±0.315 (2.22-3.171)**	**11.98%**	**p<0.05**
FSH (mlu/ml)	**3.872±0.152 (3.67-4.086)**	**3.93%**	**p<0.05**
Liver Albumin (g/l)	**6.723±3.775 (3.10-13.97)**	**56.15%**	**p<0.05**
Muscle Albumin (g/l)	**5.110±1.365 (3.29-7.10)**	**26.71%**	**p<0.05**

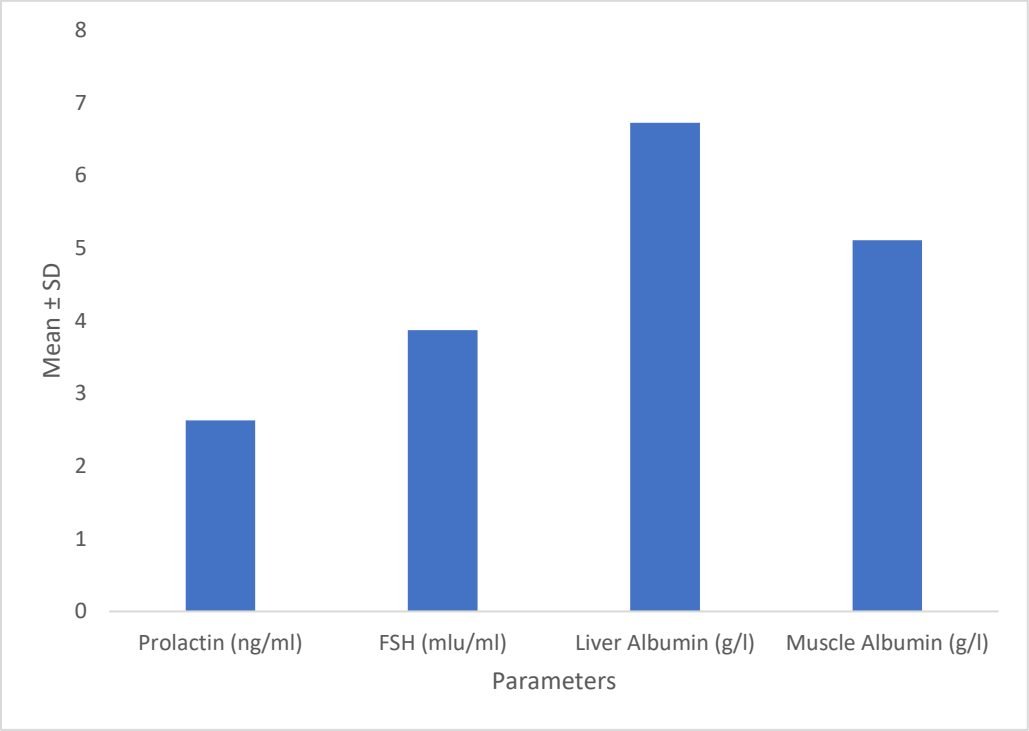

Fig 4.1: Effect of Imidacloprid on Prolactin, FSH, and Albumin Levels in C. gariepinus

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	**73.39686**	**3**	**24.46562**	**6.027083**	**0.002664**	**2.946685**
Within Groups	**113.6598**	**28**	**4.059278**			
Total	**187.0567**	**31**				

Since $p < 0.05$, there is a statistically significant difference among the four groups (Prolactin, FSH, Liver Albumin, and Muscle Albumin).

This suggests that at least one of the parameters differs significantly from the others.

4.2 Percentage change in albumin level (Table 4.3)

- Control Group: The albumin concentration in the control group was 5.935 g/dL, serving as the baseline for comparison.
- IA (Highest Concentration): The albumin level increased significantly to 9.535 g/dL, representing a 60.66% increase compared to the control. This suggests that imidacloprid at this concentration may stimulate albumin production, possibly as a physiological response to stress or toxicity.
- IB (Moderate Concentration): A rise in albumin levels was also observed in this group, reaching 7.92 g/dL, which corresponds to a 33.45% increase relative to the control. This indicates a dose-dependent response, though the increase is lower than that observed in IA.
- IC (Lowest Concentration): Unlike the previous groups, albumin levels dropped significantly to 3.50 g/dL, reflecting a 41.03% decrease. This suggests that at this exposure level, imidacloprid may be suppressing albumin synthesis,

possibly due to liver dysfunction or stress-related metabolic disruption.

Table 4.2 Percentage change in albumin level

Parameter	ALBUMIN	% CHANGE
CONTROL	5.935	-
IA	9.535	60.66
IB	7.92	33.45
IC	3.50	-41.03

4.3 Toxicity- induced albumin reduction factor (Table 4.3)

- Control Group: Albumin level was 5.935 g/dL, serving as the baseline for comparison. Since there was no imidacloprid exposure, TIARF is not applicable ("-").
- IA (Highest Concentration): The albumin level increased to 9.535 g/dL, and the TIARF value was 0.62. A TIARF value below 1 indicates that albumin levels rose rather than decreased, suggesting that at this high concentration, imidacloprid might be triggering an acute stress response leading to albumin overproduction.
- IB (Moderate Concentration): The albumin concentration was 7.92 g/dL, with a TIARF of 0.75. This is also below 1, meaning that although there was an increase in albumin, the effect was less pronounced compared to IA. This suggests that at this lower concentration, imidacloprid negatively affects albumin synthesis, possibly due to liver damage or metabolic suppression
- IC (Lowest Concentration): Albumin dropped to 3.50 g/dL, with a TIARF of 1.70. Since this value is greater than 1, it indicates a toxicity-induced reduction

Table 4.3 Toxicity- induced albumin reduction factor

Parameter	ALBUMIN	TIARF
CONTROL	5.935	-
IA	9.535	0.62
IB	7.92	0.75
IC	3.50	1.70


IC

4.4Liver-to-Muscle Albumin Ratio (LMAR) (Table 4.4)

In IA (highest imidacloprid concentration), the liver albumin was 9.535 g/dL, and muscle albumin was 5.785 g/dL, resulting in an LMAR of 1.65. This indicates that albumin synthesis and retention in the liver were elevated, likely as a response to high exposure levels. In IB (moderate concentration), liver albumin was 7.92 g/dL, and muscle albumin was 6.35 g/dL, with an LMAR of 1.25, showing a decline compared to IA but still reflecting a predominance of albumin in the liver. However, in IC (lowest concentration), liver albumin dropped to 3.50 g/dL, while muscle albumin was 4.6 g/dL, leading to an LMAR of 0.76, indicating a reversal where albumin levels in muscle exceeded those in the liver.

Parameter	LIVER/MUSCLE ALBUMIN	LMAR
IA	**9.535/5.785**	**1.65**
IB	**7.92/6.35**	**1.25**
IC	**3.50/4.6**	**0.76**

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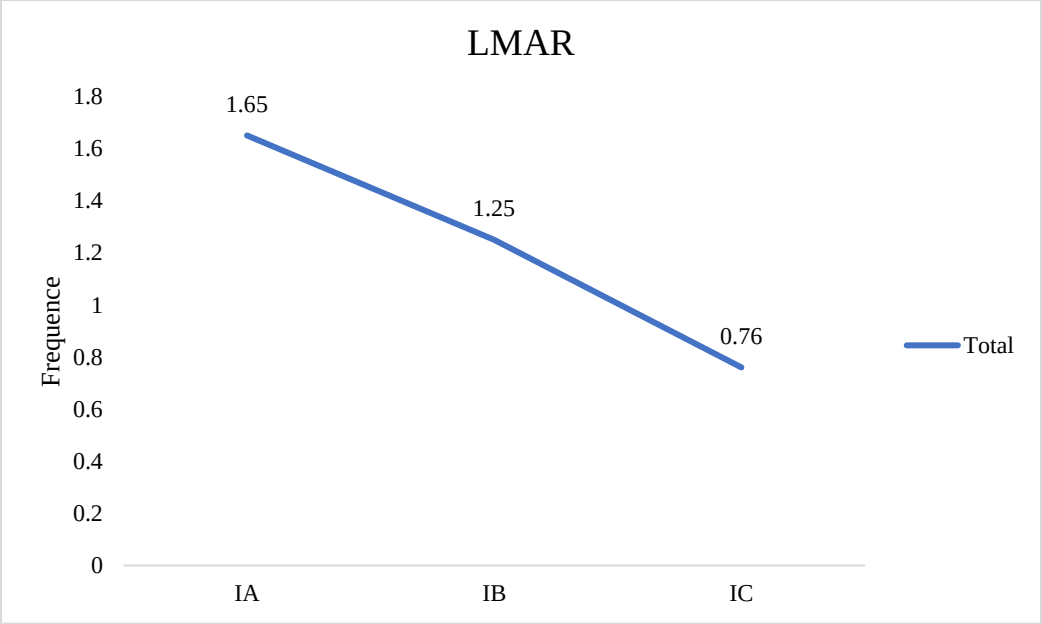
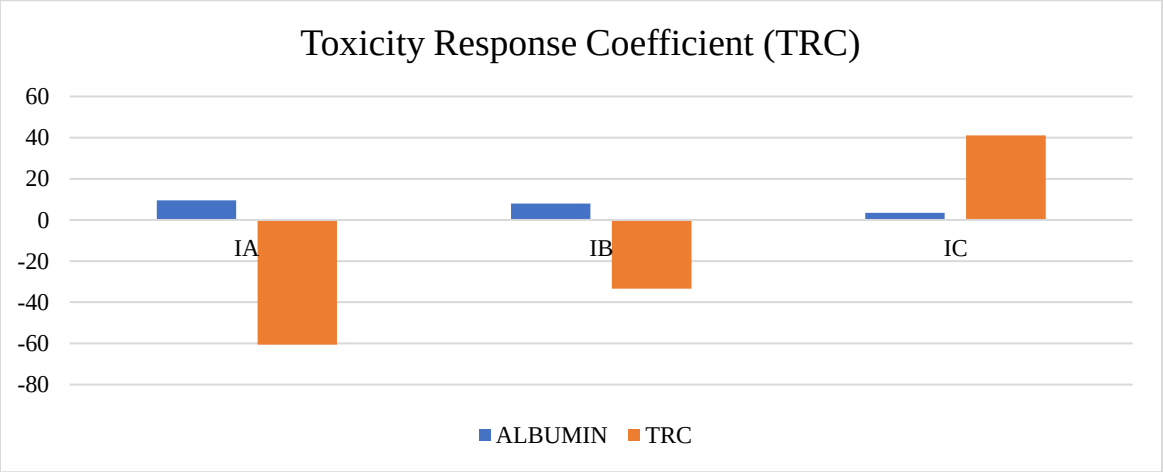


4.5 Different concentrations of Toxicants (TRC) (Table 4.5)

In the control group, the albumin level was **5.935 g/dL**, serving as the baseline with no TRC value assigned. In IA (highest imidacloprid concentration), albumin increased to **9.535 g/dL**, resulting in a TRC of **-60.66**, indicating a substantial rise in albumin levels, possibly as a compensatory response to stress or toxicity. IB (moderate concentration) also showed an increase in albumin to

7.92 g/dL, with a TRC of **-33.45**, suggesting a similar but less pronounced effect. However, in IC (lowest concentration), albumin dropped significantly to **3.50 g/dL**, with a TRC of **41.03**, indicating a toxicity-induced reduction.



Parameter	ALBUMIN	TRC

CONTROL	**5.935**	**-**
IA	**9.535**	**-60.66**
IB	**7.92**	**-33.45**
IC	**3.50**	**41.03**

Table 4.5: Different concentrations of Toxicants (TRC)

CHAPTER 5
DISCUSSION

Albumin which is synthesized in the liver constitutes a major part of the proteins in the body. Functions of albumin includes distribution of extra cellular fluid, regulation of osmotic pressure acts as transport agent for hormones,lipids and vitamins. Increased levels are found in dehydration and decreased levels are found in liver disease,malnutrition and kidney disorders. The results of this study demonstrate the effects of imidacloprid on liver albumin, and muscle albumin levels. The data revealed significant differences in these parameters among the treatment groups.

5.1 Effects of Imidacloprid on Liver Albumin and Muscle Albumin

Liver albumin had a mean concentration of 6.723 ± 3.775 g/L, with a high coefficient of variation (CV% = 56.15%) indicating substantial variability among samples. The significant p-value ($p < 0.05$) suggests that the differences observed are statistically meaningful.

Muscle albumin had a mean concentration of 5.110 ± 1.365 g/L, with a moderate coefficient of variation (CV% = 26.71%). The significant p-value ($p < 0.05$) indicates that the differences in muscle albumin levels are statistically significant.

5.2 Biochemical assessments

5.2.1 Percentage Change in Albumin Level

The results show that imidacloprid exposure led to significant changes in albumin levels. The control group had an albumin concentration of 5.935 g/dL. In contrast, the IA group (highest

concentration) showed a significant increase in albumin levels to 9.535 g/dL, representing a 60.66% increase.

The IB group (moderate concentration) also showed an increase in albumin levels to 7.92 g/dL, corresponding to a 33.45% increase. However, the IC group (lowest concentration) showed a significant decrease in albumin levels to 3.50 g/dL, reflecting a 41.03% decrease.

5.2.2 Toxicity-Induced Albumin Reduction Factor (TIARF)

The TIARF values indicate the extent of albumin reduction due to imidacloprid toxicity. The control group had a TIARF value of "-", indicating no exposure to imidacloprid.

The IA group had a TIARF value of 0.62, indicating that albumin levels increased rather than decreased. The IB group had a TIARF value of 0.75, also indicating an increase in albumin levels.

In contrast, the IC group had a TIARF value of 1.70, indicating a toxicity-induced reduction in albumin levels. This suggests that at lower concentrations, imidacloprid may negatively affect albumin synthesis, possibly due to liver damage or metabolic suppression.

5.3 Recommendations

Wastes from imidacloprid should be disposed properly or dropped off in a landfill approved for pesticide disposal or according to the Federal Governments rules.

5.4 Conclusion

Studies have shown that exposure to imidacloprid can Imidacloprid can impact the physiological processes in *Clariasgariepinus*. it may affect their respiratory function, leading to decreased oxygen, bioaccumulation of imidacloprid in the tissues of *Clariasgariepinus*. This means that if these fish are consumed by predators or humans, the chemical could accumulate in higher concentrations, posing health risks. Also the runoff of imidacloprid into aquatic ecosystems can lead to harmful impacts, affecting*Clariasgariepinus* and other aquatic organisms thereby diminishing the ecosystem.In conclusion, imidacloprid poses significant risks to *Clariasgariepinus*, affecting their behavior, physiology, and survival. Understanding these impacts is needed for the future of aquatic ecosystems and practicing safe use of pesticides in agriculture.

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