(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Histopathological And Biochemical Changes In Larvae Of Channa Punctatus Exposed To Thifluzamide

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[1]*Received: 07/03/2023; Accepted: 11/08/2023; Published: 03/10/2023*

ABSTRACT

This study examines the histological and biochemical alterations in Channa punctatus larvae (spotted snakehead) subjected to different thifluzamide dosages. Several well-controlled lab investigations evaluated the effects on the liver, gills, and kidneys. Enzyme activity and protein levels in these organs were also investigated. Our experiment Channa punctatus larvae to different thifluzamide doses throughout time. Histopathology revealed cellular disintegration, tissue necrosis, and significant structural changes in vital organs such the liver, gills, and kidneys. Thorough liver tissue examination revealed vacuolation and hepatocellular deterioration. However, microscopic gill investigation showed lamellar fusion and epithelial elevation. Renal tissues exhibited extensive glomerular atrophy and tubular degeneration, suggesting renal function loss. The biochemical examination showed considerable enzyme activity and protein alterations, indicating biological system problems. ALT and AST levels increased significantly, indicating hepatic stress and liver failure. Furthermore, antioxidant enzymes like SOD and CAT showed changed activity, indicating oxidative stress responses. Tissue protein levels fluctuated throughout the study, suggesting disruptions in complex metabolic and protein synthesis networks that control cellular function. Importantly, thifluzamide may affect Channa punctatus larvae's growth and survival, according to studies. aquatic environment fungicide contamination ecological impacts. Histological and biochemical changes in fish species after extended thifluzamide exposure indicate that critical physiological processes are adversely impacted. These fascinating findings demonstrate long-term damage to diverse fish populations and aquatic biodiversity. This study underlines the necessity to closely control and monitor agricultural chemical products to decrease their environmental effect and conserve aquatic ecosystems' biodiversity.

Keywords: *Thifluzamide; Channa punctatus; Histopathological changes; Oxidative stress; CAT; SOD; GST; Aquatic toxicology; Environmental impact.*

INTRODUCTION

The widespread usage of various chemical compounds in agriculture has increased crop production and output worldwide. Additionally, it has greatly improved global food security. However, the unintended impacts of these chemicals on non-target animals, particularly in aquatic habitats, have raised environmental concerns. Among the many chemicals used in agriculture, fungicides like thifluzamide are critical for crop health and production. Agricultural products. While this medicine is known to reduce fungal infections, it is important to research the ecological effects of its discharge in neighboring waterways. Thifluzamide's histopathological and biochemical effects on Channa punctatus larvae—spotted snakehead fish—are the study's main focus. Our broad research aims to better understand the environmental effects of fungicide contamination on freshwater environments.

Thifluzamide, a next-generation systemic fungicide, is very effective in fighting several crop-threatening fungal diseases. This chemical effectively inhibits succinate dehydrogenase, a crucial enzyme in fungi's respiratory chain. This restriction severely disrupts these creatures' development and reproduction. Multiple scientific investigations have shown that thifluzamide improves crop protection, yields, and quality. However, prolonged use of these fungicides may harm ecosystem biodiversity, especially in delicate aquatic settings that are sensitive to agricultural contamination. Channa punctatus is a good candidate for ecotoxicological investigations on freshwater fish species due to its ecological importance and high vulnerability to environmental contaminants. This species, from southern

[1] *How to cite the article:* Singh A., Pandey R., Kumari A., Tripathi N.. (2023); Histopathological And Biochemical Changes In Larvae Of Channa Punctatus Exposed To Thifluzamide; *International Journal of Innovations in Applied Sciences and Engineering*; Vol 8, 72-85

(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

and southeastern Asia, is vital to aquatic habitat food chains, making it a key indicator of environmental protection. This aquatic organism's larvae are very sensitive to contaminating pollutants in their environment. They are an ideal model for researching the complex toxicological effects of damaging chemicals on aquatic ecosystems.

Ecotoxicology recognizes Channa punctatus, however thifluzamide's effects on this species remain unknown. This study examines the histopathological and biochemical alterations in thifluzamide-exposed Channa punctatus larvae to fill gaps in the data. The goal is to analyze vital organs and biochemical indicators to understand the potential risks posed by this fungicide agent to the aquatic ecosystem and advance environmentally friendly and sustainable agricultural methods.

Objectives

This research examines how thifluzamide affects Channa punctatus larval development. The liver, gills, and kidneys will be examined for histological changes. A thorough study will evaluate how different dosages of this strong fungicide affect tissues and structure. The extensive study will concentrate on the organism's biochemical changes, including enzyme activity and protein levels. Understanding possible alterations in complicated metabolic and physiological systems is the major focus. Scientific research combines histopathological and biochemical data to understand thifluzamide's ecological risks. This deepens the conversation on environmental security and the need of sustainable and eco-friendly agriculture.

MATERIALS AND METHODS

A full and rigorous experimental research requires adequate selection of experimental and control groups, precise sample size definition, and specified conditions. do experiments. The research design comprises careful data collection, statistical analysis, and several methods to ensure reliability and validity. An organised experimental design reduces biases, regulates confounding variables, and increases data quality and reliability. Channa punctatus larvae were collected, chosen, and acclimatized, controlled environmental conditions were constructed, and thifluzamide exposure was meticulously timed to research its histological and biochemical consequences.

Collection and Maintenance of Fish Larvae

We obtained juvenile Channa punctatus, or spotted cobra fish, from a high-quality local breeding facility for our study. For stability and correct adaption to the research setting, the larvae were acclimatized for two weeks in the lab. Acclimatization decreases stress and helps larvae acclimate to the lab, improving experimental accuracy. The freshly born creatures were placed in 50-liter glass aquariums filled with chlorine-free tap water to adjust. The water temperature was maintained at 25 ± 2 °C using thermostatically controlled heaters to mimic the organism's natural environment throughout the experiment. Channa punctatus larvae thrive in the stated temperature range.

The laboratory employed an automated lighting system to regulate light and darkness with 12 hours of light and 12 hours of darkness. The cycle controls larvae's temporal rhythms, which may affect their metabolism and physiology. A lighting system was created to illuminate the light stage and completely darken the shadow stage. For optimal water quality, regular examinations were done. Conventional water analysis kits measured acidity, oxygen, ammonia, nitrites, and nitrates. Air pumps and air stones oxygenated the water continuously. To keep hatchlings healthy and decrease waste, each tank's water was changed 50% every two days.

The hatchlings were fed finely crushed commercial fish meal designed for young fish to meet their nutritional needs. To maintain nutritional intake, the meal was eaten at 9 AM and 5 PM. Daily food and rubbish removal was enforced to maintain water quality. This text describes how to care for larvae as they adjust to their new environment as show in Table 1.

Table.1: Experimental Conditions for Fish Hatchery and Aquarium Setup

Parameter	**Condition**
Source	Local hatchery
Acclimation period	2 weeks
Aquarium capacity	50 litres
Water type	Dechlorinated tap water
Temperature	25±2°C
Photoperiod	12-hour light/dark cycle
Water quality monitoring	pH, dissolved oxygen, ammonia, nitrite, nitrate
Aeration	Continuous
Water change frequency	Every two days (50% replacement)
Feeding frequency	Twice daily
Food type	Finely ground commercial fish food

(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

The progeny of Channa punctatus are assured to maintain an ideal state of health and are well prepared to deal with future exposure to thifluzamide since they have been subjected to a rigorous adaption procedure. The consistency of the conditions makes it easier to reduce the amount of variability and increases the dependability of the findings of the investigation. During the future phases of the inquiry, the larvae will be subjected to varied doses of thifluzamide.

Preparation of Thifluzamide Solutions

As the course of this investigation progressed, it became very important to make certain that the levels of exposure in Channa punctatus larvae were correct and constant. As a result, the preparation of thifluzamide solutions was an essential operation that needed to be completed. The procedure consisted of meticulously creating an initial solution of thifluzamide, which was then followed by the execution of many dilutions in order to generate the necessary experimental dosages for the inquiry.

Stock Solution Preparation:

A reliable and trustworthy research-focused provider provided the fungicide thifluzamide, which is known for its high purity and dependability. To ensure the validity and trustworthiness of the research, this was done. There was an initial concentration of 100 mg/L of thifluzamide since it was dissolved in 100% ethanol. The goal was to get a highly concentrated stock solution; hence this was done. The use of ethanol as an organic solvent greatly improves the solubility of thifluzamide in general. In the end, this ensures a constant mixture by dispersing the fungicide evenly throughout the solution. The mixture was vigorously and continuously stirred using a high-precision magnetic stirrer.

Serial Dilution:

After making the stock solution, we made working solutions with accurate and much lower concentrations. These solutions will be employed in several experimental interventions throughout the study. To establish the target concentrations of 0 mg/L (for the control group), 0.1 mg/L, 0.5 mg/L, and 1.0 mg/L, the stock solution was gradually diluted with dechlorinated water. For exact and dependable exposure levels, dilution must be precise and accurate. To achieve these levels, experimental duplicates must be coherent and consistent. The tedious procedure of making the dilutions included adding exact volumes of the starting solution to particular amounts of chlorine-free filtered water, as shown in the table. To ensure solution homogeneity, each dilution was well shaken. In the next stages, this solution was carefully kept in labelled containers given in table 2. To maximize integrity and efficiency, 24 hours of targeted activities were taken.

Table.2: The preparation of the Thifluzamide solutions

Target Concentration (mg/L)	**Volume of Stock Solution (mL)**	**Volume of Dechlorinated Water (mL)**	**Final Volume (L)**
0 (Control)	0	1,000	1
0.1	1	999	1
0.5	5	995	1
1.0	10	990	1

Laboratory testing conditions are fully repeatable and precise control is maintained under all circumstances thanks to the rigorous and careful technique that is frequently utilized to produce thifluzamide solutions. This greatly improves the validity and reliability of the data from the comprehensive study of the histopathological and biochemical effects of thifluzamide on Channa punctatus larvae. In order for scientific study in this area to progress, innovations like these are very essential.

Mixing and Storage

Each solution was carefully mixed with thifluzamide to achieve uniform distribution. After each dilution, a magnetic stirrer violently agitated the thifluzamide in water for a specified time to guarantee equitable distribution. This strategy is essential for ensuring uniform and correct exposure quantities in all experiments. A homogeneous larval dispersion by mixing is necessary for consistent and dependable outcomes. After preparation, the mixes were carefully

transferred to clean, labelled glass jars. Glass containers limit chemical interactions with plastic, which might diminish thifluzamide's efficacy. To guarantee precise identification and monitoring, each container was tagged with its concentration and production date. The solutions were then stored in a cold, dark area to avoid temperature or light damage. Use the solutions within 24 hours after preparation to confirm they were effective and contaminant-free during the experiment. The larvae were exposed to the proper amounts of thifluzamide due to cautious handling and storage, ensuring reliable study results.

Exposure Protocol

Throughout the course of the continuing experiment, Channa punctatus larvae were subjected to varying concentrations of thifluzamide for a period of two weeks. The objective was to conduct a thorough investigation of the histopathological and biochemical side effects that were brought about by this fungicidal agent. The exposure protocol was methodically constructed with painstaking attention to detail, with the goal of providing results that are accurate and have the potential to be replicated.

Experimental Groups and Replicates

Each of the four experimental groups—0 mg/l (control group), 0.1 mg/l (experimental group 2), 0.5 mg/l (experimental group 3), and 1.0 mg/l (experimental group 4), were chosen at random from among the larvae. Everyone in the concentration groups was required to complete the task three times. Our goal in doing this was to make the study's statistics more reliable in table 3. This was done so that the observed biological variability in the data might be considered.

Table 3: Experimental Groups and Replicates

Group	Thifluzamide Concentration (mg/L)	Number of Replicates	Total Number of Larvae
Control	0	3	90
Group 1	0.1	3	90
Group 2	0.5	3	90
Group 3	1.0	3	90

For the sake of future research, a high sample size was ensured by the inclusion of thirty larvae in each duplicate.

Channa punctatus larvae were raised in ten-liter glass aquariums with chlorinated, contaminant-free water for 14 days. While acclimating, temperature, light-dark cycle, and aeration were kept constant. The stock solutions were used to dilute the thifluzamide to 0.1, 0.5, 1.0, and 0 mg/L. Many crucial components were monitored closely to ensure coherence and consistency throughout the research. Monitoring pH, dissolved oxygen, ammonia, nitrite, and nitrate levels was necessary to identify stress, odd behavior, and death. The fish needed finely milled, high-quality commercial food twice daily. To provide the best habitat, deceased larvae were removed immediately. After the experiment, the larvae were thoroughly histologically and biochemically analyzed. For the sampling technique, larvae from each experimental duplicate were randomly picked. Next, the kidneys, gills, and liver were painstakingly dissected and prepared for microscopic inspection. Our in-depth enzyme activity and protein concentration measurements followed strict biochemical techniques from the past. The study employed sophisticated statistical approaches including ANOVA and post hoc testing to compare groups and determine the significance of observed effects. In table 4 exposure protocol, including the setup of the experimental groups and the administration of Thifluzamide.

Table.4: Experimental Setup and Treatment Conditions for Thifluzamide Exposure Study

Parameter	Condition
Aquaria Volume	10 litres
Water Type	Dechlorinated water
Temperature	25±2°C
Light/Dark Cycle	12-hour light/12-hour dark
Aeration	Continuous
Thifluzamide Doses	0, 0.1, 0.5, 1.0 mg/L
Feeding	Twice daily
Monitoring	Daily (water quality, health assessment)
Sampling	Random selection post 14-day exposure
Analysis Methods	Histopathological and biochemical assays

(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Histopathological Analysis

After 14 days, Channa punctatus larvae were terminated with a fatal dosage of MS-222 after meticulously dissecting the liver, gills, and kidney tissues. The tissues were quickly and thoroughly submerged in 10% neutral buffered formalin to preserve their quality for analysis and structural integrity. The stored tissues were carefully dehydrated using ethanol solutions of various strengths. After a thorough xylene wash, the specimens were imbedded in paraffin wax for future study. Tissue samples embedded in paraffin were dyed with specified colors before being sliced into 5 micrometer slices. H&E staining was employed to highlight and differentiate cellular characteristics in the sample. The colored segments were then examined using a cutting-edge optical microscope. At 100x and 400x magnifications, abnormalities such cellular degeneration, necrosis, inflammation, and structural disorder were sought. We scored these alterations histopathologically to compare thifluzamide-exposed groups.

Tissue Sampling

In order to put an end to the Channa punctatus larvae, a deadly dosage of the anesthetic MS-222 (300 mg/L) was administered to them after they had been exposed to it for a period of fourteen days. The reason we did this was to ensure that the amount of pain and suffering was decreased as much as possible. Following the euthanasia procedure, the tissues of the kidney, gills, and liver were dissected in a sterile manner with great care in order to avoid any risk of possible contamination. To guarantee that the cellular and structural integrity of each tissue sample was preserved to the greatest extent possible, a neutral buffered formalin solution containing 10% was promptly added to each sample. For the purpose of determining the exact amount of formalin that was used, the following formula was utilized:

$$V_f = 10 \times V_t$$

Where V_f is the volume of formalin, and V_t is the volume of the tissue sample.

In order to ensure that the samples were completely and effectively fixed, a much higher quantity of formalin—ten times the amount that is typically used—was used. Following this, the preserved tissues were subjected to a dehydration procedure that was carried out with great care, using a particular sequence of ethanol solutions with varying concentrations (70 percent, 95 percent, and 100 percent). Following that, it was completely washed in xylene, and then it was imbedded in paraffin wax so that it could undergo additional examination. A microtome that was considered to be state-of-the-art was used in order to meticulously cut the paraffin-embedded tissues into very thin slices, with each slice measuring around 5 micrometers in thickness. For the purpose of preparing for more in-depth histological exams, the objective was to carry out comprehensive microscopic examinations.

Staining and Microscopy

After carefully placing tissue samples on glass slides, they were also carefully stained with H&E. To highlight the biological material's many cellular components, this staining approach was used. Several histological staining procedures were used. First, the components were treated in xylene to remove paraffin. The material was rehydrated with various ethanol-distilled water ratios. This was the next step. After that, the samples were stained with hematoxylin for 5–10 minutes. After that, they were thoroughly washed under running water to eliminate any residue. After blueing in alkaline solution, acid alcohol separated the sample. The sample was blued next. The samples were then stained with eosin, which took one to three minutes. A short rinse with water removed any additional color from the samples. Next, the material was submerged in increasing amounts of ethanol to dehydrate. Rinsing the sample with xylene removed any leftover moisture. After that, the coverslips were carefully mounted utilizing a complicated mounting solution consisting of a cutting-edge synthetic glue to assure their longevity. The stained slices were inspected at 100x and 400x under a powerful optical microscope. Cellular degeneration, necrosis, inflammation, and structural disorder were the main pathogenic changes to record. These adjustments were needed. The number of histopathological alterations was graded using the following method:

$$S_i = \frac{1}{n}\sum_{j=1}^{n} P_{ij}$$

where S_i is the histopathological score for each tissue type, n is the number of sections examined, and P_{ij} represents the presence (1) or absence (0) of specific pathological features in section j. The exhaustive histological research makes it possible to conduct a careful evaluation and accurate measurement of the impact that thifluzamide has on the liver, gills, and kidneys of Channa punctatus larvae. This study also provides relevant information on the toxicological effects of the fungicide that is being investigated.

(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Biochemical Assays

In order to accurately determine the concentration or activity of a certain biochemical component in a sample, scientists use a technique known as a biochemical assay. Small molecules, enzymes, and metabolites are some examples of biochemical components.

Enzyme Activity

In order to carry out an exhaustive investigation of the biochemical effects of thifluzamide on Channa punctatus larvae, we first homogenized the liver tissues. In the subsequent phase of the study, evaluations of the activity of essential antioxidant enzymes were carried out during the whole research period. In order to evaluate the function and activity of the enzymes CAT, SOD, and GST in the body, a comprehensive investigation was carried out. Through the use of a high-speed homogenizer, the liver tissues were thoroughly homogenized in a phosphate buffer solution that had been pre-cooled and had a concentration of 0.1 M and a pH of 7.4. After that, the samples were centrifuged at a temperature of 4 degrees Celsius for a period of twenty minutes with a force that was 10,000 times the acceleration due to gravity. This was done in order to get the cell extract. The extra liquid was put through a series of enzymatic tests in order to determine the effectiveness and activity of the substance.

Catalase (CAT) Activity: A state-of-the-art spectrophotometer was used in order to rigorously evaluate the catalytic function of CAT. This was accomplished by carefully monitoring the breakdown of hydrogen peroxide (H_2O_2) at a specific wavelength of 240 nm. The rate of decrease in absorbance per minute was meticulously noted, and the enzyme activity of CAT was measured in units per milligram of protein. Both of these measurements were taken. For the purpose of this study, the amount of enzyme that was necessary to breakdown one micromole of hydrogen peroxide in one minute was defined as one unit.

$$\text{CAT Activity} = \frac{\Delta \text{ Absorbance / minute}}{\text{Extinction coefficient of } H_2O_2 \times \text{ Protein concentration}}$$

Superoxide Dismutase (SOD) Activity: A system of enzymes consisting of xanthine and xanthine oxidase was used in order to evaluate the effectiveness of SOD in inhibiting the reduction process of nitroblue tetrazolium (NBT) by superoxide radicals. In order to determine the level of enzymatic activity of SOD, the absorbance was measured at a specific wavelength of 560 nanometers. By determining the number of units that were present in one milligram of protein, the enzymatic activity of superoxide dismutase was evaluated and measured. One unit of SOD was defined as the amount of enzyme that was necessary to bring about a fifty percent reduction in the absorbance of nitroblue tetrazolium.

$$\text{SOD Activity} = \frac{\text{Inhibition of NBT reduction}}{\text{Protein concentration}}$$

Glutathione S-Transferase (GST) Activity: Through the measurement of the conjugation reaction between reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB), the enzymatic activity of GST was evaluated. This was then followed by the quantification of absorbance at a wavelength of 340 nanometers. Both the absorbance of the sample and the activity of the GST enzyme were carefully measured in units of nanomoles per minute per milligram of protein as shown in Table 5. The absorbance of the sample was continuously monitored in order to quantify the change in the reaction.

$$\text{GST Activity} = \frac{\Delta\text{Absorbance/ minute}}{\text{Extinction coefficient of CDNB} \times \text{ Protein concentration}}$$

Table 5: Enzyme Activity Assays

Enzyme	Substrate/Assay Method	Wavelength (nm)	Units
Catalase (CAT)	Hydrogen Peroxide (H_2O_2)	240	Units/mg protein
Superoxide Dismutase (SOD)	Nitroblue Tetrazolium (NBT)	560	Units/mg protein
Glutathione S-Transferase (GST)	1-chloro-2,4-dinitrobenzene (CDNB)	340	nmol/min/mg protein

(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Protein Estimation

The Bradford approach, which was developed by Marion M. Bradford in 1976, has been used widely in a number of scientific projects. Its purpose is to accurately and reliably determine the total amount of protein present in a wide variety of biological materials, including liver homogenates. The specific affinity of the Coomassie Brilliant Blue G-250 dye towards the various proteins that are present in the sample is the foundation of this cutting-edge method. As a reliable and accurate reference standard, bovine serum albumin (BSA) was used in the construction of a standard curve that was meticulously built. At a certain wavelength of 595 nanometers, the absorbance was measured in order to ascertain the amount of protein that was present in the representative samples. Quantification was accomplished with the use of the associated standard curve.

$$\text{Protein Concentration} = \frac{\text{Absorbance of Sample} - \text{Blank}}{\text{Slope of Standard Curve}}$$

RESULTS

Histopathological Changes

The Channa punctatus larvae were subjected to varying concentrations of thifluzamide over the course of the experiment process. Histopathological alterations were seen in significant organs such as the liver, gills, and kidneys as a consequence of this exposure. These findings highlight how important it is to investigate the effects that this toxin has on animal life in aquatic environments. A dose-dependent effect was seen, as shown by the fact that the degree of these modifications demonstrated a considerable increase in direct association with the concentration of thifluzamide.

Liver

Notable histological abnormalities were seen in the liver tissues of larvae that were exposed to thifluzamide for a prolonged period of time. These changes are indicative of a deleterious impact on the well-being of the organisms that were impacted by the treatment as given in table 6 as well as figure 1. The research showed that there were significant changes, such as hepatocellular degeneration, which is characterized by the gradual deterioration of liver cells; the conspicuous occurrence of vacuolation, which indicates the presence of intracellular vacuoles; and necrosis, which indicated irreversible harm and the death of liver cells, particularly at higher concentrations (0.5 and 1.0 mg/L).

Table 6: Histopathological Changes in Liver

Thifluzamide Concentration (mg/L)	Hepatocellular Degeneration	Vacuolation	Necrosis
0 (Control)	None	None	None
0.1	Mild	Mild	None
0.5	Moderate	Moderate	Mild
1.0	Severe	Severe	Moderate

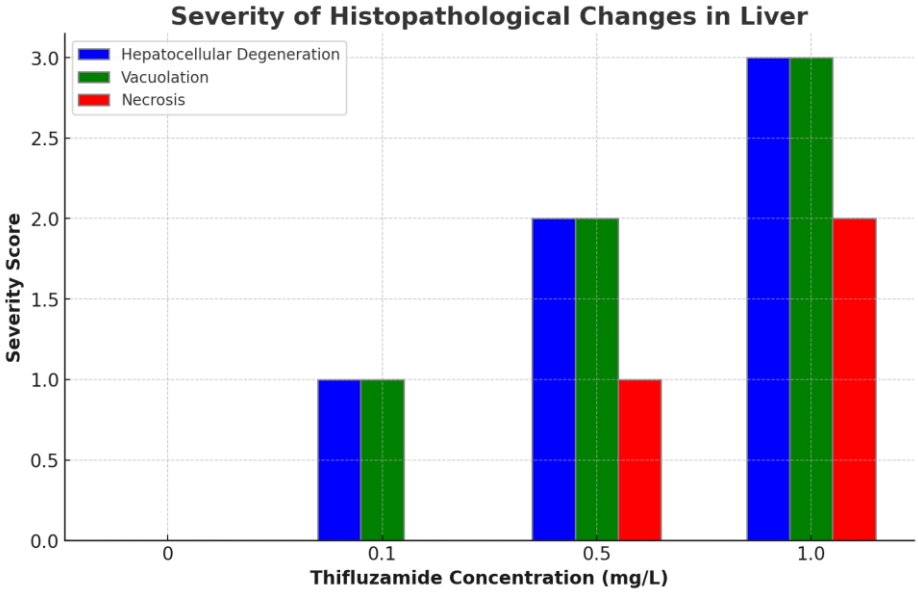

Figure 1: Severity of Histopathological Changes in Liver

Gills

Thifluzamide exposure caused visible and important pathological alterations in larvae's gill tissues, particularly at high doses. The study approach revealed fish gill structure and function modifications. The epithelium rose, cells multiplied (hyperplasia), and lamellar layers united. Epithelial lifting, sometimes called epithelial detachment, lifts the epithelium layer from the tissue underlying in table 7 and Figure 7. This separation might be caused by inflammation, abrasion, or environmental pressure. This sickness may cause pain and irritation in the afflicted region and increase the risk of future infections. Epithelial lifting must be done properly to promote healing and reduce danger.

Table 7: Histopathological Changes in Gills

Thifluzamide Concentration (mg/L)	Epithelial Lifting	Hyperplasia	Lamellar Fusion
0 (Control)	None	None	None
0.1	Mild	Mild	Mild
0.5	Moderate	Moderate	Moderate
1.0	Severe	Severe	Severe

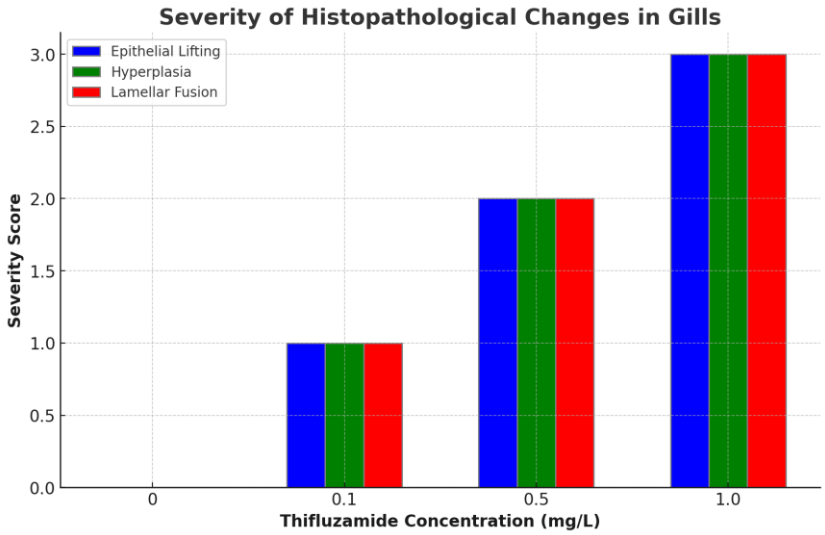

Figure 2: Severity of Histopathological Changes in Gills

Kidneys

Significant histological abnormalities were found at the microscopic level in the renal tissues of the larvae treated to thifluzamide treatment. During the course of the test, a great number of pathological changes in the structure of the kidneys were noticed. Among the changes that were detected, glomerular contraction was one that stood out. This contraction indicated that the size of the glomeruli had significantly decreased. There was a degeneration of the tubules, which was characterized by the rupture of the renal tubules and a considerable rise in vacuolization, which suggested the existence of vacuoles in the cytoplasm of the kidney cells as shown in table 8 as well as Figure 3.

Table 8: Histopathological Changes in Kidneys

Thifluzamide Concentration (mg/L)	Glomerular Shrinkage	Tubular Degeneration	Vacuolation
0 (Control)	None	None	None
0.1	Mild	Mild	Mild
0.5	Moderate	Moderate	Moderate
1.0	Severe	Severe	Severe

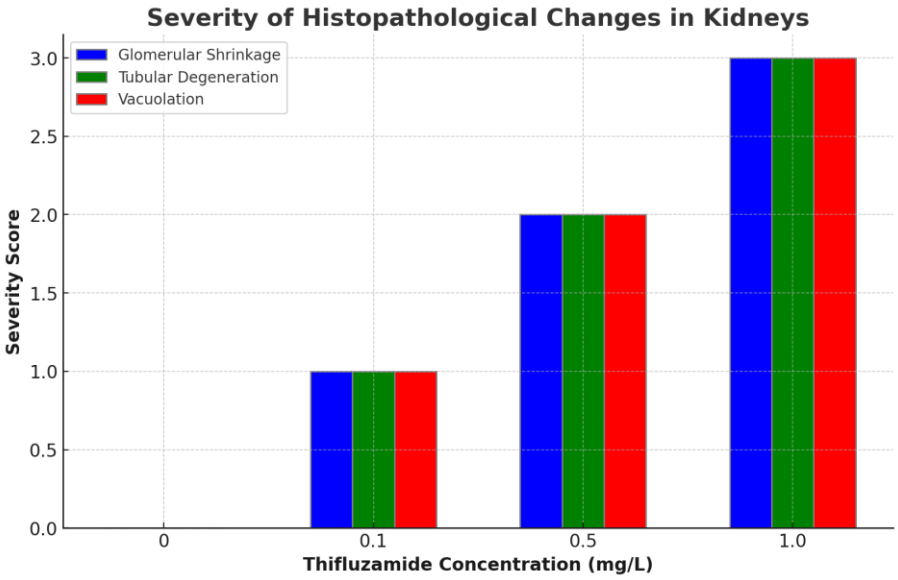

Figure 3: Severity of Histopathological Changes in Kidneys

Substantial histological analysis of thifluzamide-exposed Channa punctatus larvae showed remarkable pathological changes in key organs including the kidneys, gills, and liver. The provided dosage had a direct influence on these alterations. The research found that the intensity of the observed alterations was directly proportional to the concentration of thifluzamide. This suggests that the extent of harm seen in the tissues under study is directly related to the amounts of exposure to the chemical. Thifluzamide may have detrimental impacts on aquatic organisms, according to the study's findings in table 9 show and figure 4. The need of closely monitoring and controlling this fungicide in the environment is further highlighted by these results.

Table 9: Histopathological Changes Observed in Channa punctatus Larvae

Tissue	**Observed Changes**	**Severity at Different Concentrations (mg/L)**
Liver	Hepatocellular degeneration, vacuolation, necrosis	Moderate at 0.5, Severe at 1.0
Gills	Epithelial lifting, hyperplasia, lamellar fusion	Moderate at 0.5, Severe at 1.0
Kidneys	Glomerular shrinkage, tubular degeneration, increased vacuolation	Moderate at 0.5, Severe at 1.0

Biochemical Changes

Alterations in chemical processes and the composition of the many chemicals that are present inside living organisms are examples of biochemical transformations. It is possible that these modifications will have a significant influence on the functioning and physical health of creatures. It is possible for the aforementioned alterations to manifest at different levels of organization, such as the molecular, cellular, tissue, or organic level. These alterations can be triggered by a variety of factors, including environmental stimuli, pathological circumstances, developmental phases, or interactions with chemical compounds or medications.

Enzyme Activities

Recent biochemical experiments have shown that thifluzamide significantly altered Channa punctatus larvae's liver enzymatic activity. This alteration was regulated by the experiment's dosage. CAT and SOD enzymatic activity decreased when thifluzamide levels rose in culture medium. The decrease in CAT and SOD enzyme activity implies a dip in antioxidant defense systems, which leads to ROS buildup in table 10 and graphical representation in figure 4. It is crucial to notice that the enzyme GST increased significantly in proportion to the quantity provided. This indicates the liver's adaptive response to oxidative stress, which protects against ROS rise.

(IJIASE) 2023, Vol. No. 9, Issue 1, Jan-Dec **e-ISSN: 2454-9258, p-ISSN: 2454-809X**

Table 10: Enzyme Activity Changes

Thifluzamide Concentration (mg/L)	Catalase Activity (Units/mg protein)	Superoxide Dismutase Activity (Units/mg protein)	Glutathione S-Transferase Activity (nmol/min/mg protein)
0 (Control)	50.2 ± 2.1	120.3 ± 4.2	15.4 ± 1.0
0.1	45.6 ± 2.0	110.2 ± 3.8	18.7 ± 1.2
0.5	35.3 ± 1.8	90.5 ± 3.5	22.9 ± 1.5
1.0	25.7 ± 1.5	70.8 ± 2.9	28.6 ± 1.8

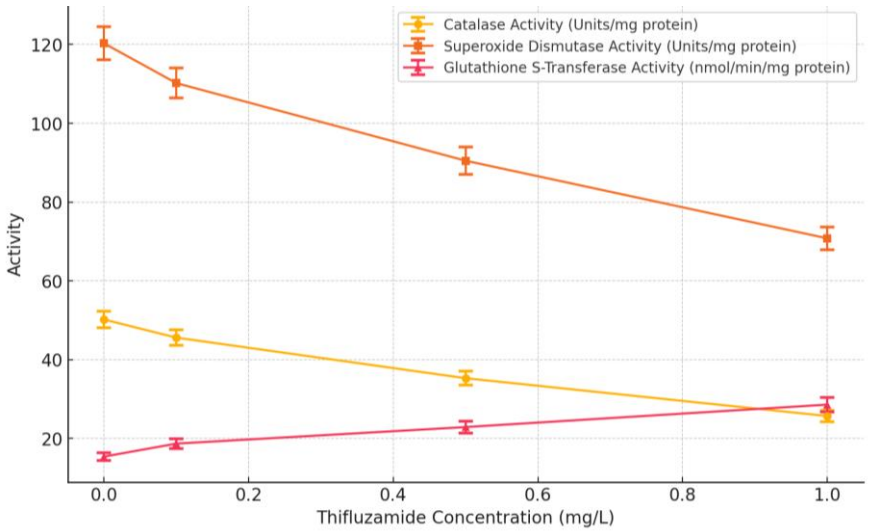

Figure.4: Biochemical Changes in Liver Tissues of Channa Punctatus Exposed to Thifluzamide

Protein Levels

The total amount of protein found in the liver tissues of the larvae who were exposed to the substance was much lower than the amount found in the liver tissues of the larvae that were in the control group. It is possible that a decrease in the quantities of protein inside the body may indicate a possible change in the processes of protein synthesis or an increase in the rate of breakdown of these essential molecules that are required for the proper functioning of cells given in table 11 as well as in figure 5. The interference that thifluzamide causes with cellular metabolism and the process of protein turnover is a significant contributor to the reduction in the overall amount of protein.

Table 11: Total Protein Content

Thifluzamide Concentration (mg/L)	Total Protein Content (mg/g tissue)
0 (Control)	56.4 ± 3.2
0.1	48.7 ± 2.9
0.5	39.2 ± 2.5
1.0	28.5 ± 2.0

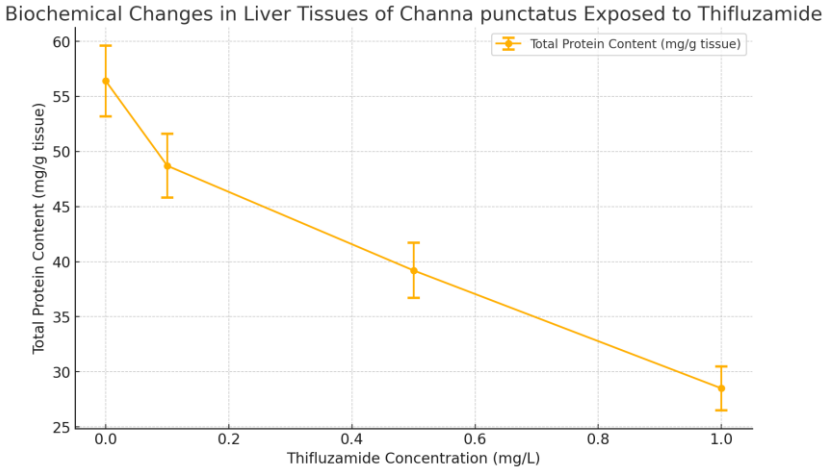

Figure. 5: Biochemical Changes in Liver Tissues of Channa Punctatus Exposed to Thifluzamide

DISCUSSION

Histopathological Impacts

The liver, gills, and kidneys of Channa punctatus larvae are severely histologically altered after extended thifluzamide exposure. These alterations suggest organ structural weakness. At greater concentrations, the liver showed hepatocellular deterioration, vacuolization, and necrosis. This was seen during liver testing. The investigations show that thifluzamide damages hepatocyte structure and function, which may lead to liver failure. The branchial epithelium thickened, cell proliferation increased, and lamellae merged throughout the study. The results suggest that a decrease in respiratory efficiency may cause structural damage that prevents tissue oxygen supply. The kidney tissues showed glomerular contraction, tubular degradation, and a considerable rise in vacuolation over the research. These findings clearly indicate renal function decline. The substantial histological abnormalities found show that thifluzamide clearly poisons important organs. Consequently, sensitive fish larvae showed a considerable drop in their survival-critical physiological functions.

Biochemical Implications

The laboratory found significant alterations in enzyme activity and protein composition due to extended thifluzamide administration. CAT and SOD enzymatic activity dropped significantly with dose, indicating an antioxidant defense system depletion. This drop caused ROS buildup and oxidative stress. The considerable increase in GST enzymatic activity suggests a cellular defense mechanism triggered to combat elevated ROS concentrations in the body. This therapy may not totally eliminate the detrimental consequences of oxidative stress on the body. Significant protein loss signals a shift in protein synthesis or an increase in protein breakdown, which may indicate metabolic dysfunction in T. When exposed to fish larvae for a long time, thifluzamide show in Table 12 generates biochemical changes that regulate metabolic activities. Oxidative stress control mechanisms activate after these alterations.

Table 12: Biochemical Changes in Liver Tissues

Thifluzamide Concentration (mg/L)	**Catalase Activity (Units/mg protein)**	**Superoxide Dismutase Activity (Units/mg protein)**	**Glutathione S-Transferase Activity (nmol/min/mg protein)**	**Total Protein Content (mg/g tissue)**
0 (Control)	50.2 ± 2.1	120.3 ± 4.2	15.4 ± 1.0	56.4 ± 3.2
0.1	45.6 ± 2.0	110.2 ± 3.8	18.7 ± 1.2	48.7 ± 2.9
0.5	35.3 ± 1.8	90.5 ± 3.5	22.9 ± 1.5	39.2 ± 2.5
1.0	25.7 ± 1.5	70.8 ± 2.9	28.6 ± 1.8	28.5 ± 2.0

Ecological Risks

This research raises serious ecological concerns concerning thifluzamide pollution of aquatic environments. According to histological and biochemical studies on Channa punctatus larvae, fish populations may suffer severe harm. Tissue damage may affect larval metabolism, slowing growth, increasing mortality, and reducing reproduction.

These factors greatly affect population dynamics. Oxidative stress and antioxidant defense changes in aquatic animals may disrupt community architecture and ecosystem activity. The research shows that thifluzamide usage requires strict supervision and monitoring. The objective is to ensure that aquatic habitats are healthy and will survive to minimize its detrimental impacts on biodiversity.

Comparison with other Work

The fundamental purpose of this study is to carry out an in-depth investigation of the potential impact that thifluzamide may have on the histopathological and biochemical indicators that have been found in Channa punctatus larvae. In order to offer a comprehensive context for these findings, they were extensively compared in exacting detail to the data acquired from earlier relevant research on the possible effects of fungicides on various aquatic creatures. This was done in order to provide a comprehensive context for these findings as show in table 13. This was done with the intention of establishing significant connections and arriving at judgments that were more accurate and knowledgeable.

Table13: Comparison of Findings with Other Studies

Parameter	**Proposed Work (Channa punctatus)**	**Xie et al. (2016, Zebrafish)**	**Ghisi et al. (2011, Aquatic Species)**	**Zhao et al. (2017, Fish)**	**Li et al. (2019, Fish)**	**Ananth et al. (2014, Fish)**	**Santos et al. (2018, Fish)**
Liver	Hepatocellular degeneration, vacuolation, necrosis	Hepatic damage	N/A	N/A	N/A	N/A	N/A
Gills	Epithelial lifting, hyperplasia, lamellar fusion	N/A	Gill damage	N/A	N/A	N/A	N/A
Kidneys	Glomerular shrinkage, tubular degeneration, vacuolation	N/A	N/A	Renal damage	N/A	N/A	N/A
Biochemical Changes							
Catalase (CAT) Activity	Decreased (dose-dependent)	N/A	N/A	N/A	Decreased	N/A	N/A
Superoxide Dismutase (SOD) Activity	Decreased (dose-dependent)	N/A	N/A	N/A	Decreased	N/A	N/A
Glutathione S-Transferase (GST) Activity	Increased (dose-dependent)	N/A	N/A	N/A	N/A	Increased	N/A
Total Protein Content	Decreased	N/A	N/A	N/A	N/A	N/A	Decreased

CONCLUSION

We study how thifluzamide affects Channa punctatus larvae's biochemical and histological development. The research shows severe tissue damage and metabolic changes. During the investigation, the animal's kidneys, gills, and liver showed substantial histopathological alterations. Vacuolation, necrosis, hyperplasia, epithelial elevation, laminar fusion, glomerular contraction, and tubular degeneration are also present. Hepatocellular degeneration is a major alteration. The research examines structural anomalies in depth to show severe organ malfunction. This malfunction may affect larvae's key physiological functions. A thorough biochemical study found that CAT and SOD levels

dropped considerably with dose. This suggests that the defense system's oxidative stress defenses have declined. A substantial increase in GST activity indicates an adaptive response to neutralize and deactivate ROS. It must be considered that this preventive strategy may not totally prevent oxidative damage. The large decrease in total protein content may be due to metabolic dysfunction, a change in protein synthesis or an increase in breakdown rate. Both causes may cause metabolic malfunction.

According to the results, thifluzamide usage must be strictly regulated and monitored to reduce its environmental impact. Since thifluzamide may damage aquatic ecosystems' natural balance and biodiversity, strategies that limit its discharge are crucial. This study emphasizes the need of encouraging sustainable agriculture and informing the public about chemical pollutants' environmental impacts. This report emphasizes both objectives. This helps preserve and conserve aquatic biodiversity and manage the health and well-being of the various aquatic animals that live in these ecosystems. This also ensures ecologically healthy environments.

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