ACUTE TOXICITY OF CYPERMETHRIN EXPOSURE ON THYROID HORMONE LEVELS IN JUVENILE *Clarias gariepinus* (Burchell, 1822)

BY

Thalia Rukevwe KUTE (Miss)
LSC1906359

DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENIN CITY

APRIL, 2024.

CERTIFICATION

This is to certify that this project work was successfully carried out by **Thalia Rukevwe KUTE (Miss)** in the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin.

______________________________ ______________________________

DR. P. A. OPUTE DATE
Project Supervisor

______________________________ ______________________________

PROF. M.O. OMOIGBERALE DATE
Head of Department

DEDICATION

This work is dedicated to God Almighty and to my loving parents and siblings for their unwavering love and support.

ACKNOWLEDGEMENTS

I owe the success of this work to the invaluable contributions and collaborative efforts of numerous individuals. I extend my heartfelt appreciation; Firstly, to my project supervisor, Dr. Prosper A. Opute, who has worn many hats and provided insightful comments to ensure that I and my colleagues turn in a very excellent report. My deepest thanks go to my beautiful parents, Mr. and Mrs. Kute, who have lovingly provided for me all the resources needed for this study. I appreciate my siblings, Kute Tovia and Kute David for their words of assistance and encouragement to keep going.

To my project mates and research colleagues; Abhulimen Donatus, Aimufua Vera, Obiuwevbi Sarah and Omeni Genevieve, thank you for your cooperation and assistance during the course of this study. To all my course mates and friends who were on this tasking journey with me and still acted as supporters, educators, and occasionally gist partners, your boundless enthusiasm, patience, invaluable information, and unrelenting flow of ideas have been instrumental in my progress. Without the unwavering support and guidance, I have received, this comprehensive report would not have been as attainable. I am truly fortunate to have such exceptional people in my life.

I express profound gratitude to Professor M.O. Omoigberale, the Head of Department, for his concern and contributions towards the seamless organization of this project. Once again, my heartfelt thanks to each of you for your pivotal roles in bringing this study to fruition.

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ABSTRACT

This study was conducted to investigate the dose relationship between thyroid hormone parameters in exposed juveniles of *Clarias gariepinus* and Cypermethrin using Thyroid function tests. Measuring the levels of Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH) are not only critical for diagnosing thyroid problems, but also in differentiating between a primary and secondary cause of a thyroid condition, for instance, an exposure to cypermethrin leading to hypothyroidism. The experimental fishes were exposed to 0, 15, 30, and 45µg/l of Cypermethrin fo96 hours in an acute toxicity experiment. At the end of the 96 hours, muscle tissue samples were harvested and prepared for biochemical analyses using standard procedures. Findings from this study confirm that cypermethrin is an Endocrine Disrupting Chemical (EDC) in fish and alters the thyroid hormone levels in *C. gariepinus* by targeting the hypothalamus-pituitary-thyroid axis. Furthermore, it provokes the inhibition of thyroid function in the fish so that the activity levels of T3 and T4 reduced significantly ($p < 0.05$). The levels of TSH increased in a dose-dependent trend, CYP 3 (0.85±0.05) > CYP 2 (0.81±0.01) > CYP 1 (0.75±0.05). A disruption in the normal thyroid function of the fish could impede the regulation of liver glycogen, protein metabolism, cell respiration, antioxidant system and the excitability of neurons and muscles, ultimately leading to deformities in juvenile fish and eventual death.

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INTRODUCTION

1.1. Background to the study

The African Catfish (*Clarias gariepinus*) is a freshwater fish indigenous to the inland waters of Africa and Asia but has been widely distributed to other parts of the world (Pharvez, 2022). It is a choice food fish species and has been cultured both intensively and extensively in African countries (Temitope, 2017) due to its beneficial characteristics, which includes high growth rate reaching market size of 1 kg in 5–6 months under intensive management conditions, adaptability and resistant to handling stress, reliable mass supply of fingerlings and it commands a very high commercial value with good meat quality making it highly cherished as food in Nigerian homes and hotels (Olaleye *et al.,* 2005, Eyo *et al*., 2015)

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A number of problems confront the production of catfish species in Nigeria; prominent among these are: poor management skills, inadequate supply of good quality seed, lack of capital, high cost of feed, faulty data collection, and a lack of environmental impact considerations such as the introduction of pollutants like pesticides into the water bodies (Adewumi, 2011). The increasing use of pesticides in aquaculture is raising concerns about the risks of environmental toxicity (Chang, 2018). Pesticides are initially applied in the control of crop pests but eventually end up in the environment as toxic pollutants of soil, surface water, and groundwater (Anton et al., 2014). Pesticides tend to enter surface waters through run-off, wastewater discharges, atmospheric deposition and spills (Cessna, 2009) adversely affecting the water quality of polluted waters. Pesticides like Cypermethrin, $C_{22}H_{19}Cl_{2}NO_{3}$, a Type II pyrethroid and an active compound of many insecticides (Biswas et al., 2019) commonly used against agricultural and domestic pests (Shaikh et al., 2020) is banned in most countries because it causes high toxicity and bioaccumulation in non-target organisms (Sabra and Mehana, 2015). Since the 1970s, pyrethroid pesticides have been applied to agriculture and aquaculture to replace traditional pesticides (James *et al*., 2017). However, pyrethroids are approximately 1000 times more toxic to fish than other organisms (Changwon *et al.,* 2020).

Aquatic species are typically exposed to pesticides in one of three ways; through their skin—because aquatic species are in direct contact with water, they quickly absorb pesticides through their skin pores while eating and breathing; through breathing—because they breathe through their gills, and lastly through eating food polluted with pesticides resulting in secondary poisoning. For instance, if a fish eats a prey that has been exposed to pesticides, the fish can die if the prey takes in a lot of the toxin (Kumar et al., 2021). Depending on the exposure duration, and exposure type, which can be lethal or sub-lethal, the toxic impacts brought on by exposure to these hazardous chemicals differs. Short-term exposure is defined as lasting less than 96 hours, whereas long-term exposure is defined as lasting longer than 96 hours (Kumar et al., 2021).

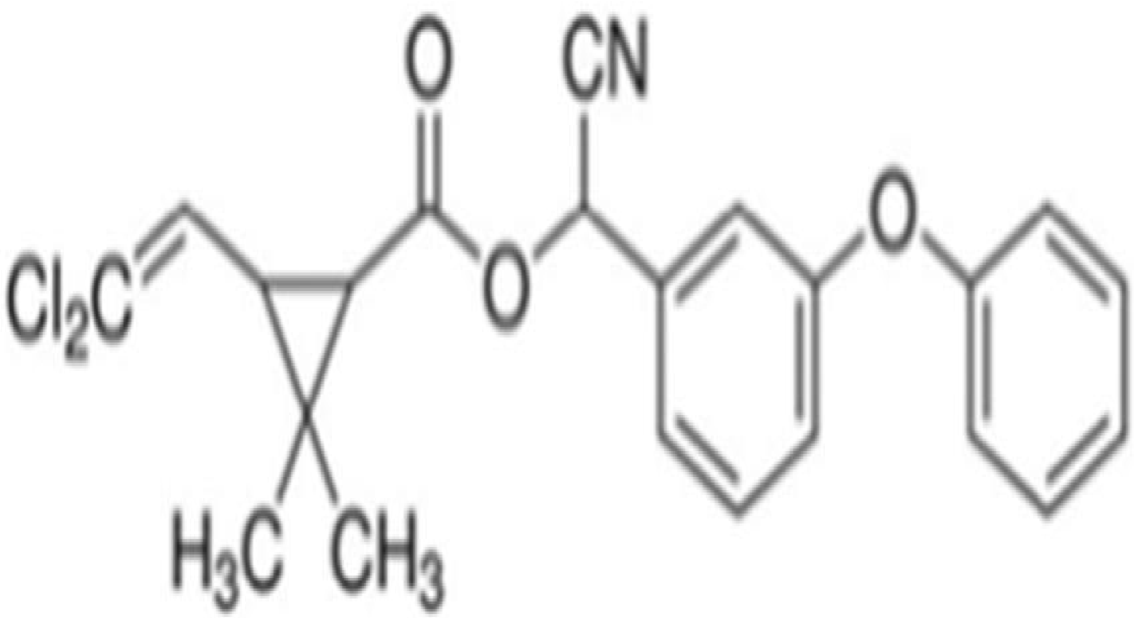

Figure 1: Chemical structure of Cypermethrin (Makwinja, 2020)

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Cypermethrin [Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate] has been reportedly found in fish tissues, soil sediments, and river water samples (Jabeen et al., 2015; Mahboob et al., 2015). Fish are highly susceptible to the toxicity of pesticide chemicals, for instance, many chemicals found in the environment affects the thyroid system of fishes (Singh, 2014). Thyroid hormones are generally involved in many physiological processes, including growth, development, behavior, and stress (Yasaman, 2023). The thyroid hormones in the gill and liver of fish play an important role in the control of osmoregulation, metabolism, and growth performance. The production of thyroid hormones in fish and other vertebrates is under the control of the hypothalamic-pituitary–thyroid (HPT) axis (Tousson *et al.,* 2011; Duarte-Guterman *et al.,* 2014; Kang *et al.,* 2020). Thyroid Hormones consist of two forms, thyroxine (or tetraiodothyronine, T4) and the biologically active triiodothyronine (T3). Thyrotropin (TSH) is the prime stimulatory hormone for the thyroid gland/follicle, from thyrotropes of the anterior pituitary. It modulates the release of T3 and T4 from thyroid follicular cells. Around 80% of the thyroid hormone is released as T4. T4 is de-iodinated to T3, which is a more potent thyroid hormone—T4 is the predominant circulating form, while T3 is more biologically active (Yasaman, 2023).Thyroid Hormones are crucial for normal brain development as well as metabolism and heartbeat rate, bone and central nervous system (CNS) maturation; any disruptions of TH levels in the blood stream and within specific tissues can potentially result in altered brain structure and behavior. The significant increase in thyroid hormone levels in fish samples following exposure to cypermethrin demonstrates this potential effects (Michelle *et al*., 2019).

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1.2. Justification of Study

Cypermethrin is a widely used synthetic insecticide with potential ecological implications, including adverse effects on aquatic organisms. Even at very low concentrations, it has been known to be hazardous to a variety of fish and aquatic invertebrates (Prusty *et al*., 2015). Among these organisms, fish species such as Clarias gariepinus (African catfish) are particularly vulnerable due to their direct exposure to contaminated water bodies and they suffer negative impacts from this exposure (Kumar *et al*., 2017).

There has been a critical concern on the number of pesticides with the potential to interfere with the developing nervous system and brain, notably with thyroid hormones which plays a significant role in the metamorphosis, brain development, and metabolism in fishes and other aquatic animals. For example, Cypermethrin exposure has negative impacts on the thyroid hormones of *Clarias gariepinus* fish which plays an important role in the regulation of various physiological processes during development, including growth, metabolism, and reproduction (Michelle *et al*., 2019). Therefore, this study is aimed at providing insight into the dose relationship between cypermethrin exposure and thyroid hormone parameters, measuring the levels of T3, T4, and TSH in the tissues of the exposed juveniles to diagnose the thyroid problems following the exposure.

This research will aid in evaluating the susceptibility of *C. gariepinus*, and provide valuable insights for regulatory decisions concerning cypermethrin usage and environmental preservation.

1.3 Aims and Objectives

The aim of the study is to evaluate the dose relationship between thyroid hormone parameters in the exposed juveniles of *Clarias gariepinus* and Cypermethrin using Thyroid function tests. The specific objectives are to:

1. Evaluate the response of thyroxine hormone (FT4) to Cypermethrin exposure in the juveniles of *Clarias gariepinus*. .
2. Determine the acute effects of Cypermethrin exposure on tri-iodo-thyronine hormone (FT3) in *C. gariepinus* juveniles.
3. Assess the acute toxicity effect of Cypermethrin on the thyrotropin hormone (TSH) in juveniles of *C. gariepinus.*

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CHAPTER TWO

LITEREATURE REVIEW

2.1 *Clarias gariepinus* as a vertebrate model test species

Fish is one of the most important sources of animal protein and has been widely accepted as a good protein source and other essential nutrients necessary for the maintenance of a healthy body (Fagbenro *et al.,* 2005; Akinneye *et al.,* 2007). It is also the cheapest source of animal protein consumed by the average Nigerian, accounting for about 40% of the total protein intake (Atanda, 2007). The fish *Clarias gariepinus* is a dominant freshwater fish belonging to the Clariidae family, and it is often referred to as the African catfish or sharptooth catfish because of its fine, pointed bands of teeth (Skelton, 1993).

In recent years, aquaculture of the African catfish *Clarias gariepinus* has fast gained global attention in Africa, Asia and Europe. In Africa, especially Nigeria as it is one of the most cultured and widely accepted species, owing to its rapid growth rate, ability to thrive under high density culture, enormous production and its unique taste, flavor and texture (Alfa *et al.,* 2014). The fish also has excellent marketability profile (Ajah, 2022). Fish has reportedly been known to be one of the most studied aquatic animals in the aquatic environment, and *Clarias gariepinus* has played a fundamental role as test species for scientific studies due to its resilience when compared to other fish species (Echessa, 2018).

Nigeria is the highest producer of this clariid catfish in the world, about 90% of farmed fish in the country is *Clarias gariepinus* (Adeleke *et al.,* 2007). One can easily identify the *C. gariepinus* using key characteristics such as smooth (non-scaly) and flexible body, terminal

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mouth, four pairs of barbels, dark black colouration at dorsal surface and pale white belly (Singh, 2015).

Plate 1: The African catfish, *Clarias gariepinus.*

When caught from the wild, like ocean, rivers and lakes, fish have been shown to be highly contaminated by heavy metals and other unsafe chemicals (Ndife, 2019). The levels of contamination depend on the environmental pollution from industrial wastes, pesticides and inorganic feedstocks (Olujimi *et al.,* 2018). This increases the need for ecotoxicology research as the relationship between land-use practices, and its contamination of fish species poses a significant threat for the health of humans and the environment. The culture and maintenance of *C. gariepinus* in the laboratory is cost effective and requires minimum expertise and physical infrastructure (Brink *et al.,* 2012). Before commencement of any sort of experiment, fish require acclimation which is done by stocking the fish in a plastic aquarium for a few days with regular feeding and renewal of water (Popoola *et al.,* 2018).

2.2 Description of Pesticides and Exposure of Aquatic Wildlife to Cypermethrin.

Pesticides are chemicals used in agriculture and aquaculture to kill or control harmful organisms that pose a threat to health or cause economic loss (Chen *et al.,* 2013). Pesticides have become an indispensable tool in large-scale agricultural development in Nigeria (Opute and Oboh, 2021). Excessive use of pesticides in agricultural areas causes the introduction of pesticides to aquatic environments. Many pesticides are developed to be stable in a harsh environment or body; therefore, they accumulate in suspended material, sediment, or aquatic organisms (Hu *et al.,*

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2010). Runoff, leaches, and spray drifts are major routes of unintended contamination of pesticides in aquatic environments.

Catfish are very suitable for studying biomagnification of environmental pollutants and toxicity from multiple exposures because they feed on plankton and have direct contact with sediments in rivers and lakes (Guiloski *et al.,* 2013). Most pesticides are designed in such a way as to disturb the physiological activities of the target organism, leading to dysfunction and reduced vitality (Akan *et al.,* 2015). Exposure to pesticides of non-target organisms causes various diseases associated with reproductive and developmental toxicity caused by endocrine disruption (Eskenazi *et al.,* 1999, 2008). Increasing use of pesticides in aquaculture is raising concerns about the risks of environmental toxicity (Chang, 2018). Chronic environmental threats caused by persistence of organochlorine and organophosphate pesticides in the ecosystem, resulted in the development of new synthetic pesticides (Yang, 2020). Introduced as a new class of pesticides in the 1970s, the use of pyrethroid pesticides has been increasing gradually with the decrease in the use of organophosphate and organochlorine pesticides (James *et al.,* 2017). Synthetic pyrethroids are currently the most promising and effective family of pesticides (Yavuz *et al.,* 2015). Due to their wide acceptability and because most of these pyrethroids end up in the aquatic ecosystem with potential negative effects on resident non-target species, high concentrations of these pyrethroids have been reported in several environmental matrices, such as high concentrations found present in the water, sediments and fish from water bodies across Nigeria, eg., in River Benue (Akan *et al.,* 2015). Despite these reports, there is limited information on the possible reproductive health and physiological effects in model tropical ecotoxicology species with significant economic and ecological relevance such as the African sharptooth catfish (*Clarias gariepinus)* (Hermandez-Moreno *et al.,* 2010).

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Pyrethroids are based on a natural compound called pyrethrin extracted from the plants of Chrysanthemum (Ensley, 2018). The sensitivity of fish to pyrethroids is thought to be due to the slow metabolism of these compounds (Glickman and Lech, 1982). The pyrethroids, owing to their properties, are easily absorbed, even at low concentrations, via the gills of fish (Datta and Kaviraj, 2003). Pyrethroids in fish are believed to act on different endpoints in various organs, including the brain, liver, muscles, kidneys, and gonads (Yang, 2020).

Cypermethrin is a cyanophenoxybenzyl pyrethroid and is classified as a restricted use pesticide by the EPA owing to high toxicity in fish (Saha and Kaviraj, 2009). It is a Type II pyrethroid pesticide used for the control of pests in cotton and soybean cultivation (Carriquiriborde *et al.*, 2007). Some studies have revealed that exposure to cypermethrin can alter thyroid gland function; and a disproportion of thyroid hormones can lead to a variety of disorders (Bretveld *et al.,* 2006).

2.3 Thyroid Hormones Function

The production of thyroid hormones in fish and other vertebrates is under the control of the hypothalamic-pituitary–thyroid (HPT) axis (Tousson *et al.,* 2011; Duarte-Guterman *et al.,* 2014; Kang *et al.,* 2020). The thyrotropin-releasing factor [thyrotropin-releasing hormone (TRH)] stimulates the pituitary to release the thyrotropic hormone (TSH), which in turn, promotes the synthesis and release of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by thyroid follicles (Yasaman, 2023). Of the two, T3 is the more biologically active thyroid hormone owing to its affinity for the nuclear thyroid hormone receptor (Carr and Patiño, 2011). The HPT axis acts parallel to the hypothalamic-pituitary–gonadal (HPG) axis, which involves a large number of hormones, including the gonadotropin-releasing hormone (GnRH) that promotes the secretion of gonadotropin hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH)

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(Schulz *et al.,* 2010; Pankhurst, 2016) which are crucial for testis development and spermatogenesis in fish (Schulz *et al.,* 2010).

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2.4 Effects of Pesticides on Thyroid Function

Thyroid disease is a global health issue that has significant adverse effects on well-being. Hypothyroidism and Hyperthyroidism are examples of such thyroid diseases, the pathology of which is thyroid hormone deficiency (Khandelwal, 2012). Thyroid stimulating hormone (TSH) and free thyroxine (FT4) is used to define thyroid dysfunction (Khandelwal, 2012). Elevated levels of TSH and simultaneously low TH indicate Hypothyroidism, whereas suppressed TSH levels and high TH suggests a Hyperthyroid condition (Koulouri, 2013).

Endocrine Disrupting Chemicals (EDCs) are chemicals with the ability to disrupt the endocrine system, including the function of the thyroid (Wachiranun, 2023). Concern has been raised that some pesticides such as Pyrethroids act as endocrine disrupting chemicals (EDCs) with the potential to interfere with the hormone systems of non-target invertebrates and vertebrates (Coudeg *et al.,* 2019). EDCs act at low doses and particularly vulnerable periods of exposure including pre- and perinatal development (Burggren *et al.,* 2015*,* Fudvoye *et al.,* 2014*)*; of critical concern is the number of pesticides with the potential to interfere with the developing nervous system and brain, notably with thyroid hormone signaling (Leemans *et al.,* 2019).

EDCs may interfere with thyroid function through several mechanisms, including disruption at the hypothalamus–pituitary–thyroid axis, interference with TSH receptors, production and distribution of THs, inhibition of sodium-iodine symporters, inhibition of the thyroid peroxidase enzyme, alteration of the binding sites of transport proteins, inhibition of deiodinase enzymes, decreased cellular uptake of thyroid hormone, and alteration during the transcription of thyroid hormone receptors (Crofton *et al*., 2008, Hauser *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Test Chemical

The test chemical used was the commercial formulation of cypermethrin, Cyperforce from Jubaili Agrotech Nigeria, containing cypermethrin (10% EC) which consists of synthetic pyrethroid used as an insecticide in agricultural applications as well as in consumer products for domestic purposes. It was purchased from Lagos Street, Ring Road, Benin City, Edo State, Nigeria. Cypeforce is used to control a wide range of insects including worms, flies, borers (Koein, 2022).

3.2 Test Water

The test water used was dechlorinated tap water. Dechlorinated water was used for the control and the experimental tanks, and was collected and sent to the laboratory for analysis. The test water was analyzed for pH, Electrical conductivity, Total Dissolved Solids, Dissolved oxygen, Temperature, Hardness, and Alkalinity.

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3.3 Experimental Procedure

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Eight hundred (800) *Clarias gariepinus* juveniles were bought from the Department of Fisheries, Faculty of Agriculture, University of Benin, Benin City, Edo state, Nigeria and transported to Animal house, Department of Animal and Environmental Biology, University of Benin.

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The juveniles were acclimatized in a big holding tank for a period of seven days to allow the fish to gradually adapt, enhancing their chances of survival in altered conditions. The water was renewed every two (2) days to remove debris and possible contaminants. The quality of the test water was carefully monitored and maintained within optimal levels throughout the experiment. This ensures that any observed effects on the fish are not due to changes in water quality parameters. They were fed 5g (2mm) of commercial fish feed (Blue crown, Olam Group, Kwara, Nigeria) twice a day at eight hours intervals.

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3.4 Morphometry

The length and weight of the fishes were measured using a metre rule and a digital scale respectively. The standard length, total length and wet weight of the fishes were measured prior to the exposure and after exposure to the pesticides. Before exposure, the juveniles had an average length of 10.6 ± 0.66 cm and an average weight of 11.14 ± 1.97 g.

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Plate 1: Measurement of standard and total length of juvenile *Clarias gariepinus* with a metre rule

Plate 2: Weighing of juvenile *Clarias gariepinus* using a scale

3.5 Exposure to Treatment Chemicals

The experiment consisted of three sublethal treatments, 15µg/l, 30 µg/l, 45µg/l, and the control 0µg/l. The chemical was measured using a micropipette into different triplicate 60L tanks containing 15 litres of dechlorinated tap water. After acclimatization, twenty juvenile fish were randomly allocated to each treatment and control tank. The tanks were labelled CYP1A, CYP1B, CYP1C, CYP2A, CYP2B, CYP2C, CYP3A, CYP3B, CYP3C and C1, C2, C3 representing the different treatments and controls in triplicates. Live fish were introduced into the experimental tanks and exposure and the acute study lasted for 96 hours. This exposure allows for comparison and assessment of the specific effects of cypermethrin exposure on thyroid function. The fish were closely monitored throughout the experiment for any behavioural changes, mortality rates, and physical abnormalities.

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Plate 3: Set-up of experimental tanks for acute toxicity exposure

3.6 Extraction of Tissue Samples

At the end of the 96 hours exposure period, the fish specimens from control and treatment tanks were dissected carefully using a dissecting kit and the liver and muscle tissues were carefully harvested from each fish. The tissue samples were placed in different sample EDTA bottles, well labelled and then preserved in an ice chest under sterile conditions to prevent contamination before being transported to the laboratory for analysis.

3.7 Biochemical Analysis

For the purpose of biochemical analysis, samples were collected per treatment and control group from the fish specimens at the conclusion of the exposure duration (96 hours). The ELISA (enzyme-linked immunosorbent assay) was used to measure the concentration levels of Thyroxine (T4), Triiodothyronine (T3), and Thyroid Stimulating Hormone (TSH) in samples.

3.7.1 Thyroxine (T4)

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Thyroxine (T4) is a hormone produced by the thyroid gland that plays a crucial role in regulating metabolism. By quantifying T4 levels, this assay indirectly assesses thyroid function and can aid in diagnosing thyroid disorders like hyperthyroidism (overactive thyroid) or hypothyroidism (underactive thyroid). (Stockigt J., 2003). Using the ELISA assay procedure for analyzing serum samples, the T4 levels in the samples were measured.

3.7.2 Triiodothyronine (T3)

A T3 thyroid function test measures the level of triiodothyronine (T3) in the blood. T3 is a hormone produced by the thyroid gland that plays a vital role in regulating metabolism. An abnormal T3 level can indicate an underlying thyroid disorder, such as hyperthyroidism (overactive thyroid) or hypothyroidism (underactive thyroid). Using the ELISA assay procedure for analyzing serum samples, the T3 levels in the samples were measured.

3.7.3 Thyroid Stimulating Hormone (TSH)

Thyroid Stimulating Hormone (TSH) is a hormone produced by the pituitary gland that regulates the function of the thyroid gland. Increased TSH is an early and sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism.

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Figure 1: TSH ELISA Test kit

Using the ELISA assay procedure for analyzing serum samples to quantify TSH (Thyroid Stimulating Hormone) levels in the samples, all reagents and controls were equilibrated to room temperature (20-25°C) and mixed gently. The following steps were involved in performing the assay procedure: (1) Equilibrate reagents and mix gently. (2) Add strips and pipette standards, controls, and samples. (3) Add conjugate and shake.- (4) Incubate plate for 60 minutes at room temperature. (5) Wash wells four times and blot dry. (6) Add substrate and incubate for 15 minutes (no shaking). (7) Add stop solution, mix, and read absorbance at 450 nm within 15 minutes.

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CHAPTER FOUR

RESULTS

4.1 Physico-chemical Parameters of Test Water

The physico-chemical properties of water were examined during the experiment using standard techniques. The recorded parameters of the test water include temperature, pH, Electrical Conductivity, Dissolved Oxygen (DO), Alkalinity, Hardness and Total Dissolved Solids. The water parameters did not significantly vary from recommended limits. The test water had a temperature of 27 °C, pH of 6.46, electrical conductivity of 42µS/cm , alkalinity of 10mg/l, hardness of 34mg/l, Dissolved oxygen of 3.7mg/l and Total Dissolved Solids of 21mg/l, respectively.

4.2 Toxicity of cypermethrin

The lethal concentration (LC50) value of cypermethrin (10% EC) based on analysis was found to be 30µg/l for 96h. The sub-lethal doses were 15, 30, and 45µg/l of cypermethrin which were denoted as CYP 1, CYP 2, and CYP 3 respecctively. Each of the concentrations and control group were maintained in triplicates in order to minimize error. No adverse behavioral changes or any mortality was recorded in the control group throughout the period of the experiment. The behaviour of the control fishes and their colour were normal. Observable changes in fish behaviour with cypermethrin exposure include lack of balance, agitated or erratic swimming, air gulping, restlessness, sudden quick movement, rolling movement and swimming on the back were observed. The colour of the skin of *C. gariepinus* changed from normal dark pigmentation in the dorsal and lateral parts to very light pigmentation in the dorsal and lateral part (S.O. Ayoola, 2008).

4.3 Biochemical analysis

Changes in thyroid hormone levels were assayed by measuring the levels of FT4, FT3, TSH in control and cypermethrin exposed juvenile *C. gariepinus* showing significant observable changes and trends for T4, T3, TSH. The findings of this study showed a declining trend of both T4 and T3 levels of cypermethrin exposed fish in contrast to the control group. A different trend was observed for TSH levels, where there was a significant elevation amongst the different samples in contrast to the control group.

4.3.1 Effect of cypermethrin on T4 level

The effect of cypermethrin on T4 levels in *C. gariepinus* exposed for 96 hours is represented in Figure 4.1. There was no significant difference observed in the T4 levels here. However, the CYP 2 group had a statistically significant increase when compared to the other samples. The lowest T4 level was found in CYP 1 (4.6±0.96), while the highest T4 was seen in CYP 2 (8.5±1.08).

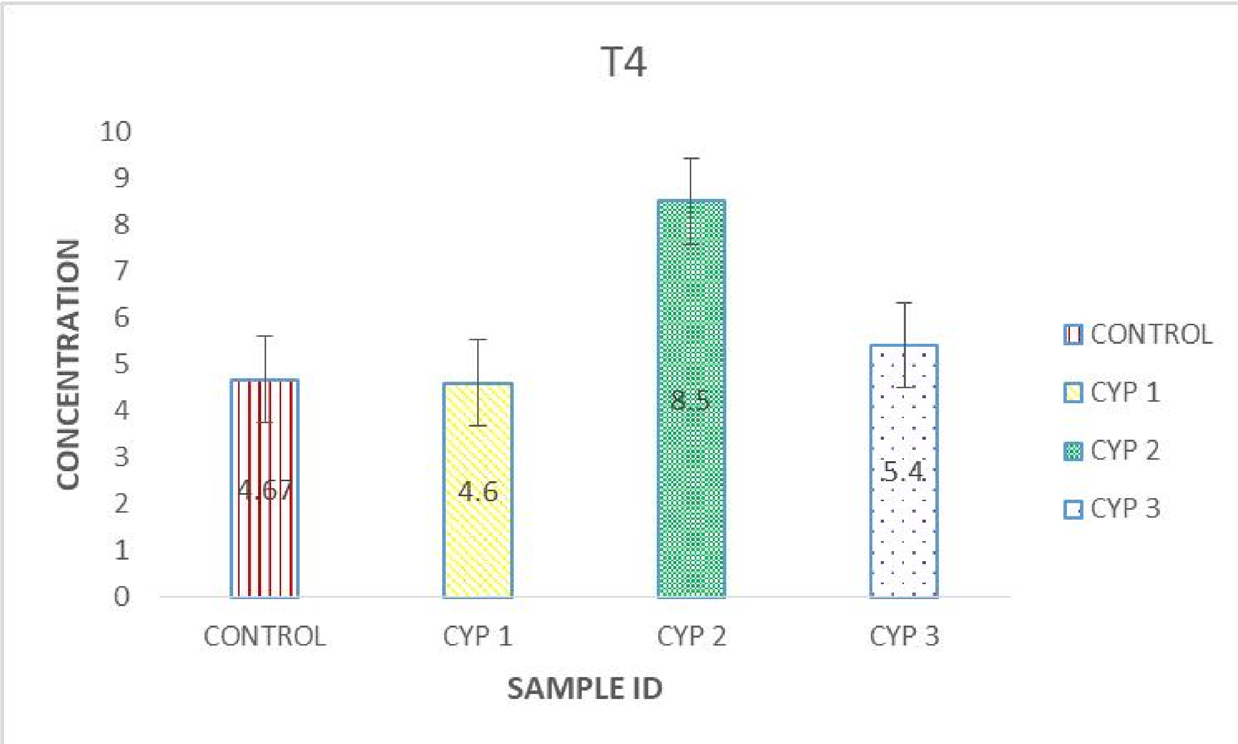

Figure 4.1: Effect of cypermethrin on T4 level in *C. gariepinus*

4.3.2 Effect of cypermethrin on T3 level

The T3 levels in the juvenile *C. gariepinus* were not significantly altered by the 96 hours cypermethrin exposure as shown in Figure 4.2. The lowest T3 level of the cypermethrin exposed *C. gariepinus* were found in the control, while the highest T4 was seen in CYP 1 (0.97±0.15) followed by CYP 2 (0.95±0.17), and CYP 3 (0.94±0.13).

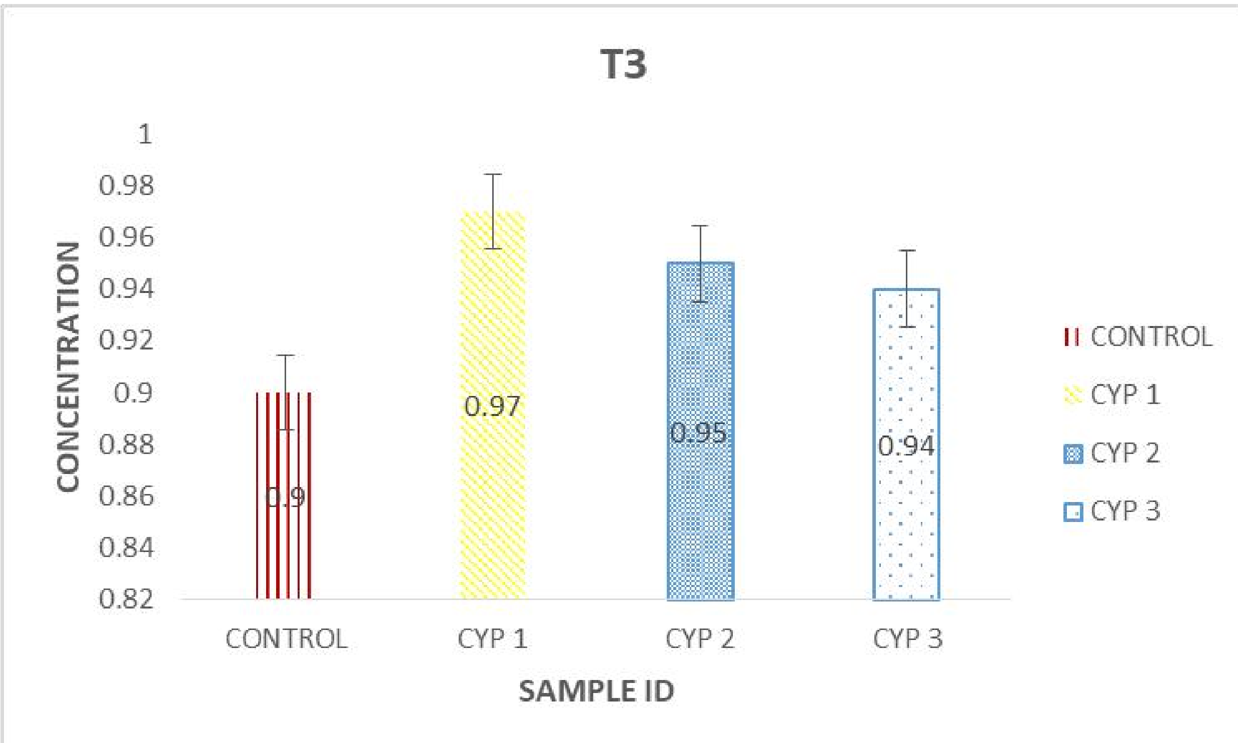

Figure 4.2: Effect of cypermethrin on T3 level in *C. gariepinus*

4.3.3 Effect of cypermethrin on TSH level

The TSH level observed in the juvenile *C. gariepinus* showed a significant trend across the cypermethrin exposed groups. There was an elevation in the different samples in contrast to the control group as represented in Figure 4.3, with CYP (0.85±0.05) as the highest, followed by CYP 2 (0.81±0.01), and lastly CYP 1 (0.75±0.05) as the lowest.

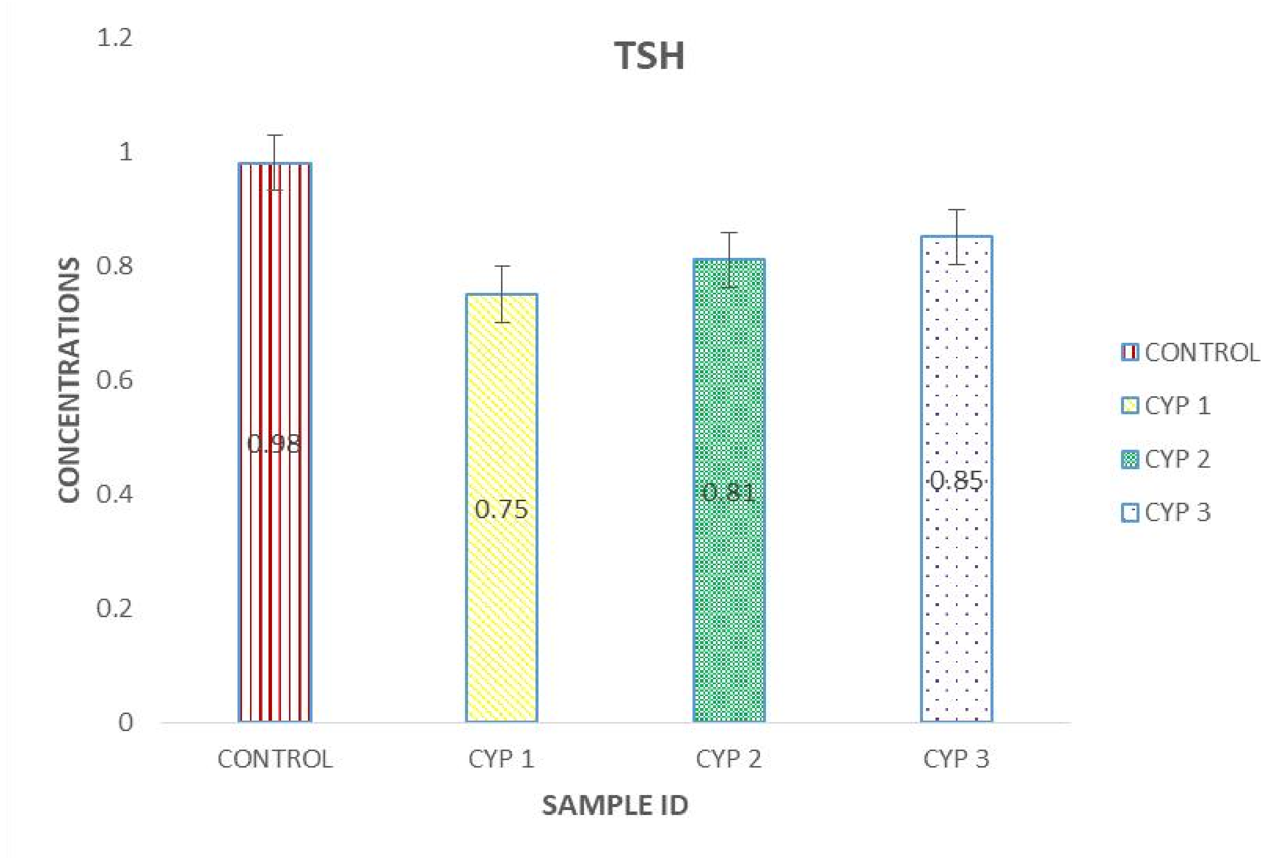

Figure 4.3: Effect of cypermethrin on TSH level in *C. gariepinus*

CHAPTER FIVE

DISCUSSION

5.1 Physico-chemical Parameters of Test Water

The water used in the experiment had physico-chemical parameters suitable for the growth and survival of *Clarias gariepinus*. The successful productivity of fish using ponds depends on the physico–chemical and biological characteristics of water used for aquaculture and the nutrition management of the fish. The quality of test water used in this experiment had a temperature of 27 °C, Dissolved oxygen of 3.7mg/l and pH of 6.46 which falls within the typical range for water quality in aquaculture. The optimum conditions for increased fish productivity have been reported to be warm temperature range of 20-30 °C, adequate DO level of 4 mg/L and an appropriate pH range of 6-9 (Edema, 2008, Ntengwe, 2008, Davies, 2014).

5.2 Toxicity of Cypermethrin Dosage

Cypermethrin toxicity in this study was found to increase with increased concentration. This observation is in agreement with the reports of Shuklan (2023), according to his findings, the intensity of toxic level of cypermethrin is dose- and time- dependent, with significant effects on the higher dose-treated group animal than that of the lower dose-treated group animal. In addition, the pesticides also possess bioaccumulation property and bioaccumulate in the tissues of aquatic organisms (Maurya and Malik, 2016, Yadav et al., 2018). The level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reaction of the animal receiving the toxicant (Ayoola, 2008).

5.3 Effects of Cypermethrin on Thyroid Hormone Levels

Exposure to cypermethrin in this study resulted in the disruption of thyroid hormone production or secretion in *Clarias gariepinus*. This could be due to the impact of cypermethrin on the hypothalamus-pituitary-thyroid axis, which regulates thyroid hormone production (Kongtip, 2021). The level of T4 observed in the juvenile *C. gariepinus* was not significantly altered by the 96 hours cypermethrin exposure as shown in Figure 4.1, but a slight decreasing trend was observed in the T3 levels of the cypermethrin exposed fish; this correlates with the report of Scott (2009) where an exposure of *C. batrachus* catfish species to the pesticide malathion showed a decrease of T3 levels and an unchanged level of T4 suggesting a lower conversion of T4 to T3.

Whilst the TSH level observed in the juvenile *C. gariepinus* showed a significant elevation trend in the different samples compared to the control group as represented in Figure 4.3, with CYP 3 (0.85±0.05) as the highest level, followed by CYP 2 (0.81±0.01), and CYP 1 (0.75±0.05) as the lowest. This result is in agreement with the report of Bhanu (2016) and Ghayyur (2021) in *C. carpio* treated with cypermethrin, according to the findings of their study, there was a significant elevation observed in the TSH levels of pesticides exposed fish compared to the control group, and a decline in the T4/T3 levels of the pesticides exposed fish. Cypermethrin provokes the inhibition of thyroid function in the fish so that the activity levels of T3 or T4 become decreased. The increasing trend in TSH levels might be the compensatory response of the pituitary gland to stimulate the thyroid gland to produce more thyroid hormones in response to the decreased circulating levels of T4/T3 (Yasaman, 2023), as minor changes in T4/T3 leads to significant changes in TSH (Shahid *et al.,* 2023).

The level of thyroid hormones affects the metamorphosis and development of the fish, a disruption of its proper function could lead to deformity in juveniles, which is frequently observed in hatchery (Yamano, 2005). The regulation of liver glycogen, protein metabolism, cell respiration, antioxidant system and the excitability of neurons and muscles would also definitely be affected in the fishes (Lacasana *et al.,* 2010, Yu *et al.,* 2015).

The thyroid-stimulating hormone (TSH) is the most delicate marker (Michelle *et al.,* 2019), it adjusts the amount of hormone production by the thyroid and aids in assessing the thyroid condition (Zhang *et al.,* 2017). Elevated levels of TSH (and most often) simultaneously low T3/T4 indicates the condition of Hypothyroidism (Shokripour *et al.,* 2022)

RECOMMENDATIONS

Pesticide use can pose a significant threat to *Clarias gariepinus* and other aquatic life. To address this concern, exploring alternative pest control methods like Integrated Pest Management (IPM) or biocontrol is recommended. Promoting responsible pesticide application practices that discourages pesticide use near water bodies is crucial for safeguarding aquatic ecosystems. Additionally, Monitoring and Evaluating effectiveness of the current regulatory policies and risk assessment methods for the long-term ecological impact of cypermethrin is recommended.

CONCLUSION

After exposing juvenile *C. gariepinus* to a sublethal dose of Cypermethrin for 96 hours, substantial changes in thyroid hormone parameters were observed, revealing that pesticides, such as cypermethrin, even at low concentrations, are associated with thyroid axis disruption and alter thyroid hormone levels in *C. gariepinus* and other aquatic fish. The results from the study shows that the pesticide exposure interferes with the normal thyroid function of *C. gariepinus* which negatively affects the metamorphosis, growth and development of the fish.

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