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Analysis of the biochemical and histopathological impact of abamectin in Oreochromis sp Fish

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Dedication

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To all those who have contributed to my education and to all those I love and who love me, with the expression of all my feelings and respect, may God accept our work.

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List of Abbreviations

GOD	GLUCOSE OXIDASE
GK	GLYCEROL KINASE
GPJ	DAILY WEIGHT GAIN
GPO	GLYCEROL PEROXIDASE
H_2O_2	HYDROGEN PEROXIDE
HDL	HIGH-DENSITY LIPOPROTEINS
HSR	THE HEPATOSOMATIC RATIO
LDL	LOW-DENSITY LIPOPROTEINS
O2	DIOXYGEN
pH	POTENTIAL HYDROGEN
POD	PEROXIDASE
TIE	TOXICITY IDENTIICATION EVALUATION

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Abstract


Introduction

Pesticides, while beneficial for enhancing agricultural productivity and pest control, pose significant threats to aquatic ecosystems. Herbicides and pesticides often enter watercourses through runoff and spray drift from agricultural fields, presenting serious risks to both wildlife and human health **(Rahman, 2002)**. This contamination results in bioaccumulation in fish and other aquatic organisms, often reaching toxic levels detectable through short-term mortality tests **(Schäfer *et al*., 2012; Silva *et al*., 2019)**. The presence of these chemicals in aquatic environments not only disrupts the delicate ecological balance but also leads to long-term adverse effects on biodiversity and ecosystem services **(Tang *et al*., 2021)**.

Despite the benefits of pesticides in agriculture, there is often a lack of urgent action to preserve the integrity and biodiversity of aquatic ecosystems, which are increasingly strained by rapid industrialization, urbanization, and population growth. These developments exacerbate pollution, including from pesticides, further stressing natural resources **(Deneke *et al*., 2020).**

Fish, a crucial protein source in developing countries, is extensively cultured in inland water bodies. Its nutritional value, which depends on its biochemical composition, is adversely affected by water pollution **(Prado *et al*., 2009)**. Numerous studies have documented the alterations in biochemical components and histological structures in response to environmental stress, such as pesticide exposure **(Ramesh *et al*., 2015; Ghazala *et al*., 2019).**

Numerous studies have documented the negative impacts of pesticides on aquatic environments, including growth depression, restriction of larval and embryonic development, altered hemato-biochemical indices, modified erythrocyte cellular and nuclear structures, and disrupted major organ functions **(Aktar *et al*., 2009; Hedayati *et al*., 2014; Mostakim *et al*., 2015; Pala *et al*., 2016; Uddin *et al*., 2016; Sadiqul *et al*., 2017; Shahjahan *et al*., 2017; Mukti *et al*., 2018; Shahjahan, 2019; Majumder *et al*., 2019; Akter *et al*., 2020; Ritu *et al*., 2020; Islam *et al*., 2021; Sultana *et al*., 2021; Al-Emran *et al*., 2022; Hasan *et al*., 2022; Uddin *et al*., 2022).**

The extent of fish poisoning varies widely depending on factors such as the type and form of pesticides, which can lead to mass deaths or destruction of organisms that serve as food sources for aquatic creatures **(Rohani, 2023)**. Among pesticides, abamectin is widely used for

its effectiveness against a variety of pests, but its impact on aquatic ecosystems is concerning due to its potential toxicity to non-target organisms **(Rahman *et al*., 2019).**

Tilapia species, which have become predominant in commercial fish farming across Africa **(FAO, 2012, 2014).** These species hold significant economic and ecological importance in African lakes and rivers **(Ahouansou *et al*., 2008; Adebo *et al*., 2008; Sirima *et al*., 2009; Tanoh *et al*., 2013)**. Tilapia are also highly valued by fish farmers and consumers, being extensively exploited through both fishing and aquaculture **(Ouattara *et al*., 2009; Toguyeni *et al*., 2009)**. Among these species, red tilapia (Oreochromis spp.) has gained popularity due to its appealing color and higher marketability, along with its high salinity tolerance which allows it to be raised in brackish and seawater **(Watanabe *et al*., 1990)**. Well-pigmented red tilapia produced in saline water can achieve higher prices in local markets due to its resemblance to high-valued marine species **(Thodesen *et al*., 2013)**.

Given the significant market potential of red tilapia, establishing good management practices is crucial to ensure high-quality and substantial production. Stress in fish, which triggers the neuroendocrine system and leads to a cascade of metabolic and physiological changes, is an important factor to consider in maintaining homeostasis and overall fish well-being **(Barton, 2002).**

This study aims to investigate the effects of abamictin on red tilapia in freshwater, exploring its biochemical and histopathological impacts. The work is organized into three chapters:

1. A bibliographic review presenting the study species and target pesticide.
2. An outline of the tools and procedures used in the research.
3. A summary of the results and their discussion.

The work concludes with a conclusion and future prospects.

Chapter 1 literature review

I. The study Species

I. 1 Biology of the red tilapia species oreochromis sp

The tilapia sub-family (Tilapiinae) belongs to the Cichlidae family and comprises around a hundred species grouped into three genera: Oreochromis, Sarotherodon and Tilapia, which differ in particular in their reproductive behaviour and feeding regimes. The only genus that has so far shown potential for aquaculture is Oreochromis, of which three species are now being bred on a significant scale: Oreochromis niloticus, Oreochromis aureus, Oreochromis mossambicus and their hybrids. It is now common practice to refer to these genera and species by the generic and common name of tilapia **(Lazard, 2009)**.

The red tilapia has garnered significant traction in the market, emerging as a top-selling commodity and gaining popularity among growers. Its inception traces back to genetic manipulation efforts, with the first red tilapias originating in Taiwan during the late 1960s. This breakthrough involved crossing a transgenic red-orange Mozambican tilapia with a standard indigo tilapia, resulting in the creation of the Taiwanese Red Tilapia. Subsequently, in the 1970s, another variant of red tilapia was developed in Florida by mating a regular- colored Zanzibar female tilapia with a red-gold Mozambican male tilapia. A third strain of red tilapia emerged in occupied Palestine through the combination of a pink transgenic Nile tilapia and a wild blue tilapia **(Vajargah ,2021)**

The complicated evolutionary biology of cichlids causes confusion in their classification and naming, which is subject to frequent change. Among these species, the Oreochromis species out for its distinctive trait; the female is the primary caregiver for the young in this mouth-brooding species. On the other hand, egg incubation in a built-in "nest" at the bottom of a lake or pond **(R.S.V. Pullin *et al*., 1982)**.

I. 2. Systematics and Physical features

I.2.1. Systematics

Tilapia is the name of a group of cichlid fish living in Africa. This group of cichlids includes 3 important genera in terms of human breeding ability: Oreochromis goldfish, Sarotherodon: sawdust, and Tilapia: (tilapia). Tilapias belong to the Tilapiinae subfamily within the larger Cichlidae family, encompassing four species commonly known as tilapia. They are classified within the order Perciformes. An identifiable trait of tilapias is their

discontinuous lateral line **(FAO, 2002; Ambre, 2009)**. The red tilapia, commonly known as such, holds the following systematic position within the classification hierarchy **(Linnaeus, 1758; Peters, 1852)**

Table 01: Systematic position of Oreochromis Sp

Kingdom	**Animalia**
Phylum	**Cordata**
Subphylum	**Vertebrata**
Superclass	**Osteichtyes**
Class	**Acteinopterygii**
Subclass	**Neopterygii**
Order	**Perciformes**
Sub-order	**Labroidei**
Family	**Cichlidae**
Subfamily	**Tilapinae**
Genus	***Oreochromis***
Species	***Oreochromis sp***

I.2.2. Physical features

The appearance of the tilapia is very similar to sunfish or crappie, but may be distinguished from other fish species by their interrupted lateral line. Pure bread species are a uniform red color and pinkish ventrally, but fade to a reddish-white upon death. They have a deep body and are laterally compressed. They have a small head and mid-sized eyes. Their anal fin has 3 spines and 9 or 10 rays; their caudal fin is rounded; and, their dorsal fin has 15 to 18 spines and 10 to 13 rays with a long base. Typically, fry, fingerlings, and even adults have broad vertical bars running along their sides **(Peyrin *et al*, 2013).** They have 16 to 22 gill rakers on their first arch. Their body is covered with cycloid scales. The Red Tilapia reaches a maximum 38 cm (15 inches) in length and 4.3 kg in weight. Its intestine, jaw teeth, and pharyngeal teeth are specialized adaptations tailored to its specific dietary needs., jaw teeth, and pharyngeal teeth are structural adaptations for a specific diet. The degree of coarseness

and movability varies among species, as they prefer coarse vegetation, unicellular algae, and bacteria **(Lowe-McConnell, 1959).**

The guidelines in **Figure. 01** can be used to characterize the morphology of tilapia. There is no sexual dimorphism among the species of tilapia, and their primary morphological traits include a compressed body, cycloid scales, and a small upper jaw length. Reddish- colored pectoral, dorsal, and caudal fins are acquired during the spawning season. The genital entrance, which varies in shape depending on the gender, is situated behind the anus **(Alhassan *et al*, 2013)** .

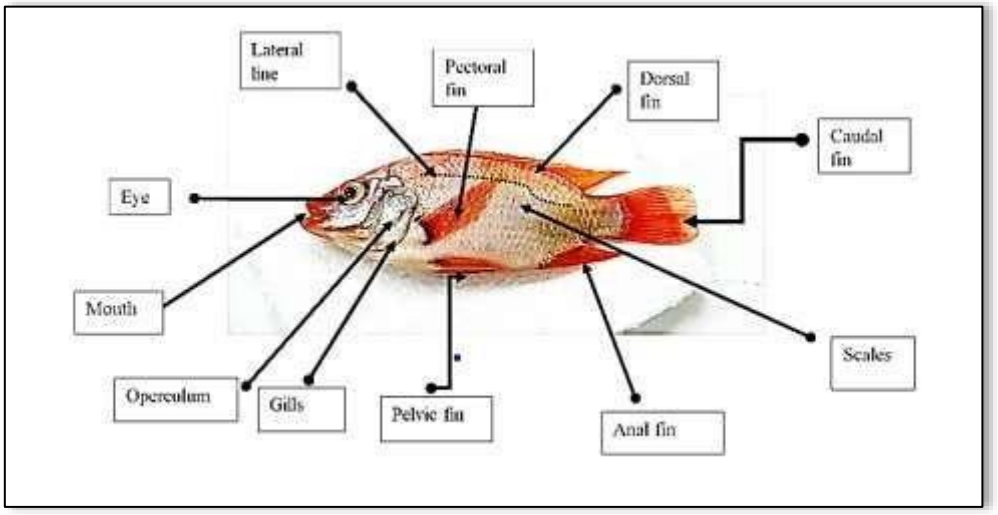


Figure 01: General morphology of red hybrid tilapia (*Oreochromis sp*.) **(Research work, 2024)**

I.3. Reproduction

The male of every Oreochromis species excavates a nest in the pond bottom, usually in water that is shallower than three feet), mating with many females. The female lays two to four eggs per gramme of brood female after a brief mating ritual. The male fertilizes the eggs, which she then retains and incubates in her mouth (buccal cavity) until they hatch. Through the absorption of the yolk sac, fry stay in the female's mouth and frequently seek shelter there for several days after they start feeding. Tilapia sexual maturity depends on environmental factors, age and size. Compared to the Nile tilapia, the Mozambique tilapia reaches sexual maturity at a smaller size and earlier age as well as blue tilapias. Large lake tilapia populations reach bigger sizes and mature later than small farm pond tilapia populations. The Nile tilapia, for instance, reaches maturity at around 10 to 12 months and 3/4 to 1 pound (350 to 500 grams).in a number of lakes in East Africa. In agricultural ponds, the same species

will attain sexual maturity at 5 to 6 months of age and 5 to 7 ounces (150 to 200 grams) when growth conditions are favorable. Nile tilapia that grow slowly take a month or two longer to reach sexual maturity, although stunted fish can breed at less than one ounce (20 grams) in weight. Males develop almost twice as fast as females (**SRAC, 2003).**

I.4. Growth

Tilapia (Oreochromis sp.) has become a popular choice in aquaculture because of its rapid growth and favorable size **(Molina *et al.*, 2005)**. In nursery ponds, one-gram fish are raised to one to two ounces (20 to 40 ounces) under ideal development circumstances grams in seven to eight weeks, after which they are supplied in grow out ponds. Males typically attain a weight of 1/2 pound (200 + grams) in 3 to 4 months, 1 pound (400 + grams) in 5 to 6 months, and 1.5 pounds (700 grams) in 8 to 9 months in mono sex grow out ponds with ideal temperature regimes **(Popma & Michael , March 1999).**

I.5. Diet

In general, all tilapias share a diet that is mostly herbivorous, unlike most other fish species, which primarily consume small invertebrates or young, small fish **(Lazard, 2009).**

Tilapia indeed have a varied diet, consuming a wide range of natural food items. This includes plankton, aquatic macrophytes, and various aquatic invertebrates found both in the water column and on the bottom (benthic). While they primarily rely on natural food sources, when supplementary feeding is provided, it can significantly contribute to their development, typically comprising 30 to 50 percent of their diet.

Their feeding behavior is notable for their ability to filter feed, efficiently extracting plankton from the water. Additionally, they possess specialized pharyngeal plates with fine teeth, which allow them to grind plant tissues for digestion.

An interesting aspect of their digestion process is the low pH environment of their stomachs, below 2. This acidic condition aids in breaking down food matter, causing bacteria and algae to burst their cell walls, facilitating digestion **(Temesgen *et al.*, 2022).** Understanding the feeding behavior and dietary preferences of tilapia is crucial for their management and cultivation in aquaculture settings.

I.6. Ecological requirements

Compared to most cultivated freshwater fish, tilapia can withstand a greater range of environmental variables, including salinity, dissolved oxygen, temperature and pH. Although each species has a different tolerance to salinity, dissolved oxygen, temperature and Ph **(Popma *et al*, 1999).**

I.6.1. Temperature

Red tilapia (Oreochromis sp.), like other tilapia species, thrives in warm water temperatures. They are typically found in natural environments with temperatures ranging from 14°C to 33°C. However, they can tolerate temperatures outside this range, in laboratory setting a tolerance range of 7°C to 41°C. For optimal growth and development, temperatures between 25°C and 30°C are considered ideal for red tilapia **(FAO, 2013)**.

I.6.2. Salinity

Tilapia are usually more resistant to high salinity, in breeding water than other farmed freshwater species. Red tilapia is a relatively euryhaline species tolerating salinities of 0.015p. Mille to 30 p. mille **(FAO, 2013)**. Adult fish demonstrate greater salt tolerance than fry and juveniles. Fry and juveniles tolerated direct transfer at 19‰ with no apparent stress or mortality, but 100% mortality occurred at 27‰. In contrast, adult fish tolerated direct transfer at 27‰, with 100 % at 37‰ **(Abdel-Fattah *et al.*, 2006)**.

I .6.3 Potential hydrogen (pH)

Tilapia are known for their ability to withstand environments with extreme pH levels, as well as maintaining an optimal pH level is crucial for their growth and overall health. Typically, the recommended pH range for red tilapia is 7.0 and 9.0, with a preferred range around 7.5 to 8.5. Within this range, tilapia exhibit improved feed utilization, nutrient absorption, and immune system function. Proper pH management ensures the availability of essential minerals and nutrients for the fish, contributing to their well-being **(Romana-Eguia *et al*, 2020).**

I .6.4 Dissolved oxygen

Tilapia are capable of surviving in conditions where the concentration of dissolved oxygen is very low. In fact, tilapia can tolerate routine daybreak dissolved oxygen (DO) concentrations of less than 0.3 mg/l, which is well below the tolerance limits of most other

farmed fish. Research studies showed that Nile tilapia grew better when aerators were used to keep morning DO concentrations above 0.7 to 0.8 mg/L (as opposed to unaerated control ponds). However, a minimum level of 2 to 3 mg/l is recommended for breeding, below which a decrease in metabolic rate, growth and even disease resistance level for an extended amount of time **(Popma *et al*., 1999).**

I.6.5 Photoperiod

The action of light, although closely linked to temperature, acts on growth via the endocrine system, **(Mélard *et al*.,1986)** explain that an optimal photoperiod (18 h) stimulates the secretion of growth hormone (GH) in O. niloticus. Furthermore, larvae are more sensitive to photoperiod than fry and juveniles **(El Sayed *et al*., 2004).**

I.7 Habitat and geographical distribution

Tilapias have their origins in Africa, but they have been introduced and distributed worldwide. The majority of farmed tilapia are produced in countries with tropical or subtropical climates. The primary tilapia-producing countries are located in Asia, with mainland China, the Philippines, and Taiwan leading the world in production **(Lovshin, 1997).** Tilapia farming is also on the rise in the Americas, driven by domestic market growth and exports to the United States. In the Mediterranean region, Algeria stands out for its low production of fishery products, with a food ratio of 5.4 KG/ha/year in 2010, well below the world average of 19.2 KG/ha/year estimated in 2012 **(FAO, 2012).**

I.8 Species selection in ecotoxicology

Selecting red tilapia as a model species in ecotoxicology provides researchers with a robust and versatile platform to investigate the effects of environmental pollutants on aquatic organisms, ecosystem health, food safety and human well-being.

Numerous studies in aquatic toxicology and ecotoxicology have used tilapia species **(Wu *et al*., 2007; Aldoghachil *et al*., 2016; Nurulnadia *et al*., 2018; Hagar, 2019)**. Indeed, they are acknowledged for their early maturity, disease resistance, and wide range of tolerance to salt and density **(Watanabe *et al*., 1989; Vovener, 2012).** Most of these studies concentrate on the Oreochromis species, which is characterized by a relative tolerance to pollutants **(Gadagbui *et al*., 1996; Almeida *et al*., 2002; Shailaja *et al*., 2003)**. Additionally, Red tilapia are widely distributed and commonly used in aquaculture, making them readily available for research purposes. Furthermore, tilapia is consumed by humans worldwide, so

understanding their responses to environmental toxins is directly relevant to human health concerns. Studying red tilapia in ecotoxicology can shed light on potential risks associated with the consumption of contaminated fish.

Lastly, Tilapia species exhibit considerable genetic diversity, offering researchers the opportunity to study different populations and their responses to environmental stressors.

I.9. Assessment tools in ecotoxicology

Assessment tools in ecotoxicology encompass a wide range of methods and techniques used to evaluate the effects of contaminants on organisms and ecosystems. Some common assessment tools include:

- **Bioassays**: These are laboratory-based tests that expose organisms to various concentrations of contaminants to assess their toxicity, often measuring endpoints such as mortality, growth, reproduction, and behavior.
- **Field Studies**: Field assessments involve studying organisms and ecosystems in their natural environment to observe the effects of contaminants over time.
- **Chemical Analysis**: Analyzing water, sediment, and tissue samples for the presence and concentration of contaminants helps determine exposure levels and potential risks to organisms.
- **Biomarkers**: Biomarkers are measurable biological indicators of exposure to contaminants or their effects on organisms. They can include biochemical, physiological, and genetic markers that provide early warning signs of stress or toxicity.
- **Toxicity Tests and toxicity identification evaluation (TIE) tests:** these tests identify and assess the toxic effects of specific contaminants on organisms or biological processes, often using standardized protocols and endpoints to compare results across studies **(CSIRO, 2021)**
- **Community Indices:** These evaluate overall community health and ecological impacts (as a result of the combined effects of physical, chemical, and biological stresses operating in a system, organism communities exhibit ecosystem integrity at a level of biological organization. Changes in the structure , function and tolerance to pollutants over time can all be used to characterize community responses **(Connon *et al.*, 2012)**

I.10. Stress in fish

I.10.1. Definition

The concept of "stress" has its roots in the physiological definition proposed by **Selye (1950, 1973)**: “stress is the nonspecific response of the body to any demand placed upon it.” However, the definition we prefer and the one used throughout this book is “The physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of insult” **(Schreck, 2000).** To provide a comprehensive understanding, various definitions of "stress" have emerged in the literature over time.

- A state produced by an environmental or other factor that extends the adaptive responses beyond the normal range **(Brett, 1958).**
- The cascade of biological events that occur when the organism faces a challenge out of the normal range and the attempt to reestablish homeostatic values **(Barton, 1997).**
- The reaction of the organism aimed at regaining homeostasis **(Chrousos, 2009).**
- Stress is a condition where an environmental demand exceeds the natural regulatory capacity of an organism **(Koolhaas *et al*., 2011).**

I.10.2 Stress biomarkers

A biomarker is defined as a biochemical, cellular, physiological or behavioral variation that can be measured in a tissue, biological fluid or whole organism and that provides evidence of exposure to and/or the effect of one or more biological chemical pollutants or radiation **(Depledge, 1993; Sanchez, *et al*., 2012).**

The presence of multiple stressors associated with various environmental factors represents a great challenge to the impact assessment of pollutants on the health of aquatic organisms **(Kroon et al., 2017).** Therefore, a widely applied and adequate strategy is the multi-biomarker approach, which may involve analyzing different target organs **(Yamamoto *et al*., 2023).** According to the National Research Council **(NRC, 1987)** and the World Health Organization **(WHO, 1993)**, biomarkers can be categorized into three distinct groups:

- **Exposure biomarkers:** these cover the detection and measurement of an exogenous substance or its metabolites, or of a product resulting from the interaction between a

xenobiotic agent and a target molecule or cell. These biomarkers are measured in a specific compartment within the body.

- **Biomarkers of effect:** these biomarkers include measurable biochemical, physiological or other alterations in the tissues or body fluids of an organism. They are associated with an established state of health or a possible deficiency or disease.
- **Biomarkers of susceptibility:** these biomarkers indicate the intrinsic nature or acquired capacity of an organism to respond to exposure to a specific xenobiotic substance. This includes genetic factors and changes in receptors that modify the organism's sensitivity to this exposure.

I.10.3. Physiological and histological responses to stress

Fish responses to stress, encompassing both handling and environmental fluctuations such as temperature, salinity, and water quality variations, alongside interactions with other fish and prolonged physical exertion, induce a series of biochemical and physiological changes.

Physiological responses of fish to environmental stressors have been grouped broadly as primary and secondary **(Fig. 02)**. Primary responses, which involve the initial neuroendocrine responses, include the release of catecholamines from chromaffin tissue **(Randall and Perry, 1992; Reid *et al.*, 1998)**, and the stimulation of the hypothalamic-pituitary-interrenal (HPI) axis culminating in the release of corticosteroid hormones into circulation. Secondary responses include changes in plasma and tissue ion and metabolite levels, hematological features, and heatshock or stress proteins (HSPs), all of which relate to physiological adjustments such as in metabolism, respiration, acid-base status, hydromineral balance, immune function and cellular responses **(Mommsen *et al.*, 1999)**. Additionally, tertiary responses occur **(Fig. 02)**, which refer to aspects of whole-animal performance such as changes in growth, condition, overall resistance to disease, metabolic scope for activity, behavior, and ultimately survival **(Wedemeyer *et al.*, 1990).**

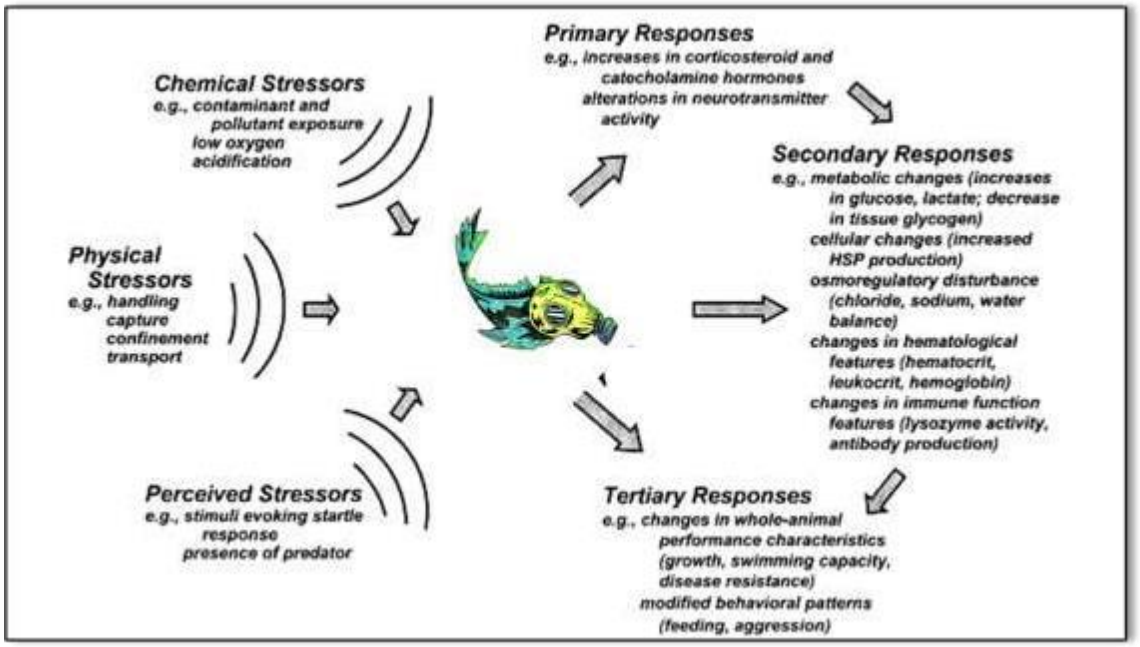


Figure. 02. Physical, chemical and other perceived stressors act on fish to evoke physiological and related effects, which are grouped as primary, secondary and tertiary or whole-animal responses. In some instances, the primary and secondary responses in turn may directly affect secondary and tertiary responses, respectively, as indicated by the arrows **(Barton, 2002)**

Fish exposure to stressors can lead to pathological changes in several internal organs, including the liver, kidneys. These changes can manifest as structural alterations, inflammation, necrosis, and other abnormalities, depending on the nature and intensity of the stressor. Histological investigation of different tissues of pesticide-exposed fish is a useful tool in monitoring pesticide effects. Organs which are highly affected by pesticide exposure are the gills, liver, kidney, intestine, spleen and gonads. ROS produced during the biotransformation process is the main causative agent of tissue damage.

However, majority of studies concerning the influence of chemical substances as well as herbicides on fish organism focus on the structure of gills and liver. Very often histological lesions are observed in gills being the site of first contact with environmental pollutants **(Bojarski, *et al*..2018).**

II. Pesticides

II.1 Definition

Pesticides are a group of chemical compounds that are extensively applied to restrict the development of wide range of harmful organisms including pests, insect, weeds, and others **(Kim *et al.*, 2017).** These chemicals encompass a broad range of substances such as insecticides, fungicides, herbicides, bactericides, larvicides etc., which are commonly available all around **(Abdelkhalek S.T *et al.*, 2018).**

II.2 Classification of pesticides

Pesticides can be classified on the basis of chemical structure, intensity of toxicity, mode of action as well as functional groups **(P.F. Garcia et al.; 2012)**. General classification of pesticides is presented in **Fig. 03.**

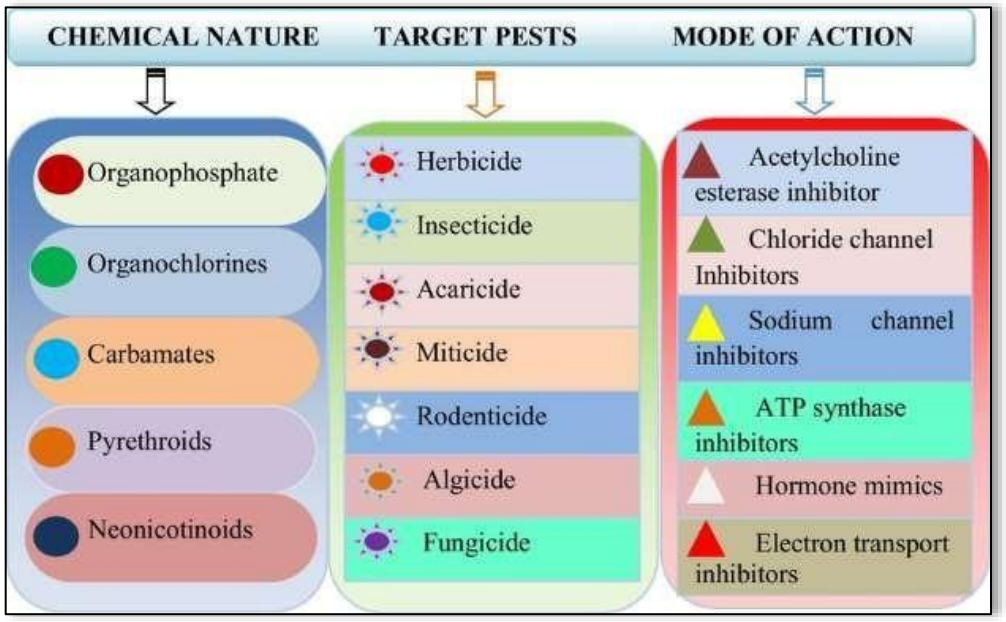


Figure 03: Major classes of pesticides based on chemical nature, target pests, and mode of action **(Dhuldhaj *et al.*, 2022).**

II.2.1. Classification based on target pests

Using this approach, pesticides are categorized according to the organism they target, with their names selected to reflect their specific actions (**Yaday *et al* ., 2017) (See**

Table 02).

Table 02: Classification of pesticides based on target organisms **(Shaw *et al* 2019)**

Pesticide	Target organsme
Insecticides	Insects
Herbicides	Weeds
Rodenticides	Rodents
Fungicides	Fungi
Acaricides an miticides	Arachnids of the order Acarina such as ticks and mites
Molluscicides	Mollusks
Bactericides	Bacteria
Avicides	Bird Pests
Virucides	Virus
Algicides	Algae

II.2.2. Classification based on mode of action

Pesticides are categorized as either systemic or contact (nonsystemic). Pesticides classified as non-systemic are those that do not significantly pierce plant tissues and are therefore not transferred into the plant. The term "contact pesticides" refers to the fact that non-systemic pesticides will only have the intended effect when they come into touch with the intended insect. Paraquat and diquat dibromide are two examples of contact insecticides. Conversely, systemic insecticides are those that efficiently enter plant tissues and flow via the vascular system of the plant to produce the intended result. 2, 4-D and glyphosate are a few examples of systemic insecticides **(Buchel, 1983).** This category also includes stomach poisons, such as rodenticides, that have the intended effect after consumption. The fumigants are those insecticides that emit vapors that destroy the bugs **(Sengupta *et al.*, 2009).**

II.2.3. Classification based on toxicity

Depending on the harmful properties of pesticides and the health risks they pose. They were divided into four groups by the World Health Organization (WHO). Numbers I through

IV, which represent the rating class from lowest to greatest toxicity, signify very poisonous, highly toxic, moderately toxic, and somewhat toxic (**Table 4)**:

Table 03. WHO classifications of pesticides **(Yadav *et al.*, 2017)**

WHO class	*Toxicity level*	*LD_{50} for the rat (mg/kg body weight)*		*Examples*
		Oral	*Dermal*	
Class Ia	Extremely hazardous	<5	<50	Parathion, Dieldrin
Class Ib	Highly hazardous	5–50	50–200	Eldrin, Dichlorvos
Class II	Moderately hazardous	50–2000	200–2000	DDT, Chlordane
Class III	Slightly hazardous	>2000	>2000	Malathion
Class IV	Unlikely to present acute hazard in normal use	≥ 5000		Carbetamide, Cycloprothrin

II.2.4. Classification based on chemical structure

According to **(Kim *et al.*,2017)**, pesticides can be classified based on chemical structure such as (a) Organic including organochlorines (DDT, Lindane, Endosulfan, Aldrin); organophosphorus (Parathion, Malathion, Diazinon) and (b) Inorganic including fungicides (Benomyl, Oxine copper). The main component of organic pesticides is carbon while inorganic pesticides composed of several inorganic substances such as copper, sulfur, copper sulphate, ferrus sulphate etc. (**D. Gunnell *et al.*, 2007)**.

II.2.5. Classification based on use

Pesticides can indeed be classified according to their intended use, yielding six categories based on the purpose of treatment: crops, residential buildings, and humans and animals. It's essential to highlight that agriculture stands as the predominant consumer of pesticides, constituting approximately 88% of the global market, while non-agricultural usage represents only 12% **(Fillatre, 2011).**

II.3. Ecosystem contamination pathways

Pesticide residues are still moving through many environmental components, even though recent developments in pesticide formulation have been concentrated on lowering the application rate and environmental exposure **(Bish *et al.*, 2017).** Following application, the

pesticides undergo transit and modification **(Fig.4).** The following are significant events related to the fate of pesticides applied in agro-ecosystems: pesticide deposition and residues near crops and vegetation; soil particle absorption and transfer through wind and water run- off; pesticide solubilization in water and uptake and assimilation into plant parts through vessels; and lastly, pesticide degradation and formation of the residual product through photo- oxidation, chemical, and microbial processes **(Meftaul *et al*., 2020).**

Pesticides in nature undergo three distinct fate processes: (a) absorption, (b) transfer, and (c) environmental degradation. These processes are contingent upon various factors such as environmental conditions, soil properties, pesticide physical and chemical characteristics, solubility in water and volatility in air, and application techniques **(Raffa *et al*.; 2021).** The importance of pesticides for the environment and whether they will be safe after application are determined by these destiny mechanisms. Adsorption, which employs various adsorbents such as activated carbon, charcoal, clay, and nanoparticles, is a popular, economical, and environmentally beneficial technique for eliminating pesticides from contaminated environments **(Srivastav *et al*., 2020)**. In agro-ecosystems, the movement and destiny of pesticides have been well demonstrated **(Dhuldhaj *et al*., 2022).**

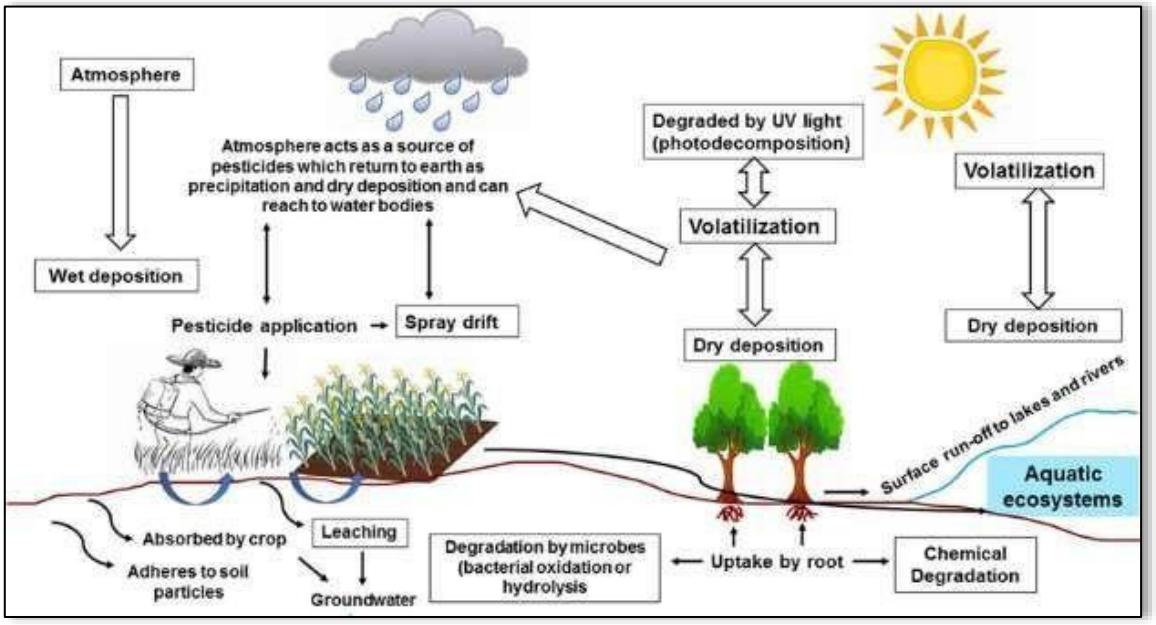


Figure 04: Fate and transport of pesticides in the environment **(Dhuldhaj *et al* ., 2022)**

II.4. Water contamination by pesticides

Herbicides and pesticides enter water systems mostly through agricultural and urban runoff, where they seep into the soil or are discharged directly as polluted wastewater. The physical and chemical characteristics of pesticides cause them to interact with water in various ways. The primary components of all pesticides are combined or dissolved in inert substances (such as solvent) to change the concentration. Thus, the presence of fillers, contaminants, and/or intermediates throughout the degradation process may also be the cause of water contamination in agricultural systems.

The primary methods by which pesticides are transferred through soil and water are diffusion, dispersion, and penetration. There isn't much information in the literature about the complicated phenomena of pesticide interaction with soil and/or water.

Pesticide half-lives are often correlated with pesticide stability. Despite the fact that a significant volume of wastewater from industries and cities is dumped into water bodies, 70% of water abstraction globally is related to agriculture. The health of people, aquatic ecosystems, and plants are all at risk due to the large-scale release of agrochemicals.

According to research done in the mid-1990s by the U.S. Geological Survey (USGS), pesticides were found in 90% of fish samples and river water in major rivers that were near agricultural and urban terrain. Among the 21 pesticides with concentrations over recommended limits found in surface and ground water across the country were the herbicides 2,4-D, diuron, and prometon. A high quantity of pesticides over their threshold levels were found in 13–30% of all surface and ground waters in Europe **(Shahzad *et al.*, 2022)**

Chapter 2 Materials and Methods

I. Practice period and location

The study was conducted over a two-month period at a pilot shrimp farm and scientific research center in Hassi Ben Abdellah, Ouargla. Blood analyses and histological sections of organs were performed at Mohamed Boudiaf Hospital in Ouargla.

II. Materials

II.1 Biological material

Red hybrids of tilapia (Oreochromis sp), derived from a cross between Oreochromis niloticus and Oreochromis mossambicus, were obtained from a pilot shrimp farm and scientific research center at Hassi Ben Abdellah, Ouargla. Thirty of these fish, with an average weight of 62.59 g and an average length of 15 cm, were reared for seven days in three 60 cm by 40 cm aquariums, each containing 80 liters of water (10 individuals per aquarium).

Figure 05: Oreochromis sp specimen **(Research work, 2024)**

II.2 Chemical material

II.2.1. Pesticide used

Abamectin (1.8% EC) is used as an antiparasitic in veterinary practice and as an insecticide in agriculture. It was purchased from an agricultural supplies store. The chemical

name, 5-O-demethyl avermectin A1a (i) mixture with 5-O- demethyl- 25-de (1-methyl propyl-25- (1-methyl ethyl) avermectin A1a. It is a mixture of avermectins containing mainly avermectin B1a and avermectin B1b. This natural insecticide and acaricide of organic origin is used to control various agricultural pests, including, biting-sucking insects and certain minor insects. It acts by ingestion and contact, interfering with the transmission of nerve influx in the target arthropods **(Ramade, 1998)**. In our study, we used two concentrations of abamectin, namely 50 µg/l and 100 µg/l.

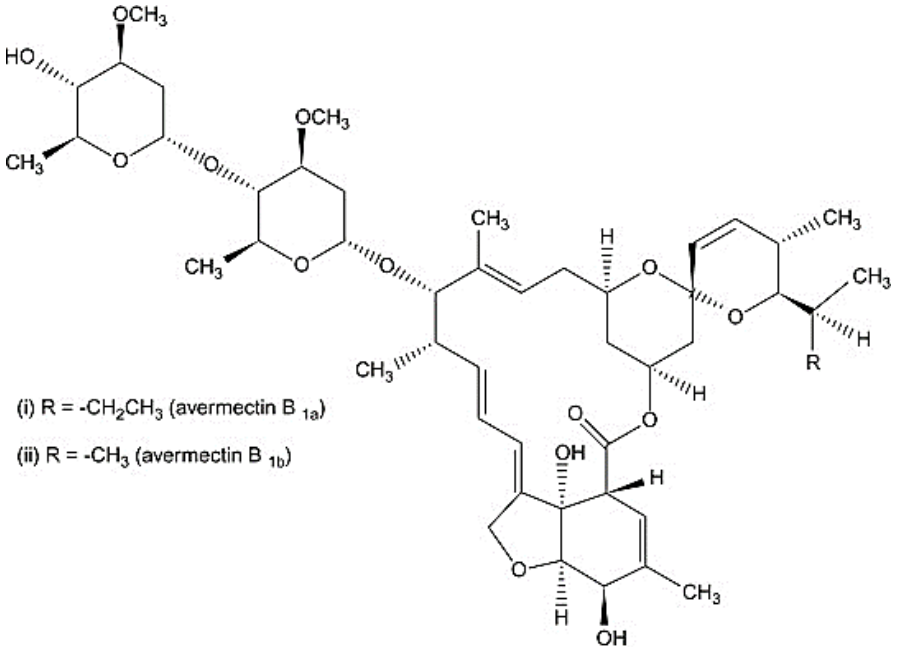


Figure 06: Chemical structure of Abamectin **(Jim *et al.*, 2010)**

II.2.2. Physicochemical characteristics and properties

Abamectin technical material was of high purity (> 98%) and was used for the determination of the physical and chemical properties of the pure active substance. Properties of abamectin (> 98% purity) and degradation in water (avermectin B1a)

Table.04: The characteristics of abamectin, compiled from the sources provided **(Ramade, 1998; Agrow, 2022).**

Characteristic	Details
Chemical Name	Abamectin
CAS Number	71751-41-2
Chemical Family	Avermectin
Mode of Action	Neurotoxin (GABA agonist), leading to paralysis and death in target organisms
Common Use	Insecticide and miticide
Physical State	Solid (commercially available as a formulation)
Color	White to yellowish
Molecular Formula	C48H72O14
Molecular Weight	873.09 g/mol
Water Solubility	0.0078 mg/L (25°C)
Vapor Pressure	2.5 x 10^-8 mm Hg (25°C)
Environmental Fate	- Low mobility in soil
	- Moderately persistent in soil and water
	- Potential to bioaccumulate
Toxicity (Mammals)	- Acute Oral LD50 (rat): 10 mg/kg
	- Acute Dermal LD50 (rat): >330 mg/kg
Toxicity (Aquatic Organisms)	- Highly toxic to fish and aquatic invertebrates

III. Methods

III.1 Fish and maintenance regimen

Thirty Tilapia were selected according to weight and length. They were then reared for an adaptation period of seven days in three aquariums of dimensions 60 cm × 40 cm × 40 cm, with a water volume of 80 liters each (ten individuals per aquarium). Each aquarium is equipped with an oxygen diffuser and a thermoregulator. Fish were fed commercial fish feed daily at a rate of 2% of the average body weight. Each day, a quantity of water equivalent to 50% of the total volume of the aquariums was renewed to ensure proper quality for the survival of the fish and to minimize any kind of stress. Water parameters were measured daily and maintained at the following values:

- ✓ Temperature: 24-27°C
- ✓ pH: 7 ± 1
- ✓ Dissolved oxygen content: (4 to 6 mg/L)
- ✓ Salinity: 3
- ✓ A 12h light / 12h dark photoperiod was maintained.

III.2 Experimental treatments and sampling

III.2.1 Long-term toxicity test

After an adaptation phase under similar experimental conditions, fish were exposed to two concentrations of abamectin (50 µg/l and 100 µg/l) for 14 days, while control fish were kept untreated. Abamectin treatment was carried out under controlled conditions by introducing the xenobiotic into the water used for all our experiments. This process was carried out daily after cleaning the aquarium of waste and renewing half the volume of water in each aquarium. A parental solution of ambectin has been prepared by diluting the commercial formulation.

After 14 days of exposure, the fish were individually collected, anesthetized, and had their weight and length assessed. Blood samples were collected from the caudal vein using heparinized syringes, then centrifuged to separate the plasma, which was aliquoted and frozen at -20°C for biochemical measurements. At the end of the experiment, the fish were euthanized. Gills, livers, kidneys, and intestine were removed and collected in 10% formalin solution. The fixed tissue specimens underwent washing, dehydration, cleaning, and embedding in paraffin beeswax. Paraffin tissue sections were obtained and stained with hematoxylin and eosin **(Bancroft *et al*., 1996)** for histopathological examination by light microscope.

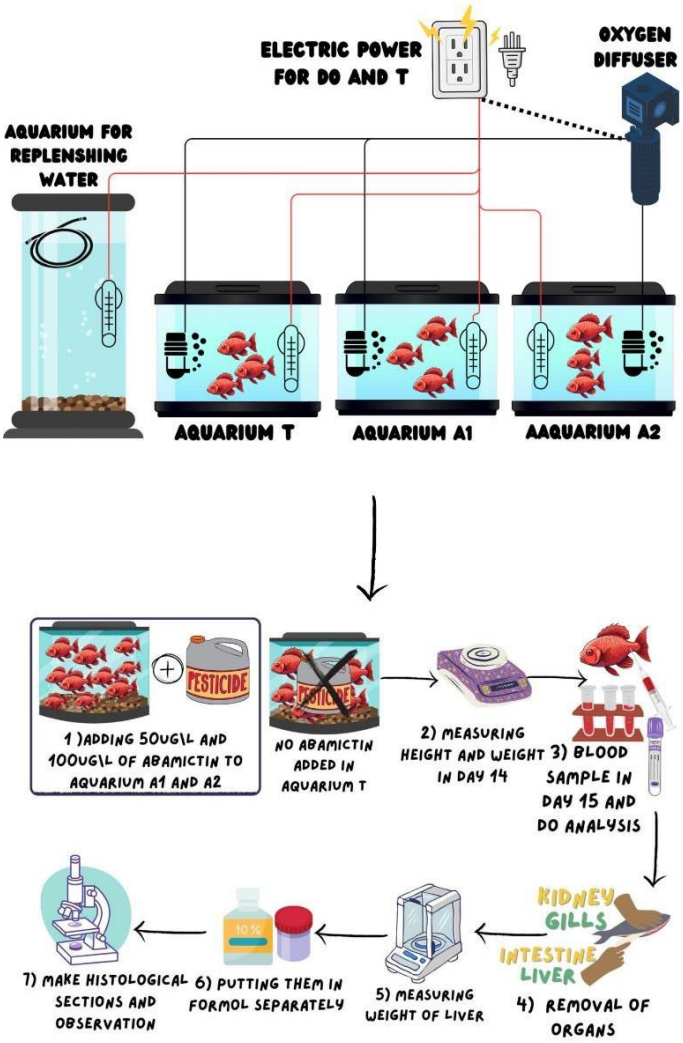


Figure 07: Schematic representation of the experimental protocol **(Research work, 2024).**

III.3. Biometric parameters

III.3.1 Daily weight gain

Daily weight gain (gram fish/day) can be calculated using the following relationship **(Glasser *et al*., 2001):**

$$GPJ_{max} = 17\times10^{-9} \times T^{4,875} \times P^{3,397} \times T \times -0,579$$

In this expression, temperature (T) is expressed in degrees Celsius and weight (P) in grams.

III.3.2 The hepatosomatic ratio (HSR)

The hepatosomatic ratio (HSR) is a parameter used in biological studies within the fields of ichthyology (the study of fish) and ecotoxicology.

HSR is an index that measures the general physiological condition of a fish by measuring the weight of its liver weight to eviscerated fish weight, expressed as a percentage. It is calculated according to the following formula **(Bogus, 1952).**

HSR = (liver weight / total body weight) x 100

III.4. Biochemical and hormonal analysis

III.4.1 Blood glucose assay

Glucose determination was carried out using a colorimetric technique based on the use of enzymes (GOD and POD) **(Trinder, 1969).**

❖ **Principle**

Glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid. The hydrogen peroxide (H2O2) produced is detached by means of a chromogenic oxygen acceptor, phenol-ampirone, in the presence of peroxidase (POD):

$$\text{B-D-Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconique Acide} + H_2O_2$$

$$H_2O_2 + \text{Phénol} + \text{Ampirone} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the concentration of glucose present in the sample tested **TRINDER P. (1969).**

❖ **Mode of operation**

1. After centrifugation, the plasma sample is separated from the other elements.
2. Enter sample into software, each met of codebar .
3. Place tubes in a device called Backmen colture AU480, which mixes 1.6 µL of sample with reagent (R1 = 40ul and R2 = 20ul). This color reflex makes.
4. After homogenization, the absorption reading of the samples is at 505nm, and the result is given according to the calibration curve.

III.4.2 Cholesterol assay

Cholesterol is a lipid (fat) that comes as part of the liver. The rest is of food origin. Total cholesterol is the level of HDL (good cholesterol) and LDL (bad cholesterol) **(Annabelle, 2024)**

- **Principle of assay**

The cholesterol present in the sample gives rise to a colored compound, according to the following reaction:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the concentration of cholesterol present in the sample tested **(Naito *et al*.,1984).**

. III.4.3 Triglyceride assay (Wahlefeld *et al*., 1974)

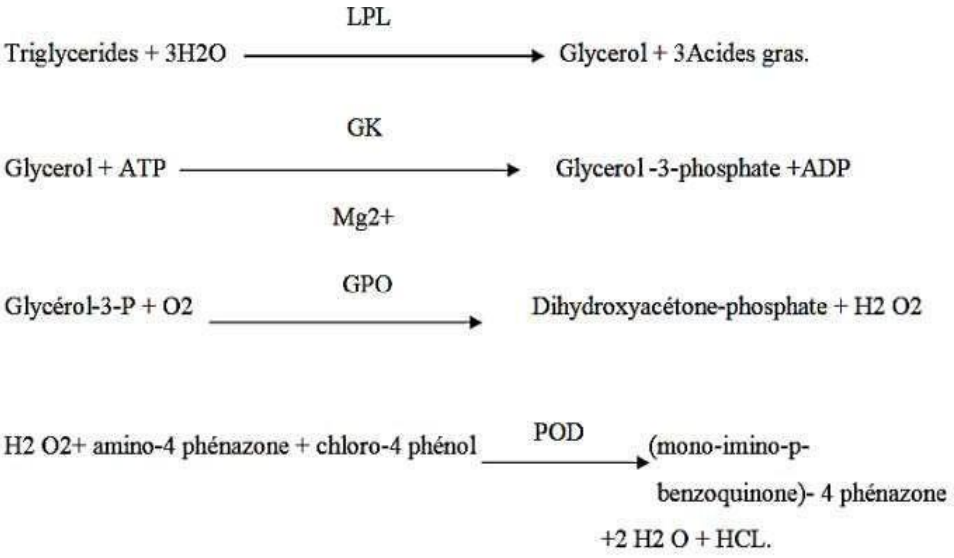
Triglycerides are esters of glycerol and three long-chain fatty acids. They are partly derived from food and partly synthesized in the liver.

- **Principle of assay**

This is an enzymatic-colorimetric test. The test is performed on a biochemistry automated system (COBAS: INTEGRA 400/800 Roche).

The assay method described below is based on the work of Wahlefeld **(Wahlefeld *et al*., 1974),** which uses the enzyme LPL (Lipoprotein lipase) for rapid and complete hydrolysis into glycerol and fatty acids. In the first reaction, this glycerol is reacted with glycerol kinase (GK) to give glycerol-3-phosphate; in the second reaction, the resulting compound is then oxidized to dihydroxyacetone-phosphate to form hydrogen peroxide, in the presence of glycerol peroxidase (GPO). The hydrogen peroxide (H_2O_2) formed reacts with 4-aminophenazone and 4-chlorophenol to form a red-colored derivative (end-point method). The intensity of the red

color developed is directly proportional to the concentration of triglycerides in the sample, and is measured photometrically.



III.4.4 Determination of plasma urea levels (Myara *et al.*, 1994)

Plasma urea is determined by a colorimetric method based on the use of diacetyl monooxine and Fe3+ ions. In the presence of Fe3+ ions and a reducing agent, urea reacts with diacetyl monooxine to give a pink-colored complex. The color obtained is proportional to the amount of urea present in the sample. The reading is taken at a wavelength of 525 nm.

III.4.5 Determination of plasma creatinine levels (Long *et al.*, 1984)

Plasma creatinine is determined by a colorimetric method based on the reaction of picric acid with creatinine in a basic medium to form a yellow-orange complex. Color intensity is measured at a wavelength of 530 nm

III.4.6 Growth hormone analyses

Measurement of growth hormone levels to evaluate the impact on growth and metabolic regulation. Knowledge of GH has increased with the purification of GH from a growing number of species, combined with the development of measurement techniques. One such technique is the radioimmunoassay (RIA), which has the advantage of being sensitive, accurate and easy to use. This technique is commonly used to detect peptides and other hormones present in small quantities, from nanomolar to picomolar **(Peake *et al.*, 1979, Lauritzen *et al.*, 1994)**.

IV. Histological Analysis procedures

The selected fish organ (liver, gill, kidney, and intestine) was placed in formalin-containing vials until they were analyzed for histopathological alterations. This microscopic analysis was carried out in the internal anatomy laboratory of Mohamed Boudiaf Hospital in Ouargla. The standardized process was conducted in two main stages: preparation of the histological sections and observation of the sections under the microscope.

IV.1 Fixing samples

After taking the organs of interest (liver, kidneys, gills and intestine), they are cut into small fragments of appropriate size (0.5-1 cm) and placed in cassettes. These cassettes are then immediately immersed in a liquid fixer, usually 10% buffered formalin, to preserve their structure.

Figure 08: Cut-off and stabilization phase **(Research work, 2024).**

IV.2 Dehydration

The aim of this phase is to prepare the tissues for embedding in paraffin. This process is carried out automatically, involving the movement of cassettes through a series of steps **(Figure 09):**

- Samples undergo gradual dehydration by passing through 5 alcohol baths of increasing concentrations (70%, 90%, 100%). This step removes all water from the tissues before embedding in paraffin.
- Samples are then immersed in 4 xylene baths followed by 3 paraffin baths. This step makes the tissue translucent and permeable to paraffin.

Figure 09: Automatic sample preparation system **(Research work, 2024).**

IV.3 Paraffin inclusion

• The samples are then impregnated with melted paraffin in a vault at 58-60°C. They are embedded in molds filled with paraffin, which, once cooled, forms a hard block that is easy to cut.

Figure 10: Paraffin inclusion center **(Research work, 2024).**

IV.4 Microtome cutting

- Using a device called a microtome, paraffin blocks are sliced into thin sections, typically 4-6 microns thick.
- The resulting sections are collected on glass slides to be colored.

Figure 11: microtome devise **(Research work, 2024).**

IV.5 Deparafinisation and coloration

The sections undergo several baths to deparaffinize them:

- Three times in xylene, each time for 5 minutes.
- Two times in alcohol, each time for 2 minutes, followed by washing.

Next, the staining process includes the following steps:

1. Immersing the slides in a solution of Harris hematoxylin for 4 minutes, which marks the nuclei in blue/violet.
2. Abundant rinsing with running water until a clear nuclear blue is obtained.
3. Differentiation for a few seconds using a solution of absolute alcohol (700ml), hydrochloric acid (10ml), and water (300ml).
4. Rinsing again with water, followed by immersion in lithium carbonate for a few minutes and washing with water.
5. Cytoplasmic coloration with eosin: Immersing the slides in an alcoholic eosin solution for 4 seconds.

6. Dehydration by quick passes in alcohol: 70%, 95%, and two baths of 100% (2 minutes each).
7. Brightening the sections by immersing them four times in xylene baths, each for 2 minutes.

Figure 12: Painting device and de-painting **(Research work, 2024).**

IV.6 Blade mounting

1. Place one to two drops of EUKITT on the blade.
2. Cover with another slide to avoid air bubbles.
3. Allow to dry outdoors or in a 60°C oven for a few hours.

IV.7 Microscopic analysis of sections

To analyze the sections, we used phase-contrast photonic microscopy equipped with a camera. Observations were carried out at 10x and 20x magnifications.

V. Statistical study

Results are given as mean and standard deviation, using Exel software. Statistical evaluation is performed by comparing the means of the Abamectin-treated groups with those of the control groups, using ANOVA analysis of variance. The data on pH, temperature, morpho-physiological characteristics, growth parameters, and semi-quantitative histological analysis were subjected to descriptive and univariate statistical analysis, including graphs, with results expressed as mean ± standard error.

Chapter 3 Results and Discussion

I. Physico-chemical parameters of farm water

In order to minimize potential stress factors such as pH, temperature and salinity, priority was given to ensuring optimal environmental conditions for rearing test fish. This was done as a preliminary step before starting the ecotoxicity tests.

The water used for fish rearing at the Hassi Ben Abdellah pilot shrimp farm is systematically monitored. Experimental data, collected on a daily basis, are presented in figures 11, 12 and 13 below.

I.1. Temperature

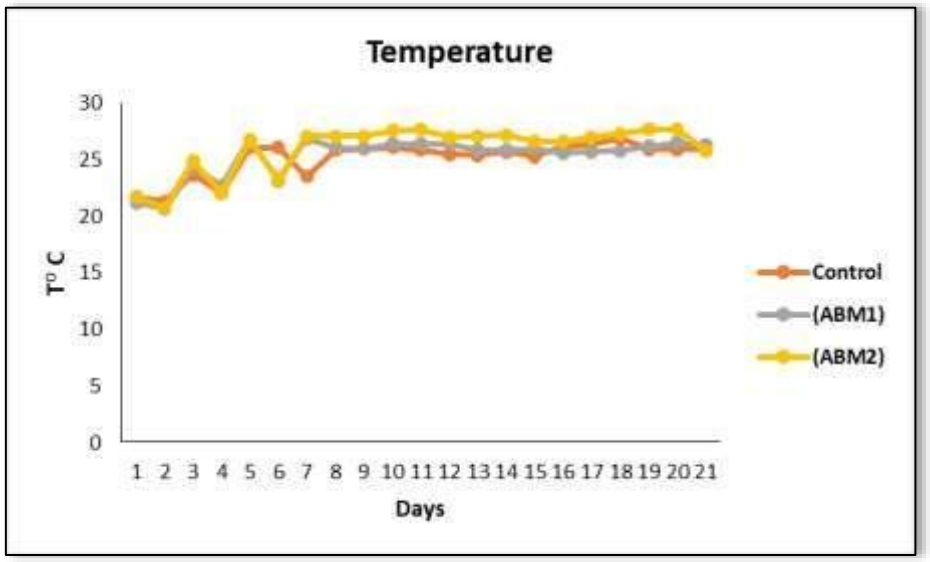


Figure 13: Temperature trends throughout the experimental cycle.

Referring to Figure 11, it is apparent that temperature fluctuation remains virtually constant for all test groups throughout the experimental period. These variations range from 23° to a maximum of 27°C.

The temperature values recorded in our experiment, ranging from 23°C to 27°C, comply with European fish water regulations **(2006/44/EEC and 2006/113/EC)**, which recommend a temperature range of 8°C to 30°C **(Daniel *et al.*, 2001)**.

I.2. Potential of Hydrogen (pH)

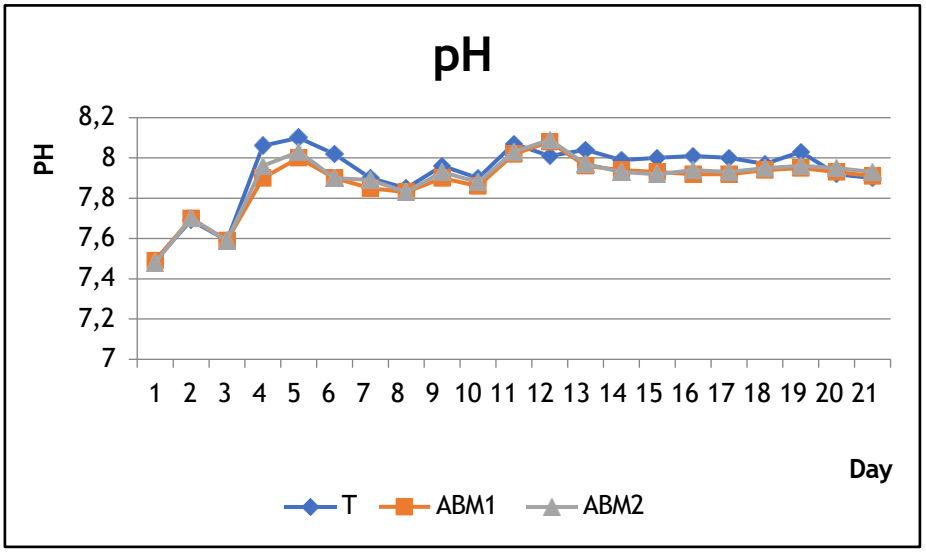


Figure 14: Evaluation of pH throughout the experimental cycle

The potentiel of hydrogen (pH) range of 7.5 to 8.1 falls within the typical range for most aquatic settings, which is mildly alkaline. Our findings are consistent with research conducted by **(Alvarez *et al.*, 2005)**, which concluded that variations in pH between 7.5 and 8.5 did not significantly affect the variety and quantity of benthic macroinvertebrates in freshwater streams.

I.3. Dioxygen (O2)

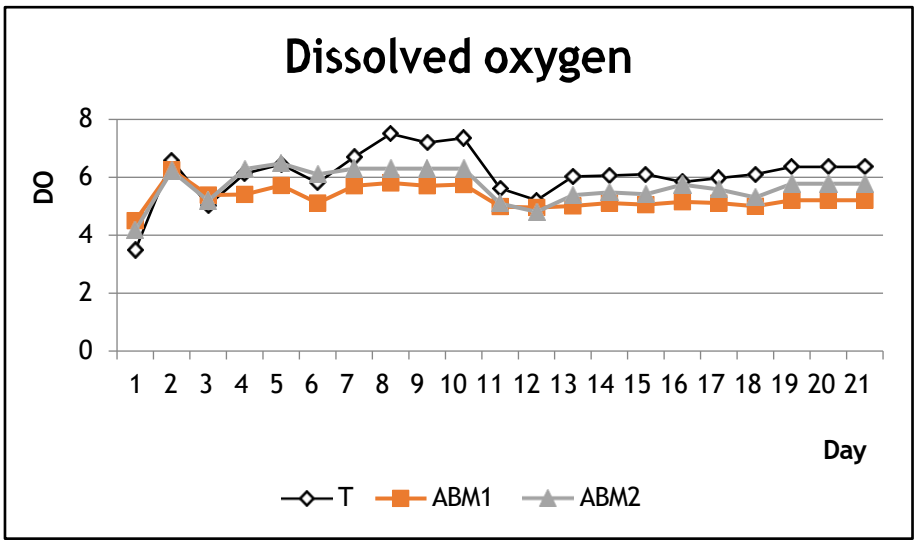


Figure 15: Dioxygen evolution throughout the experimental cycle

Regarding Figure 13, it is evident that throughout the experiment, the dioxygen variation remained almost constant across all test groups, ranging from 3 to a maximum of 7. Numerous controlled environment experiments have demonstrated that oxygen levels tend to remain relatively stable over time, typically within this range. Furthermore, earlier research by **(Stephens, *et al* 2011)** supports these findings, indicating that a dioxygen range of three to seven is common in similar experimental setups.

II. Study of the effect of abamectin on bioenergetic parameters

II.1 Daily weight gain

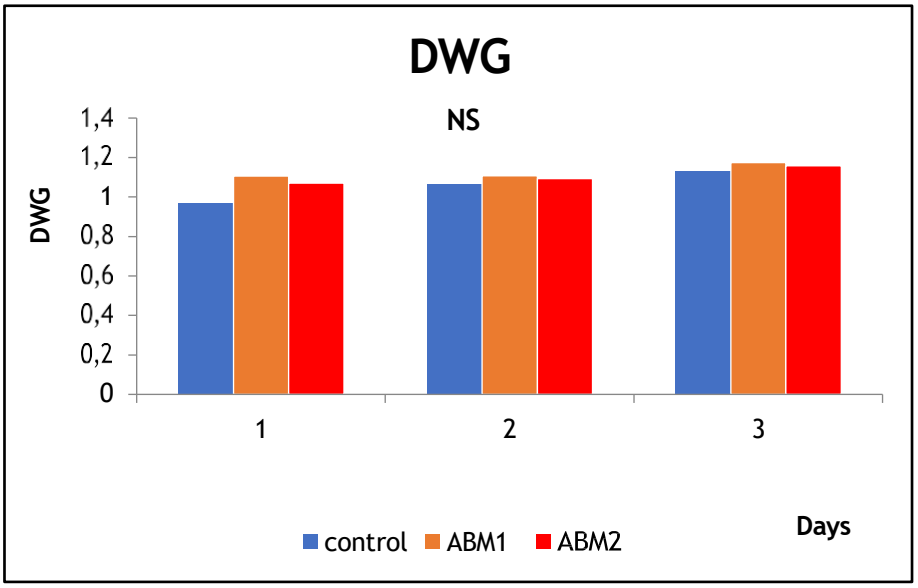


Figure 16: Average values for daily weight gain. Series 1: control. Series 2: fish group exposed to ABM1 (50 µg/L). Series 3: fish group exposed to ABM2 (100 µg/L). Each value represents the mean ± standard deviation (n = 5 per group). *P < 0.05; **P < 0.01, *** P < 0.001, Ns : non-significant differences; P > 0.01.

Our study's findings indicated that a similar trend in the calculation of daily weight gains in the different test groups both during the adaptation phase and during contamination, with no significant differences between the control and abamectin-exposed groups. This lack of variation suggests that abamectin exposure did not significantly impact the growth rates of the tilapia under the conditions tested. However, in other studies, a clear distinction has emerged between control fish and those exposed to various pollutant concentrations during the contamination phase **.** Numerous studies have shown that abamectin may interfere with the

function of some important enzymes involved in the cellular metabolism of fish **(Jones *et al.*, 2019)**. The fish exposed to abamectin may have growth, reproduction, and cellular repair imbalances as a result of this enzymatic inhibition, which might jeopardize their survival and development **(Xu *et al.*, 2018).** However, other studies align with our findings, suggesting that at sub-lethal concentrations or under specific conditions, the impact on growth might be minimal **(Santos *et al.* 2018)** found that the effects of pesticides on fish growth could vary widely depending on the species, exposure duration, and environmental conditions.

II.2 The hepatosomatic index (HSI)

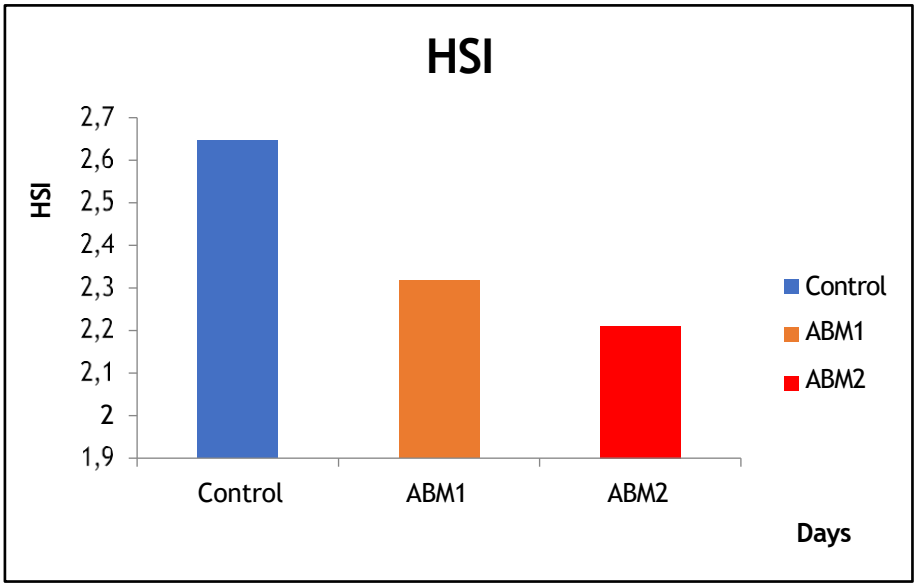


Figure 17: Average values for hepatosomatic index (HSI). Series 1: control Series 2: fish group exposed to ABM1 (50 µg/L). Series 3: fish group exposed to ABM2 (100 µg/L). Each value represents the mean ± standard deviation (n = 5 per group). *P < 0.05; **P < 0.01, *** P < 0.001, Ns : non-significant differences; P > 0.01.

The hepatosomatic index (HSI), is a metric used to assess the health and condition of fish based on the relative size of their liver to their body mass.

Our study findings reveal variations in liver index ratios among the three different groups, notably demonstrating a gradual decline in the groups exposed to abamectin compared to the control group.

This finding is supported by a study by **(Zahri *et al.*, 2022)** which observed seasonal changes in the liver somatic index of Nile tilapia (Oreochromis niloticus) in a Mediterranean

stream. This research revealed seasonal variations in the hepatosomatic index of Nile tilapia, with a progressive decrease over time .

Overall, monitoring the hepato-somatic relationship provides valuable insights into the health and condition of fish populations and can help researchers and aquaculturists assess the impact of environmental stressors, pollutants, or management practices on fish physiology and well-being.

III. Influence of Abamectin on Fish Physiology

Abamectin can impact fish physiology through various mechanisms. It can directly affect the nervous system of fish, causing significant disruptions in essential physiological processes such as respiration, osmotic regulation, metabolism, and immune function.

III.1 Plasma glucose level

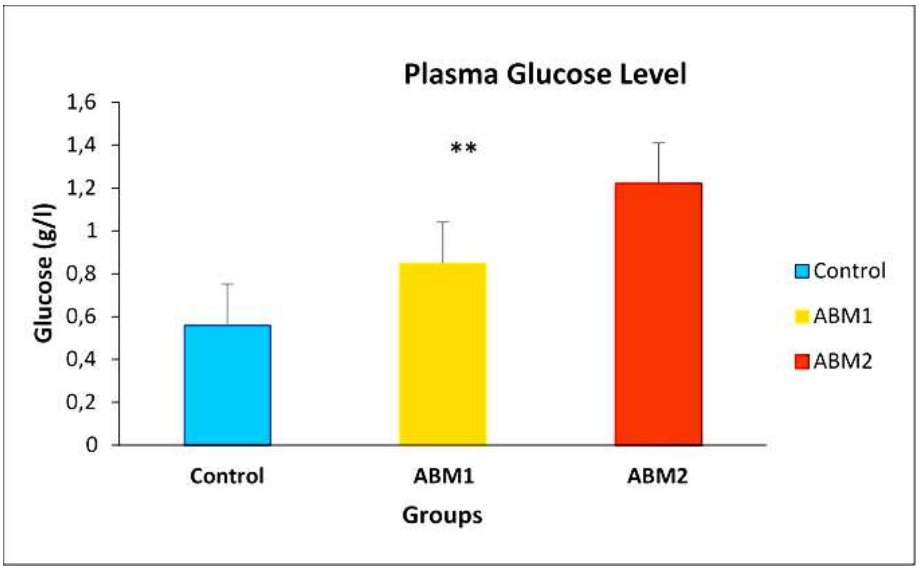


Figure 18: Influence of exposure to ABM1 (50 µg/L) and ABM2 (100 µg/L) on glucose levels in Oreochromis sp. Each value is expressed as mean ± standard deviation (n = 5 per group).*P < 0.05, **P < 0.01, *** P < 0.001, Ns: no significant difference

When tilapia is exposed to abamectin, it can trigger physiological reactions, potentially leading to alterations in blood glucose levels. Glucose serves as a vital energy source for fish, and disruptions in its metabolism can impact their well-being. As depicted in Figure 16, exposure to abamectin elicits a stress response in Oreochromis sp., potentially elevating glucose levels in their bloodstream.

Pesticide exposure, including abamectin, can induce stress in fish and trigger the release of stress hormones, such as cortisol. Cortisol plays a crucial role in the regulation of glucose metabolism by promoting gluconeogenesis **(Mommsen *et al* 1999).** Consequently, elevated cortisol levels due to pesticide stress can lead to an increase in circulating glucose levels in the bloodstream.

In a study conducted by **(Naqvi *et al.*, 2016)**, the effects of abamectin exposure on the freshwater fish Channa punctatus (spotted snakehead) were investigated. The researchers observed that when fish were exposed to sublethal quantities of abamectin, there was a significant increase in blood glucose levels along with other stress-related indicators such as cortisol and lactate dehydrogenase activity. The scientists hypothesized that the stress response triggered by exposure to abamectin, which stimulated processes like glycogenolysis and gluconeogenesis, likely contributed to the elevated glucose levels.

III.2 Plasma cholesterol level

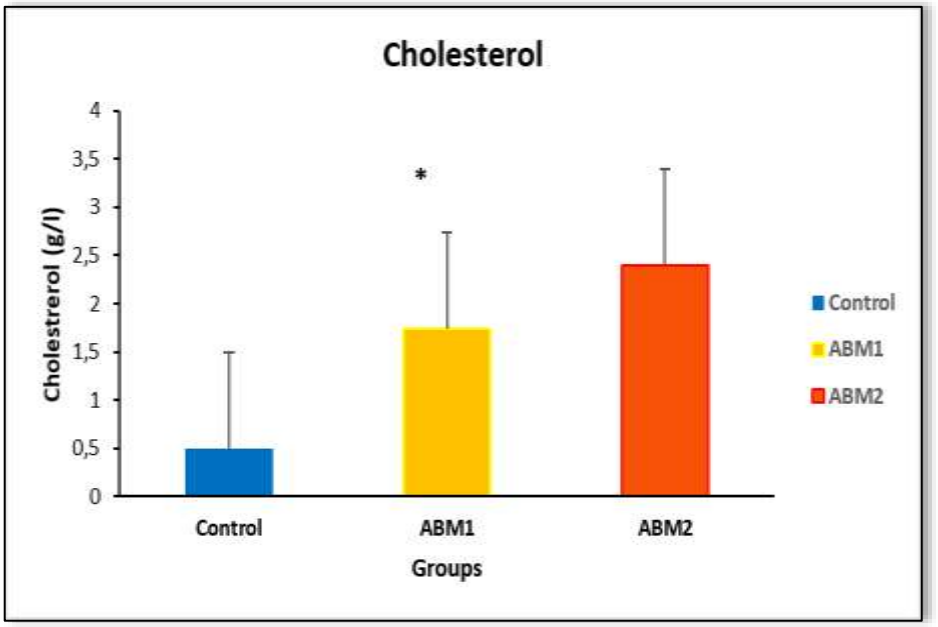


Figure 19: Influence of exposure to ABM1 (50 µg/L) and ABM2 (100 µg/L) on cholesterol levels in Oreochromis sp. Each value is expressed as mean ± standard deviation (n = 5 per group). *P < 0.05, **P < 0.01, *** P < 0.001, Ns: no significant difference

Cholesterol is an important component of cell membranes and serves as a precursor for various hormones and bile acids. Changes in cholesterol levels can indicate alterations in lipid metabolism, which may be linked to stress, toxicity, or metabolic disruption caused by abamectin exposure.

As depicted in Figure 17, exposure to abamectin elicits a significant elevation in cholesterol level in the groups exposed to abamectin compared to the control group.

Pesticide exposure can induce a stress response in fish, triggering the release of hormones such as cortisol and catecholamines. These stress hormones play a role in influencing lipid metabolism, including cholesterol synthesis and mobilization (**Kusakabe *et al* 2009)**. Elevated cortisol levels can stimulate cholesterol synthesis in the liver and adipose tissue, thereby contributing to an elevation in circulating cholesterol levels.

In a study by **(Naqvi *et al.*, 2016)** on the freshwater fish Channa punctatus, exposure to sublethal doses of abamectin resulted in a significant increase in blood cholesterol levels. The researchers proposed that the stress response triggered by abamectin, which disrupted normal lipid metabolism and cholesterol homeostasis, may underlie this observation.

Furthermore, in a separate study, serum cholesterol levels significantly increased after exposure to the herbicide fenvalerate in research conducted by **(Prasanthi *et al.*, 2005)** on the freshwater fish Cyprinus carpio. The authors hypothesized that the oxidative stress induced by the pesticide could hinder enzyme activities involved in lipid metabolism and transport, potentially leading to disturbances in cholesterol homeostasis and lipid metabolism.

III.3 Plasma Triglyceride's level

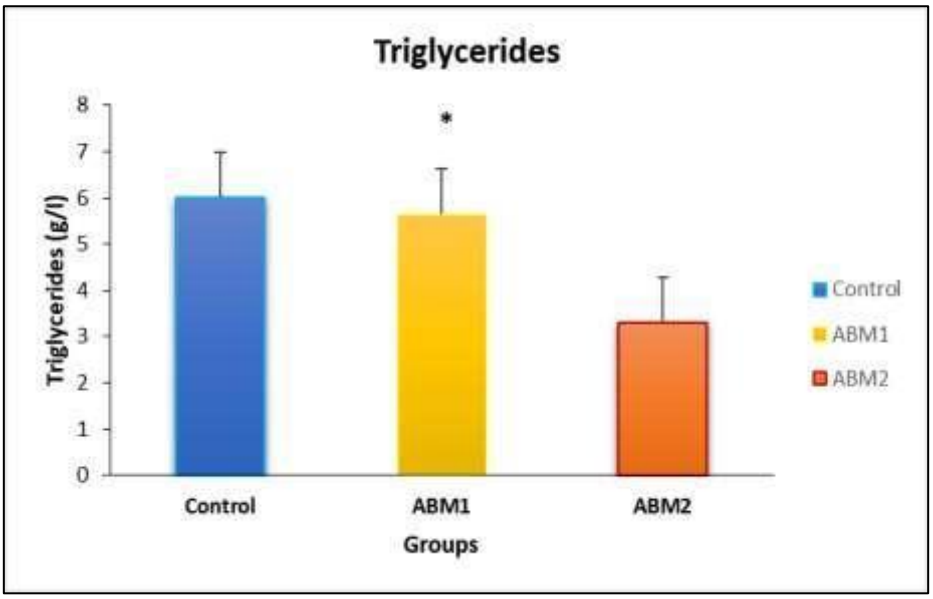


Figure 20: Influence of exposure to ABM1 (50 µg/L) and ABM2 (100 µg/L) on triglycerides levels in Oreochromis sp. Each value is expressed as mean ± standard deviation (n = 5 per group). *P < 0.05, **P < 0.01, *** P < 0.001, Ns: no significant difference

Exposure of Oreochromis sp. to abamectin can significantly affect triglyceride levels in their bodies, as evidenced by Figure 18, which demonstrates a marked decrease in plasma triglycerides in contaminated groups compared to the control group. This observation is consistent with findings from various scientific studies. For instance, the research conducted by **(Naqvi *et al* 2016)** on the freshwater fish Channa punctatus observed a significant decrease in serum triglyceride levels upon exposure to sublethal concentrations of abamectin. The authors suggested that this decrease could be attributed to altered energy utilization and lipid mobilization patterns in response to the stress induced by abamectin exposure. Under stress conditions, fish may rely more on lipids as an energy source, leading to increased triglyceride utilization and a consequent decrease in plasma triglyceride levels.

III.4 Plasma creatinine level

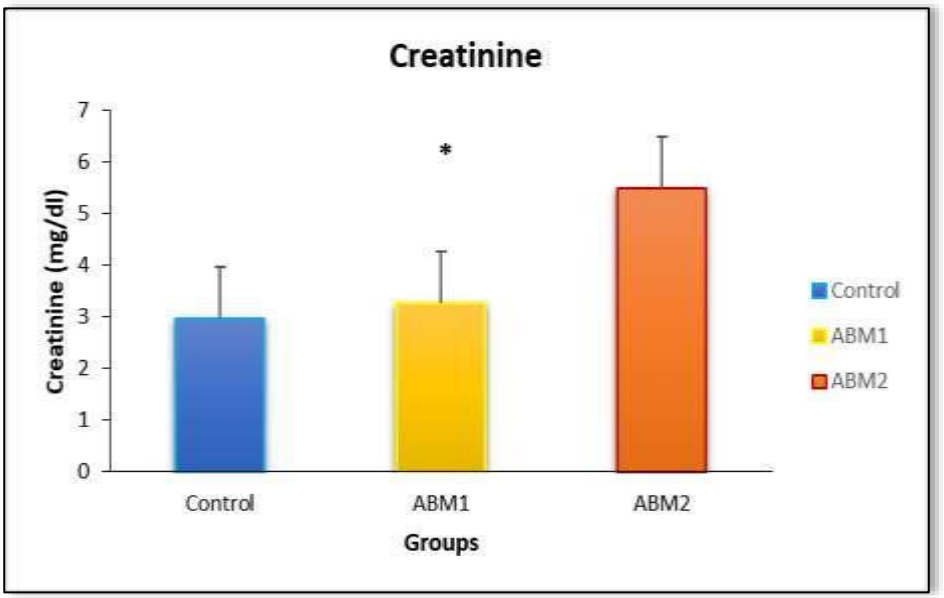


Figure 21: Influence of exposure to ABM1 (50 µg/L) and ABM2 (100 µg/L) on creatinine levels in Oreochromis sp. Each value is expressed as mean ± standard deviation (n = 5 per group). *P < 0.05, **P < 0.01, *** P < 0.001, Ns: no significant difference

Exposure of Oreochromis sp. to abamectin can significantly impact creatinine levels in their bodies, as illustrated by Figure19, which displays a notable increase in plasma creatinine in contaminated groups compared to the control group. This observation aligns with findings from several scientific studies, including research conducted by **(Prasanthi *et al.*, 2005)** on the freshwater fish Cyprinus carpio. The scientists hypothesized that this increase may be connected to the pesticide's nephrotoxic effects, which would decrease renal function and lessen the body's capacity to remove creatinine from the blood.

Furthermore, elevated plasma creatinine levels may also be a sign of increased muscle catabolism or injury, which can be brought on by oxidative stress linked to pesticide use or other toxicity processes **(Huang *et al.*, 2017).**

III.5 Plasma urea level

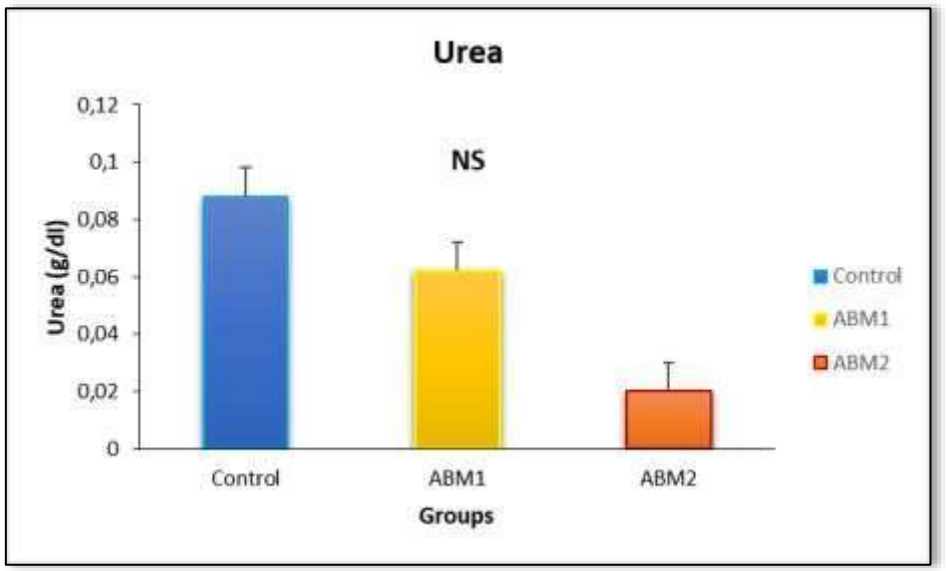


Figure 22: Influence of exposure to ABM1 (50 µg/L) and ABM2 (100 µg/L) on urea levels in Oreochromis sp. Each value is expressed as mean ± standard deviation (n = 5 per group). *P < 0.05, **P < 0.01, *** P < 0.001, Ns: no significant difference

Exposure of Oreochromis sp. to abamectin can impact urea levels in their bodies. However, as despite in Figure 20, which shows no significant difference in plasma urea between contaminated groups and the control group. This observation is consistent with findings from several scientific studies. In the research conducted by **(Ayoola *et al.*, 2008)** on the Nile tilapia (Oreochromis niloticus), exposure to the pesticide cypermethrin did not result in significant changes in plasma urea levels compared to the control group. The authors suggested that the lack of significant differences could be attributed to the efficient excretion of urea by the kidneys or the specific exposure conditions and duration of the study. While these studies indicate that exposure to certain pesticides, including abamectin, may not significantly alter plasma urea levels in some fish species, it is important to note that the specific response can vary depending on factors such as the pesticide type, exposure duration, concentration, and the species' physiology and metabolic capabilities. Further research is needed to fully understand the impact of abamectin on urea metabolism and excretion in Oreochromis sp.

Urea is a waste product of protein metabolism that is primarily filtered out by the kidneys. If the kidney function is not significantly impaired by abamectin exposure, the clearance of urea from the bloodstream may remain unaffected, leading to no significant differences in plasma urea levels between contaminated and control groups **(Huang *et al.*,**

2017). Additionally, some fish species may have efficient mechanisms for regulating urea levels, even in the presence of toxicants.

III.6 Growth hormone (GH)

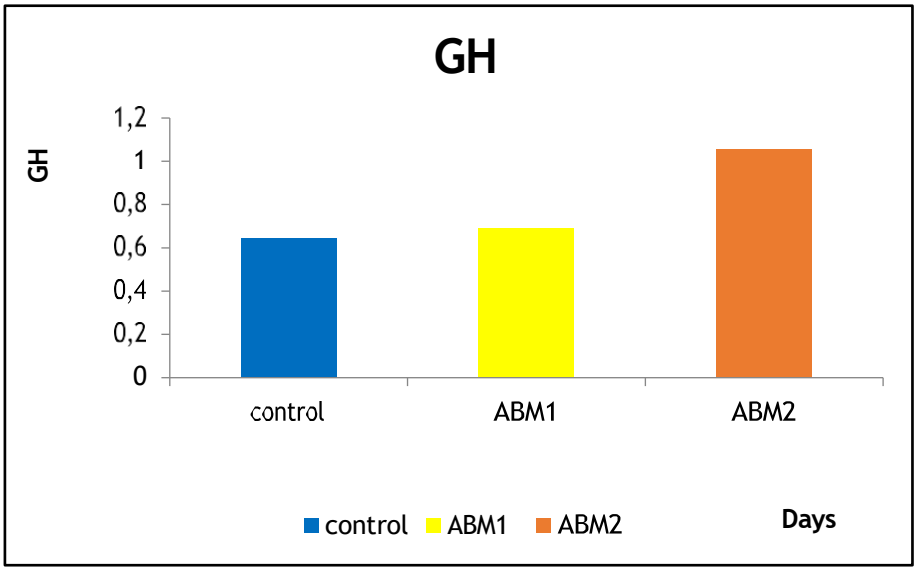


Figure 23: Influence of exposure to ABM1 (50 µg/L) and ABM2 (100 µg/L) on growth hormone levels in Oreochromis sp. Each value is expressed as mean ± standard deviation (n = 5 per group). *P < 0.05, **P < 0.01, *** P < 0.001, Ns: no significant difference

Abamectin exposure can cause physiological changes in tilapia, which may alter blood levels of growth hormone (GH). The primary role of GH in fish involves development and metabolism, regulating several physiological processes such as somatic growth, reproduction, and energy homeostasis. Studies have indicated that although abamectin may interfere with the balance of several hormones in fish, it may also raise the plasma levels of growth hormone (GH) in tilapias. According to a study done on Mozambique tilapia (Oreochromis mossambicus), exposure to abamectin significantly raised the levels of growth hormone in the blood **(Pavlov *et al.*, 2009).** In response to pesticide-induced stress, this rise in GH may represent a compensatory mechanism meant to maintain development and energy metabolism **(Reinecke, 2010).**

The tilapias' response to the stress caused by exposure to abamectin may also include an increase in growth hormone levels in their blood. An investigation on the Nil tilapia (Oreochromis niloticus) revealed that exposure to abamectin resulted in a significant increase in plasma levels of growth hormone and cortisol, indicating an activation of the hypothalamo-

hypophyso-interrenalien axis involved in the stress response **(Khalil *et al.*, 2017**). This hormone response may benefit fish in mobilizing their energy reserves and adjusting to environmental stressors **(Mommsen *et al.*, 1999).**

IV. Histopathology

IV.1. Effect of abamectin on gill

Tissue Histopathological examination results revealed that fish exposed to low concentrations of abamectin exhibited hyperplasia and necrosis of the gill filaments, accompanied by epithelial lifting and alteration of the gill lamellae **(Figure 22A, B).** Conversely, fish exposed to high concentrations of abamectin showed congestions and hemorrhages in the capillaries of the gill arch, presenting as granular acidophilic lamellae with leukocytes at the base of the filaments **(Figure 23C, D).**

Gill tissues play a crucial role in respiration by facilitating the exchange of gases such as oxygen and carbon dioxide. Damage to gill structures can impair this respiratory function, leading to reduced oxygen uptake and potentially affecting the overall health and survival of the fish.

In a study conducted by **(Ehsan et *al.*, 2014)** on the effects of acute fenitrothion insecticide exposure on Nile tilapia fingerlings (Oreochromis niloticus L.), it was observed that the respiratory epithelium and filaments in the control animals' gills were normal. However, acute exposure to fenitrothion resulted in localized epithelial and mucous cell fusion, proliferation, and infiltration of a small number of leukocytes in the gills. There was focal secondary lamellar sloughing, lymphocyte aggregations, and congestion of lamellar capillaries. diffuse growth and fusion of the respiratory epithelium accompanied with bleeding and infiltration of leukocytes.

Light micrograph of gills of Nile tilapia showing Gills of control with normal filaments and respiratory epithelium. Focal epithelial and mucous cells proliferations, fusion and few leukocytic infiltrations. Focal sloughing of the secondary lamellae with aggregations of lymphocytes and congestion lamellar capillaries. Diffuse proliferation and fusion of the respiratory epithelium with hemorrhage and leukocytic infiltration.

Furthermore, according to **(Mohammad et *al.*, 2017)** microphotographs of gills sample showed histopathological changes that resulted in lamellar fusion , hemosiderin , dystrophy in

secondary lamellar ,cirrhosis , degeneration of hepatocytes , dystrophy in lamellar , hyperplasia , necrosis and lamellar bending. These changes were observed in fish exposed to abamectin but not in the control group.

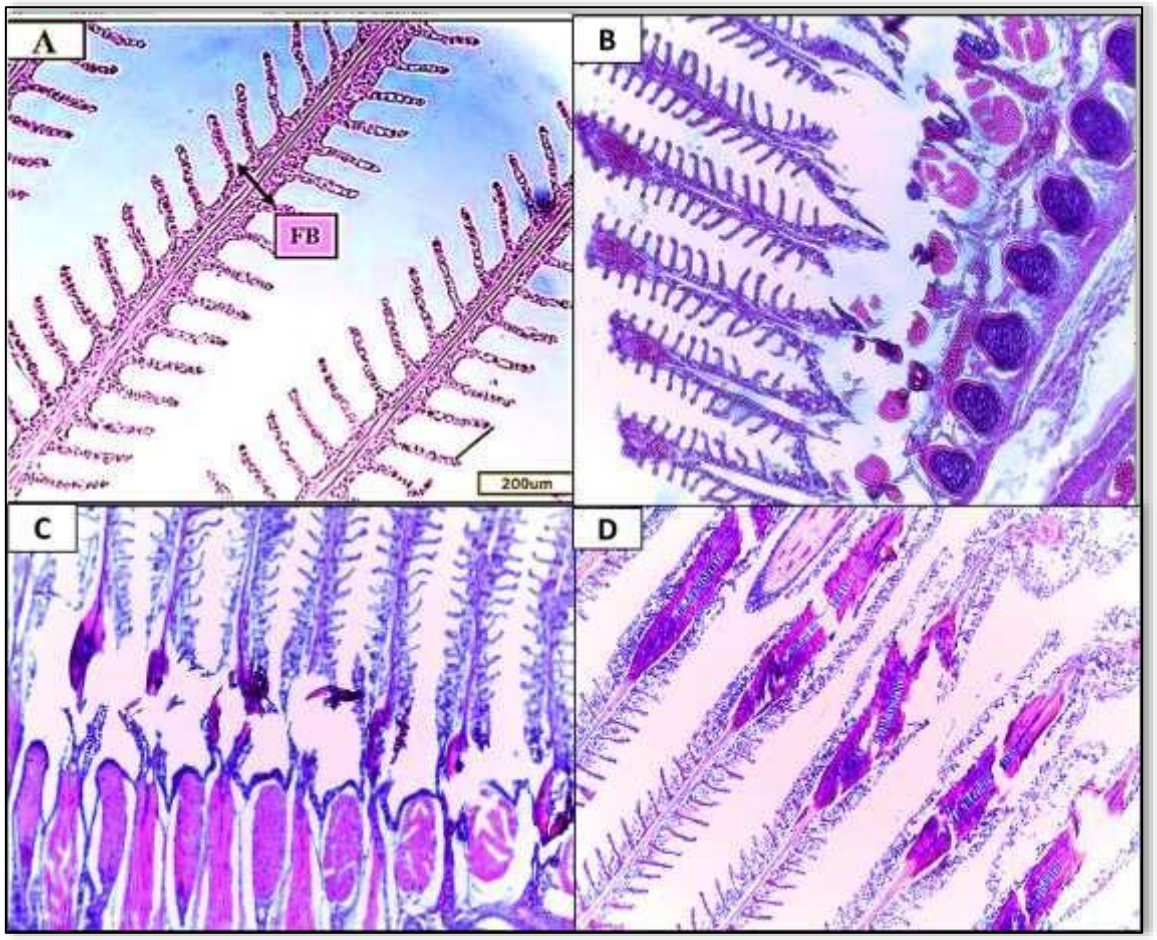


Figure 24: Photomicrographs of gill tissue sections for all experimental groups of Oreochromis sp. Experimental groups of Oreochromis sp." (A). Gills of control fish showing normal histological structure. (B). Gills of fish exposed to 50µg/L abamectin for 14 days, showing lamellar necrosis. (C and D). Gills of fish exposed to 100 µg/Lof abamectin for 14 days, showing infiltration of acidophilic cells granular cells at the base of the filling (GR X10). Where FB: branchial filament

IV.2. Effects of abamectin on the liver

Exposure to abamectin, a commonly used insecticide and acaricide, can have significant effects on the liver of aquatic organisms. The liver serves as a vital organ responsible for numerous physiological functions essential for the overall health and well-being of the organism **(Al-Kahtani *et al*. 2011)**.

Our findings have revealed that Tilapia fish subjected to abamectin exposure for a period of 14 days exhibited notable histopathological changes in the liver, including vacuolization, blood congestion, and hepatocyte necrosis. Consistent with our findings, other studies have shown that exposure to abamectin can induce various alterations in hepatic tissues, such as hepatocellular necrosis, inflammation, and fatty degeneration. In the study conducted by **(Mohammad *et al.*, 2017)** microphotographs of liver samples from Cyprinus carpio juveniles exposed to different concentrations of abamectin (2 mg/l, 3 mg/l, 6 mg/l) revealed liver lesions. These included hemosiderin, cirrhosis, degeneration of hepatocytes, nuclear karyolysis, primary biliary cirrhosis, and swelling.

Additionally, a study conducted by **(Kabir *et al*., 2019)** showed that in the control group, the hepato-pancreas, hepatic cell, and other cells were normal and grouped in a systematic manner, however liver sections subjected to sumithion pesticide revealed bleeding, necrosis, vacuole, degraded hepatic cells, and perforated hepato-pancreas. After seven days of exposure, liver tissue from tilapia subjected to 0.025 ppm revealed perforated hepato-pancreas. After seven days of exposure, a dosage of 0.10 ppm caused vacuole and bleeding, while a dosage of 0.05 ppm resulted in necrosis and a ruptured hepato-pancreas. At a dose of 0.025 ppm, ruptured hepato-pancreas, necrosis, and vacuoles were discovered after 15 days of exposure. After 15 days of exposure, vacuole and ruptured hepato-pancreas were discovered at a dosage of 0.05 ppm, and blood vessel and vacuole were also observed at a dosage of 0.10 ppm.

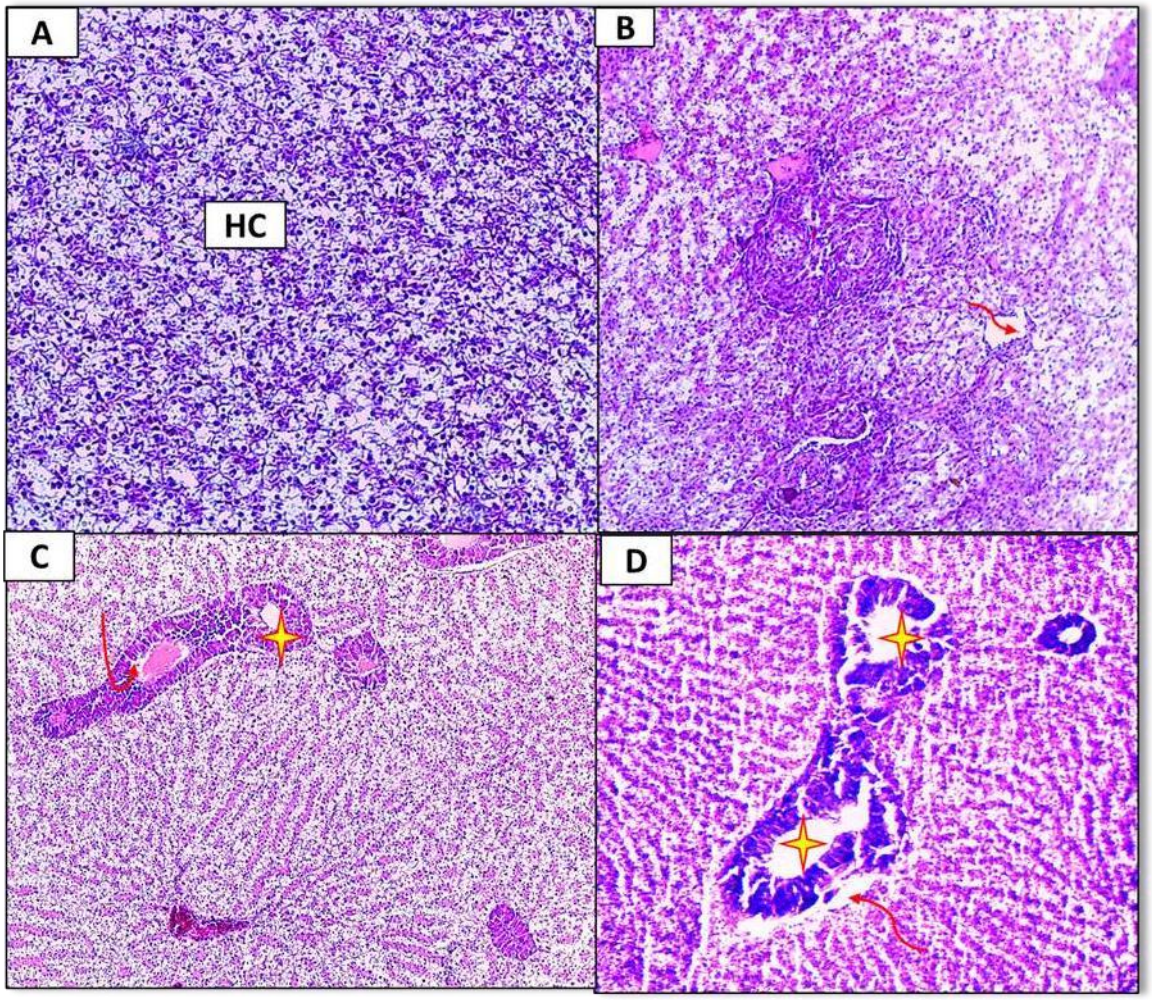


Figure 25. Photomicrographs of liver sections from all experimental groups of Oreochromis sp. The control group (A) sections showing normal hepatocytes. The group exposed to 50 µg/L abamectin for 14 days (GRX10). (B, C) sections showing degenerative (arrows) and necrotic (star) changes in most of the liver parenchyma (GRX10). The group exposed to 100 µg/L abamectin for 14 days (D) sections showing congestion of some hepatic blood vessels (arrows) and partial destruction of pancreatic acini (star) (GRX40).

IV.3. The effects of abamectin on the kidney

In fish, the kidney plays a vital role in maintaining internal homeostasis by regulating electrolyte balance, osmoregulation, and waste excretion (**Takvam *et al.*, *al* 2021)**

Fish kidneys are composed of nephrons, similar to those in mammals, which filter waste products and excess ions from the blood.

The effects of abamectin on the kidney can vary and may include histopathological alterations, disruptions in renal function, and cellular damage. Our findings have highlighted that fish exposed to abamectin for a period of 14 days exhibited degenerative and necrotic

alterations in the kidneys. These alterations include localized hemorrhages between degenerated renal tubules, dilation of Bowman's space, vacuolization of the tubules, as well as changes in the glomeruli. These observations align with numerous studies that have shown exposure to abamectin can induce histopathological changes in the kidneys, such as tubular lesions, inflammation, and interstitial fibrosis.

According to the study of **(Ehsan *et al.*, 2014**) the control group's cortex was found to be normal, displaying normal renal corpuscles with Bowman's capsules. A tuft of blood capillaries makes up the renal glomeruli by and large. Normal cuboidal epithelial lining was seen in sections of the proximal and distal convoluted tubules. Fish with acute Fenitrothion exposure had significant regions of necrosis in their kidneys, which were focally displaced with lymphocytes. The renal tubules have extensive hemorrhages visible throughout them. The majority of glomeruli had dilated Bowman's space and were constricted, while some others had modest glomerular tuft growth with lobulated capillaries. Additionally, a study conducted by **(Nannu *et al.*, 2015)** showed hematological and histo-architectural damages in the kidney and liver of Nile tilapia on exposure to kinalux in contrast to the control. Additionally, dose-dependent differences were shown in the fish treated with kinalux and their hematological characteristics. Hematopoietic cells and kidney tubules were regular and placed in a systematic manner in the controltherapy. Kidney portions subjected to Kinalux demonstrated disintegration of huge intracytoplasmic vacuoles in the lumen and epithelial cells of convoluted tubules. There were additional observations of glomerulus shrinkage and degeneration, as well as dilatation inside the Bowman's gap.

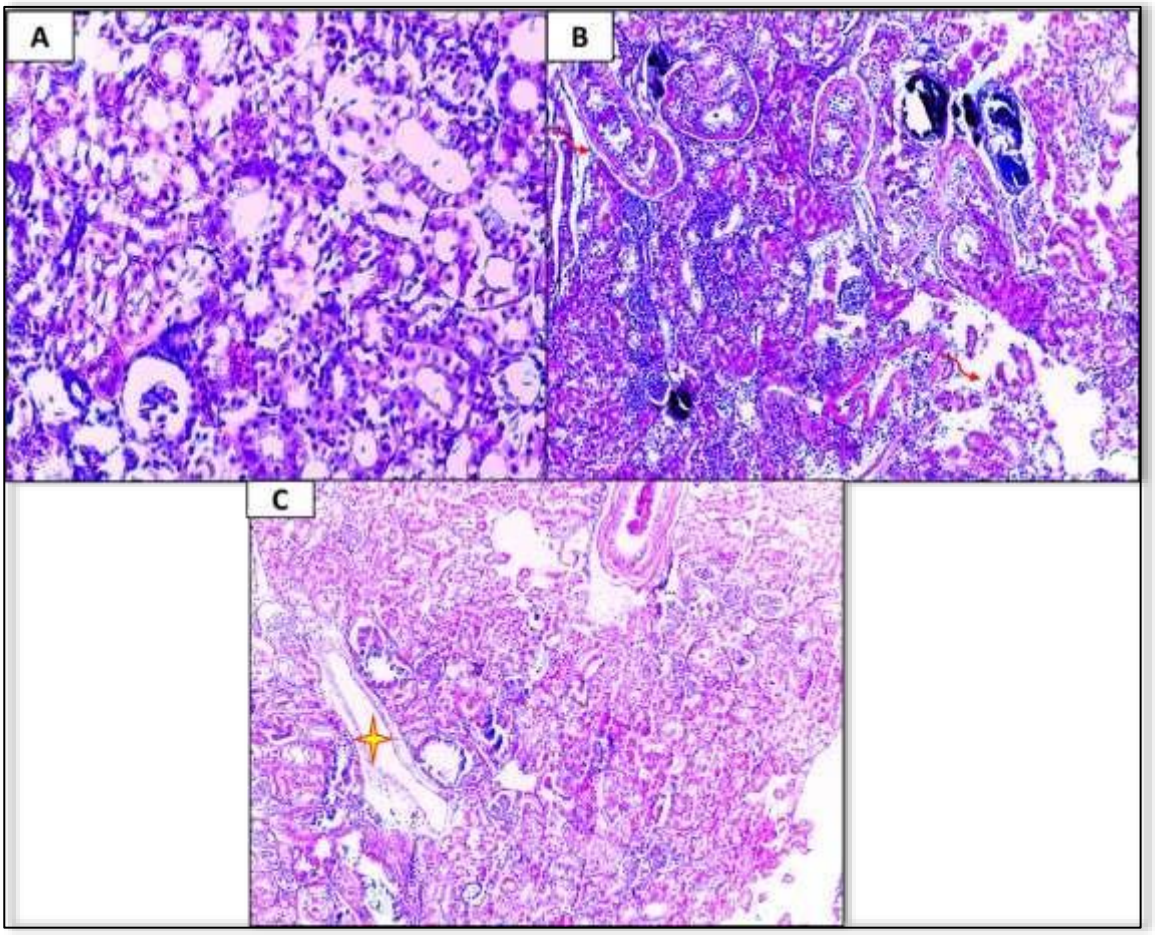


Figure 26. Photomicrographs of kidney sections from all experimental groups of Oreochromis sp. (A) Kidney of control fish showing normal structure with numerous renal tubules. (B, C) Kidneys of fish treated with 50 and 100 µg/L abamectin for 14 days show focal hemorrhages between degenerated vacuolar renal tubules (GRX10)

IV.3. The effects of abamectin on the intestine

The intestine's significance in the digestive system of tilapia and other animals cannot be overstated. Its key functions encompass digestion, water balance regulation, and immune defense.

Our findings reveal that exposure of Nile tilapia to abamectin can lead to several histological alterations in the intestine tissue. These alterations encompass epithelial damage, resulting in the distortion of tissue architecture, and the presence of hemorrhagic lesions indicative of hemorrhage.

According to **(Mohammad *et al.*, 2017)** microphotographs of intestine sample histopathological changes resulted intestine lesions after exposure to different concentration of abamectin 2 mg/l 3 mg/l 6 mg/l in Cyprinus carpio juveniles fed of dietary isomalto-oligosaccharides. Vacuolation, epithelium degeneration, necrosis, hemosiderin, villous atrophy, lymphocytes penetration, degradation of intestinal epithelium and villous degeneration were detected .

These alterations indicate that exposure of Nile tilapia to abamectin can result in several histological changes. These histological alterations reflect the detrimental effects of abamectin on the intestinal epithelium and associated tissues in Nile tilapia. In conclusion, these changes can disrupt not only normal intestinal function but also affect other organs previously mentioned.

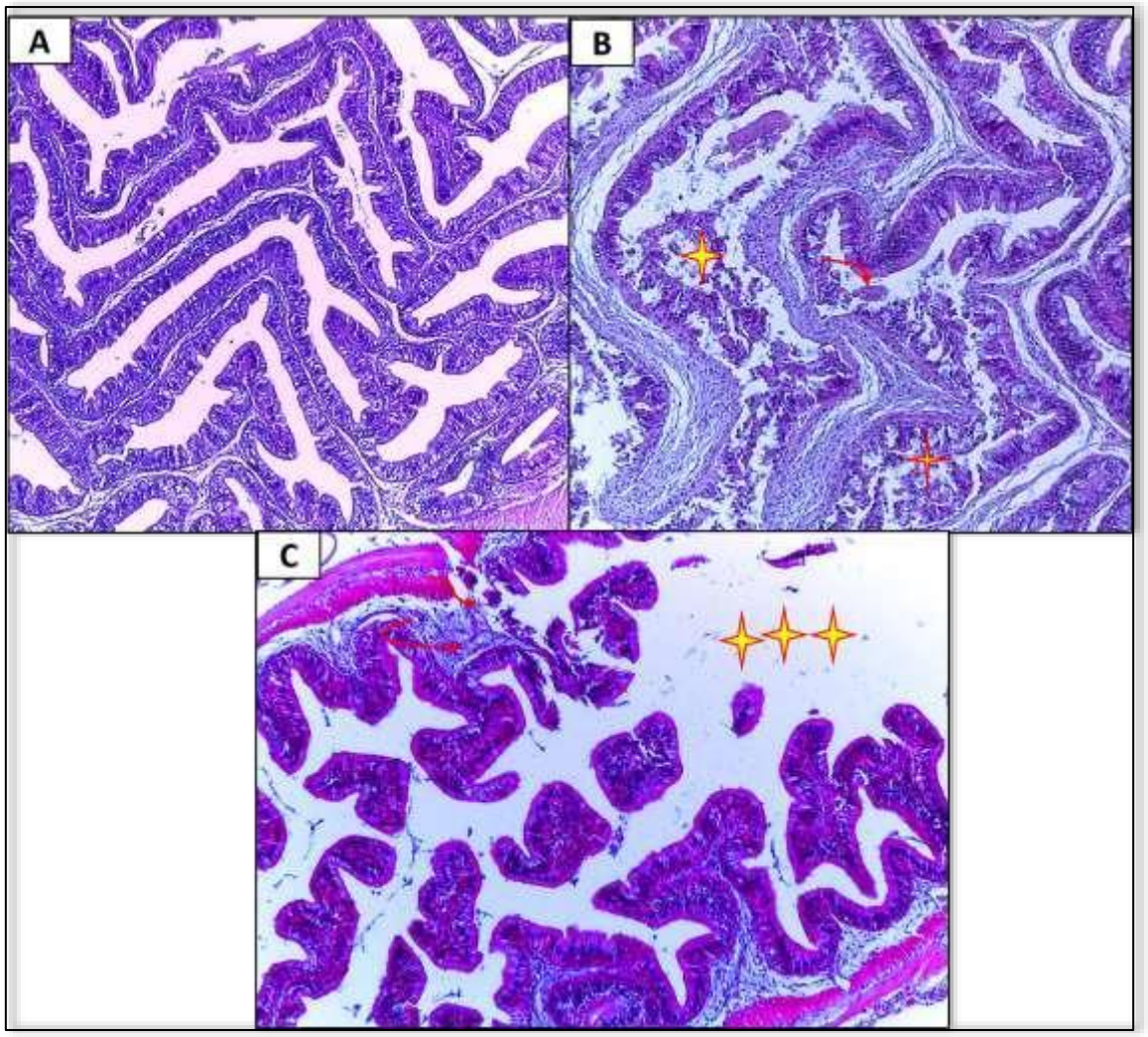


Figure 27: Histological alteration in intestine tissue of Nile tilapia in the control group (A) showing normal morphology and architecture. (B, C) Intestine of fish treated with 50 and 100 µg/L abamectin for 14 days show lesion and necrosis (GRX10)

Conclusion and prospects

In summary, our research focuses on evaluating the toxicity of abamectin using the red tilapia (Oreochromis sp.) as an animal model. The results of our study significantly highlight the toxicity of the insecticide abamectin on the physiology of tilapia (Oreochromis sp.). This toxicity is evident through alterations in biochemical parameters, including elevated glucose, cholesterol, and creatinine levels, as well as a decrease in triglycerides levels.

It is important to note that despite the exposure to abamectin, the growth and hepatosomatic ratio (HSR) of the tilapia were not affected. However, this exposure had detrimental effects on the fish's organs, causing degenerative and necrotic lesions in the liver, gills, kidneys, and intestines.

Therefore, it is crucial for policymakers to choose pesticides that are less harmful to the environment, particularly by prioritizing the use of biological pesticides, and to ensure the responsible use of these products.

In the future, it would be prudent to continue this research by:

1. Investigating the long-term effects of abamectin exposure on fish physiology and health.
2. Exploring the impact of abamectin on other aquatic organisms and ecosystems.
3. Evaluating the effectiveness and safety of alternative, environmentally friendly pesticides.
4. Implementing strategies to mitigate the adverse effects of pesticide exposure on aquatic life.

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Abstract: The presence of pesticides in aquatic ecosystems primarily results from agricultural runoff. Pesticides, being generally non-selective, exhibit toxicity towards aquatic fauna. Fish, as common dietary components, often accumulate contaminants from their aquatic habitats. This study aimed to assess the impact of exposure to two concentrations (50 and 100 µg/L) of the insecticide abamectin over a 14-day period on the metabolic and hormonal systems of freshwater fish of the species Oreochromis sp. The findings indicated that abamectin exposure induced significant metabolic and hormonal changes. Comparative analysis with control groups revealed histopathological alterations in the organs of the abamectin-exposed groups. In conclusion, our results confirm the toxicity of the insecticide abamectin in aquatic environments.

Keywords: aquatic ecosystems, abamectin, Oreochromis sp, metabolic, hormonal, histopathological alterations, toxicity.

ملخص: يرجع وجود مبيدات الآفات في النظم الإيكولوجية المائية بشكل رئيسي إلى الجريان السطحي الزراعي. وتعتبر مبيدات الآفات، التي تكون غير انتقائية بشكل عام، سامة للحيوانات المائية. وغالبًا ما تتراكم الملوثات من موائلها المائية في الأسماك، باعتبارها مكونات غذائية شائعة. كان الهدف من هذه الدراسة هو تقييم تأثير التعرض لتركيزين (50 و100 ميكروغرام/لتر) من المبيد الحشري الأبيامكتين على مدى 14 يومًا على الأنظمة الأيضية والهرمونية لأسماك المياه العذبة البلطي الأحمر .

أشارت النتائج إلى أن التعرض للأبيامكتين أحدث تغيرات استقلابية وهرمونية كبيرة. كشف التحليل المقارن مع المجموعات الشاهدة عن تغيرات نسيجية في أعضاء المجموعات المعرضة للأبامكتين. وفي الختام، تؤكد النتائج على سمية مبيد الأبامكتين الضارة في البيئات المائية.

الكلمات المفتاحية: النظم الإيكولوجية المائية، الأبيامكتين ، البلطي الأحمر، الأبيض ،الهرمونات، التغيرات النسيجية، السمية

Résumé : La présence de pesticides dans les écosystèmes aquatiques résulte principalement du ruissellement agricole. Les pesticides, généralement non sélectifs, présentent une toxicité pour la faune aquatique. Les poissons, en tant que composants alimentaires communs, accumulent souvent des contaminants provenant de leurs habitats aquatiques. Cette étude visait à évaluer l'impact de l'exposition à deux concentrations (50 et 100 µg/L) de l'insecticide abamectine sur une période de 14 jours sur les systèmes métaboliques et hormonaux des poissons d'eau douce de l'espèce Oreochromis sp. Les résultats ont indiqué que l'exposition à l'abamectine induisait des changements métaboliques et hormonaux significatifs. L'analyse comparative avec les groupes témoins a révélé des altérations histopathologiques dans les organes des groupes exposés à l'abamectine. En conclusion, nos résultats confirment la toxicité de l'insecticide abamectine dans les milieux aquatiques.

Mots-clés : écosystèmes aquatiques, abamectine, Oreochromis sp, métaboliques, hormonales, histopathologiques, toxicité.

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