

REPRODUCTIVE TOXICOLOGY OF *CYPRINUS CARPIO* IN POLLUTED AQUATIC ECOSYSTEMS

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Abstract

The purpose of the study was to check the reproductive toxicity of sub-lethal contact to Lambda-cyhalothrin (LCH) in *Cyprinus carpio*. A concentration of 0.2 µg/L of LCH was bare to healthy specimens for 1, 5, 10, 15, and 20 days. The gonadosomatic Index (GSI), histopathology of the gonadal tissues, and serum concentrations of sex steroid hormones (testosterone, 17 β-estradiol, and 11-ketotestosterone) were studied. The results showed a progressive decline in GSI in male as well as female fish under all bare conditions. Results of the histopathological analysis revealed severe structural damage in the testes with atrophy of the germinal epithelium, reduced lobular diameter, decreased spermatozoa, as well as in the ovarian tissues with cytoplasmic clumping, deformity of the follicles, and increased atretic oocytes. Structural impairments correlated with significant reductions in testosterone, 17β-estradiol, and 11-ketotestosterone concentrations in hormonal assays. The results show that sub-lethal, short-term exposure to LCH can severely disrupt reproductive physiology and endocrine functioning in *Cyprinus carpio*. The study demonstrates that synthetic pyrethroid contamination is a direct cause of reproductive decline in freshwater fish and that gonadal and hormonal parameters are sensitive early biomarkers of aquatic pollution. Therefore, this result highlights the fact that strict environmental regulations, together with long-term ecological monitoring, are required to lower pesticide-induced reproductive risk in aquatic ecosystems.

Keywords: Reproductive toxicity, *Cyprinus carpio*, Lambda-cyhalothrin, Gonadosomatic Index, Histopathology, Endocrine disruption.

INTRODUCTION

Modern agricultural practices of the pervasive use of pesticides caused the surrounding aquatic ecosystem contamination as a direct threat to the health and sustainability of non-target organisms. Fish are among the most affected groups since they are both important components of aquatic food webs and serve as bioindicators of environmental health. Over the past decades, reproductive toxicity induced by these chemicals, and synthetic pyrethroids like Lambda-cyhalothrin (LCH) (Tessema et al., 2020) have been one of the pressing concerns amongst others. Both the naturally occurring pyrethrins extracted from *Chrysanthemum cinerariaefolium* as well as synthetic analogues of the compounds are lauded for their effectiveness against pests, but are legendary for their unintended ecological effects. While these pollutants have a high lethality at high concentrations, their insidious nature is not only in their fatal ability but also in their ability to induce sub-lethal physiological and behavioural disturbances that accumulate over time and interfere with important biological processes, such as reproduction (Korkmaz et al., 2022).

The reproductive system is very sensitive to chemical insults because it is regulated by hormonal and is dependent on finely tuned physiological pathways. Reproductive success in fish is critically dependent on anatomical, behavioural, and physiological adaptations that are specially adapted to the specific ecological conditions (Korkmaz et al., 2020). This complex system is disrupted, however, and any disruption can cause massive reproductive failures, which ultimately destroy aquatic biodiversity. In this case, synthetic pyrethroids, such as LCH, have been shown to affect different endocrine glands with great effect, disturbing hormonal balances, and regulating gametogenesis and differentiation of sexual. Gonadosomatic Index (GSI), a reliable index for reproductive maturity and the general gonadal condition, becomes measurable in the presence of sub-lethal concentrations of pollutants. Earlier warnings of reproductive dysfunction include reductions in GSI accompanied by histopathological changes in gonadal tissues (Islam et al. 2019).

Analysis of histopathology has become a sensitive and inextricably important tool in toxicology studies, capable of eliciting delicate anatomical changes at the cellular and tissue levels before the manifestation of clinical signs of toxicity. Exposure to environmental pollutants in fish results in morphological changes such as degeneration of seminiferous tubules, loss of germinal epithelium, cytoplasmic clumping in oocytes, and atresia, all of which are detrimental to reproductive capacity (Wolf & Wheeler, 2018). The disruption of nerve conduction pathways, particularly through the blocking of sodium channels by toxicants, further exacerbates cellular and tissue-level damage. Such physiological insults are frequently accompanied by hormonal imbalances, evidenced by diminished circulating levels of key sex steroids such as 17 β -estradiol as well as testosterone, 11-ketotestosterone. These hormones are pivotal not only for the development and preservation of secondary sexual features but also for regulating gamete maturation and spawning behaviours (Al-Hasawi, 2022).

The reproductive health of fish serves as a sentinel measure of ecosystem integrity, with gonadal histology and hormonal profiling providing critical insights into the sub-lethal effects of environmental contaminants. Feminization of males, reduced fertility, impaired vitellogenesis, and abnormalities in gonadal structure occur when males are exposed to endocrine-disrupting compounds. While the direct effects of low-level, chronic exposure to pesticides would not prove immediately lethal, insidious deterioration leading to the progressive decline in reproductive functions has been linked to chronic, low-level exposure to pesticides and is a potential, though silent, threat to fish populations. Therefore, monitoring changes in vitellogenin production, sex steroid concentrations, and histological features of gonads has become an important strategy to assess the ecological impact of chemical pollutants (Gutiérrez-Noya et al., 2020).

The consequences of pesticide-induced endocrine disruption extend beyond that of individual organisms to population-level consequences such as a reduction in reproductive success and altered sex ratios. Steroidal regulatory orchestration of reproduction depends on a finely sensitive axis between the brain, pituitary, and gonads that are extraordinarily susceptible to exogenous chemical interference in fish. Impaired gametogenesis, reduced fecundity, abnormal secondary sexual characteristics, and, in extreme cases, population decline or collapse, are the disturbances in this axis. Additionally, histopathological findings of increased interstitial gaps between lobules in testes, degeneration of germinal epithelium, cytoplasmic vacuolation in oocytes, and atresia indicate the level of physiological stress that exposed individuals experience (Luja-Mondragón et al., 2019).

Such knowledge of the environmental pollutant pathways that affect reproductive systems is crucial to the design of effective conservation strategies and ecological damage mitigation. The study can assess both morphological and endocrinological endpoints to get a comprehensive picture of the toxicological effects of contaminants such as LCH on aquatic organisms (Chatterjee et al., 2021). As *Cyprinus carpio* is ecologically and economically important, it is crucial to understand the full extent of reproductive toxicity caused by environmental pollutants. Therefore, this study is conducted to explore the histopathological and hormonal effects of sub-lethal exposure of Lambda-cyhalothrin in *Cyprinus carpio* to provide valuable information on the mechanisms of reproductive dysfunction in polluted aquatic ecosystems. This investigation aimed to provide a comprehensive understanding of how anthropogenic activities are changing how aquatic life manifests and to inform policy for the conservation of freshwater biodiversity (Jaffer et al., 2017).

Problem Statement

With the increasing use of synthetic pesticides such as Lambda-cyhalothrin (LCH) in agriculture, there have been great concerns about the unintended impacts of these pesticides on non-target aquatic entities. Among freshwater fishes, *Cyprinus carpio* is highly dispersed and of enormous importance to aquatic ecosystem structure and a bioindicator of environmental health. However, less information is available about the sub-lethal impact of LCH exposure on its reproductive functions, which are ecologically and economically important. Chemical disturbances affecting endocrine

pathways and gonadal development are particularly sensitive to reproduction in fish. Even low concentrations of pesticides may produce reproductive impairments, hormone disruption, population declines, and others. To understand these effects yet remain subtle and very important is key to correcting ecological risk assessment and to best strategies in conservation. This study, therefore, fills a gap in the urgent need to evaluate the reproductive toxicity of LCH on *Cyprinus carpio* using integrated morphological, histological, and hormonal assessment.

Objective and Hypothesis of the Study

The purpose of this study was to comprehensively study the sub-lethal impacts of Lambda-cyhalothrin (LCH) on the reproductive health of *Cyprinus carpio*, an ecologically significant freshwater fish species. Therefore, the main goals were to assess the gonadosomatic index (GSI) changes, examine histological modifications of gonadal tissues, and look into disruptions of the important sex steroid hormones, namely testosterone, 17 β -estradiol, and 11-ketotestosterone, after exposure for various lengths of time. The study combined morphological, histological, and hormonal assessments to identify early and sensitive biomarkers of pesticides' lethal effects on the aquatic environment. Even brief exposure to Lambda-cyhalothrin at sublethal concentration would result in marked reproductive dysfunction observed as a regular decline in GSI, damage of structural tissues of gonads as well as dramatic hormonal balance marked loss of biosynthesis and cyclicity and could open the way for reproductive failure and the long-term stability of freshwater fish populations at risk.

Materials and Methods

Experimental Animal

The study was done on healthy individuals, i.e., *Cyprinus carpio* (common carp). The fish were acclimatized under laboratory conditions in well-aerated freshwater tanks before the commencement of experiments. They were acclimated at controlled temperature and photoperiod and fed a standard diet appropriate for the species.

Experimental Design

The study was designed to check the reproductive toxicity of the synthetic pyrethroid Lambda-cyhalothrin (LCH) at a sub-lethal concentration. The fish were acclimated and randomly divided into control and treatment groups with six individuals (n=6) in each group. Fish in the treatment group were exposed to LCH at a concentration of 0.2 μ g/L, while control fish were kept under identical conditions without pesticide exposure.

Exposure Regimen

The exposure protocol involved subjecting the treatment group to LCH for intervals of 1, 5, 10, 15, and 20 days. Fresh LCH solutions were prepared daily to maintain consistent exposure concentrations. Control fish were handled similarly, without the addition of LCH, ensuring that all other environmental variables were identical between groups.

Sampling and Biological Assessment

At the end of each exposure period, fish from the control as well as the treatment groups were sampled. Following humane euthanasia, gonadal tissues were carefully dissected out, weighed, and used to calculate the Gonadosomatic Index (GSI) using the formula:

$$\text{GSI (\%)} = (\text{Gonad weight/Body weight}) \times 100$$

The GSI was calculated separately for testes and ovaries to determine the impact of LCH on gonadal development and reproductive potential.

Histopathological Examination

Gonadal tissues were fixed in suitable fixatives, processed through standard dehydration and embedding protocols, sectioned, and stained for microscopic examination. Histological slides were prepared and observed under a light microscope to evaluate structural integrity.

In testes, the arrangement and condition of spermatogenic cells—spermatogonia, spermatocytes, spermatids, spermatozoa, and Sertoli cells—were assessed. Specific attention was paid to detecting pathological changes such as increased interstitial gaps, reduced lobular diameters, loss of germinal epithelium, and decreased spermatozoa presence. In ovaries, the assessment focused on the integrity of perinuclear oocytes, cortical alveolar oocytes, and vitellogenic oocytes, with abnormalities such as cytoplasmic clumping, interfollicular spaces, follicular deformation, and atresia carefully documented.

Hormonal Analysis

After exposure, samples of the blood were collected from the caudal veins of each fish. Centrifugation was used to separate the sera, and the hormones of key reproductive importance were analyzed. In males, testosterone (T) and 11-ketotestosterone (11-KT), and in females 17 β -estradiol (E2) and 11-ketotestosterone (11-KT) were determined.

In concentrations of ng/ml for testosterone and 17 β -estradiol, and pg/ml for 11-ketotestosterone, results were expressed and compared across different durations of exposure, and percentages relative to control were calculated to determine the degree of endocrine disruption.

Results

The present study assessed the reproductive poisonousness of the pesticide Lambda-cyhalothrin (LCH) in *Cyprinus carpio*, through the analysis of Gonadosomatic Index (GSI), histopathological alterations of the gonads, and changes in serum hormone levels after exposure periods of 1, 5, 10, 15, and 20 days.

The testicular GSI of male common carp exposed to LCH declined progressively throughout the experimental period. The percentage reduction in GSI values compared to the control was observed as -7.02%, -16.09%, -24.48%, -37.64%, and -42.29% after 1, 5, 10, 15, and 20 days, respectively. A similar trend was noted in female fish, where the ovarian GSI values decreased by -12.79%, -21.36%, -29.82%, -43.96%, and -46.02% over the corresponding exposure periods, indicating a pronounced impact of LCH on gonadal development (Table 1, Figure 1).

Table 1: Effect of LCH Exposure on Testicular and Ovarian GSI (%) in *Cyprinus carpio*

Exposure Duration (Days)	Testicular GSI (% Change)	Ovarian GSI (% Change)
1	-7.02	-12.79
5	-16.09	-21.36
10	-24.48	-29.82
15	-37.64	-43.96
20	-42.29	-46.02

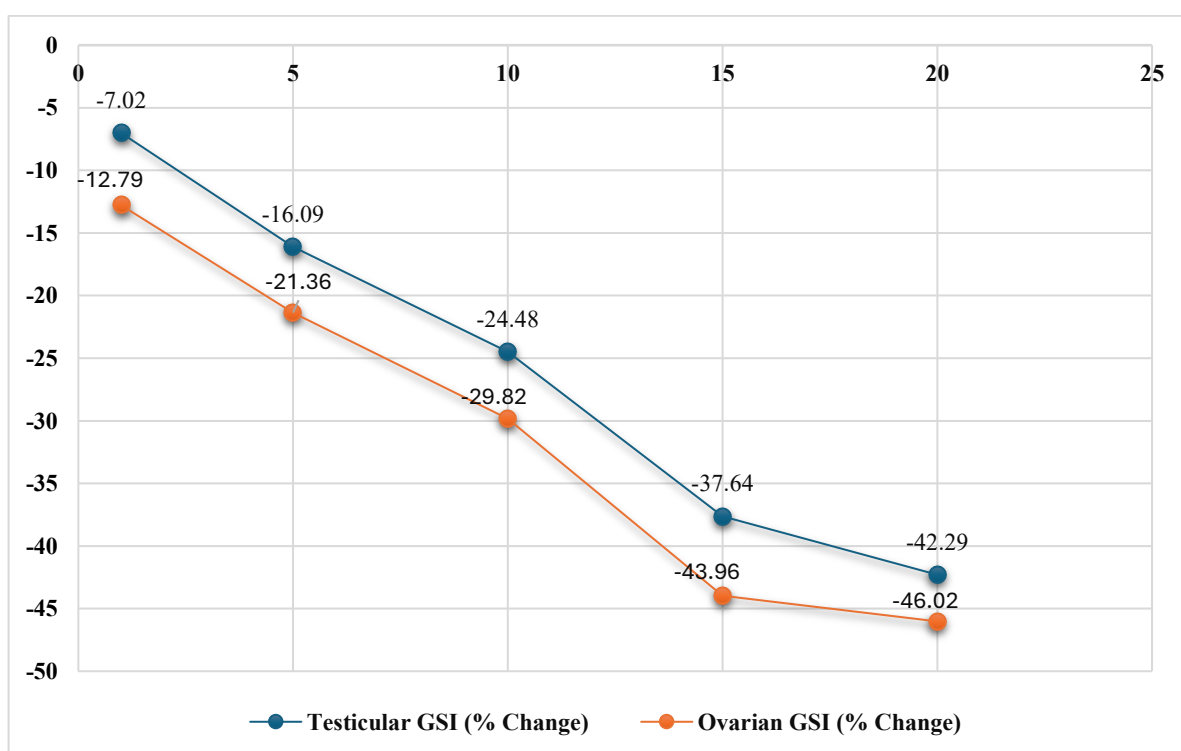


Figure 1. Effect of LCH Exposure on Testicular and Ovarian GSI (%) in *Cyprinus carpio*

Histological analysis of the testes from control fish revealed well-organized seminiferous lobules with active spermatogenic stages, including spermatogonia, spermatocytes, spermatids, spermatozoa, and Sertoli cells. Testes of fish exposed to LCH for 1 day exhibited normal histoarchitecture similar to controls. However, by 5 days of exposure, mild gaps were observed in the interstitial spaces between lobules. After 10 and 15 days of exposure, a reduction in lobular diameter, decreased spermatozoa within the luminal space, and atrophy of the germinal epithelium were evident. Prolonged exposure for 20 days resulted in extensive loss of germinal tissue and significant widening of interstitial gaps, highlighting severe gonadal impairment.

Similarly, ovarian histology of control fish showed normal oocyte development with distinct perinuclear oocytes, cortical alveolar oocytes, and early vitellogenic oocytes. Ovaries exposed to LCH for 1 day appeared largely normal; however, mild cytoplasmic clumping and the formation of inter-follicular spaces became evident after 5 days. With 10 days of exposure, ovarian follicles exhibited deformation and elongation, losing their characteristic morphology. After 15 and 20 days of exposure, pronounced cytoplasmic clumping, increased inter-follicular spaces, and a rise in atretic oocytes were noted (Table 2).

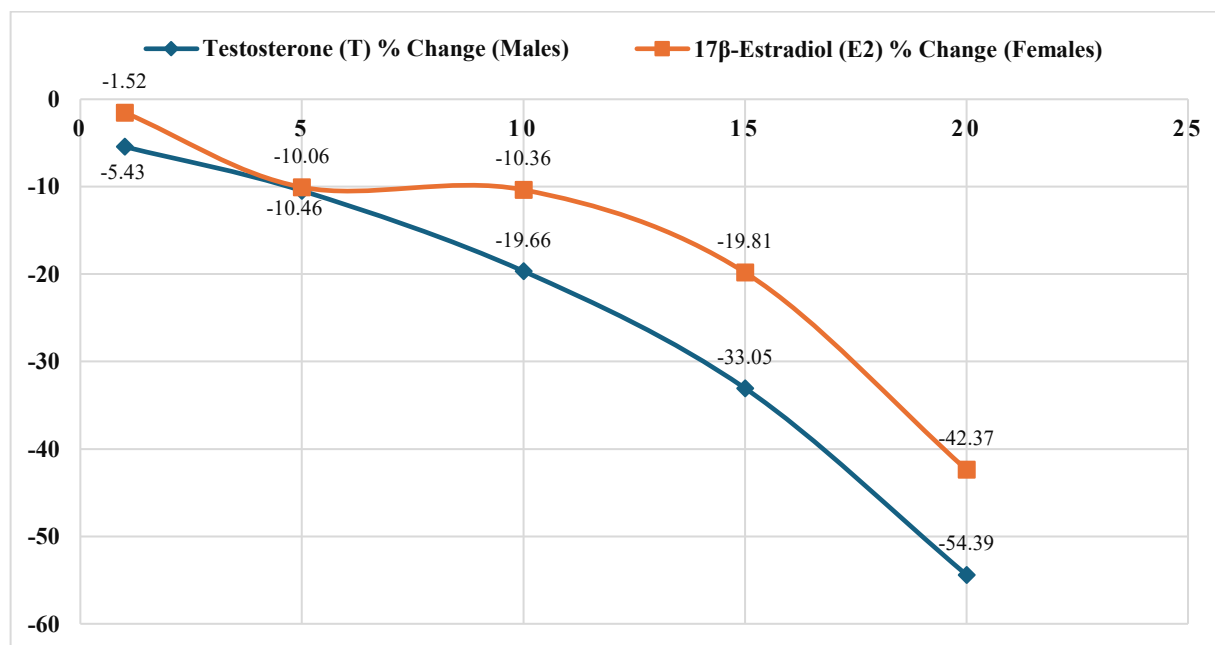
Table 2: Summary of Histopathological Alterations in Gonadal Tissues Following LCH Exposure

Exposure Duration (Days)	Testicular Changes	Ovarian Changes
1	Normal histoarchitecture	Normal histoarchitecture
5	Mild interstitial gaps	Mild cytoplasmic clumping, inter-follicular space
10	Reduced lobular diameter, fewer spermatozoa	Deformed and elongated ovarian follicles
15	Germinal epithelium atrophy, increased interstitial gaps	Cytoplasmic clumping, atretic oocytes
20	Severe germinal loss, extensive interstitial gaps	Severe cytoplasmic clumping, wrinkled oocytes

The analysis of serum testosterone (T) levels in male fish revealed a marked decline with increasing exposure duration. The percentage reductions compared to control were -5.43%, -10.46%, -19.66%, -33.05%, and -54.39% for 1, 5, 10, 15, and 20 days, respectively. In female fish, the levels of 17 β -estradiol (E2) were also significantly reduced, showing percentage declines of -1.52%, -10.06%, -10.36%, -19.81%, and -42.37% over the respective exposure periods (Table 3, Figure 2).

Table 3: Effect of LCH Exposure on Serum Testosterone and 17 β -Estradiol Levels in *Cyprinus carpio*

Exposure Duration (Days)	Testosterone (T) % Change (Males)	17 β -Estradiol (E2) % Change (Females)
1	-5.43	-1.52
5	-10.46	-10.06
10	-19.66	-10.36
15	-33.05	-19.81
20	-54.39	-42.37

**Figure 2.** Effect of LCH Exposure on Serum Testosterone and 17 β -Estradiol Levels in *Cyprinus carpio*

Further, the levels of 11-ketotestosterone (11-KT), another key androgenic hormone, were measured in both male and female fish. In males, 11-KT levels demonstrated fluctuating reductions of -1.14%, -16.44%, -10.48%, 2.15%, and 4.92% over the 1, 5, 10, 15, and 20-day exposure periods, respectively. In females, 11-KT levels exhibited a progressive decline, with per cent changes recorded as 0.47%, -5.54%, -14.10%, -22.02%, and -28.20% at the respective time points (Table 4, Figure 3).

Table 4: Effect of LCH Exposure on Serum 11-Ketotestosterone (11-KT) Levels in *Cyprinus carpio*

Exposure Duration (Days)	11-Ketotestosterone (11-KT) % Change (Males)	11-Ketotestosterone (11-KT) % Change (Females)
1	-1.14	0.47
5	-16.44	-5.54
10	-10.48	-14.10
15	2.15	-22.02
20	4.92	-28.20

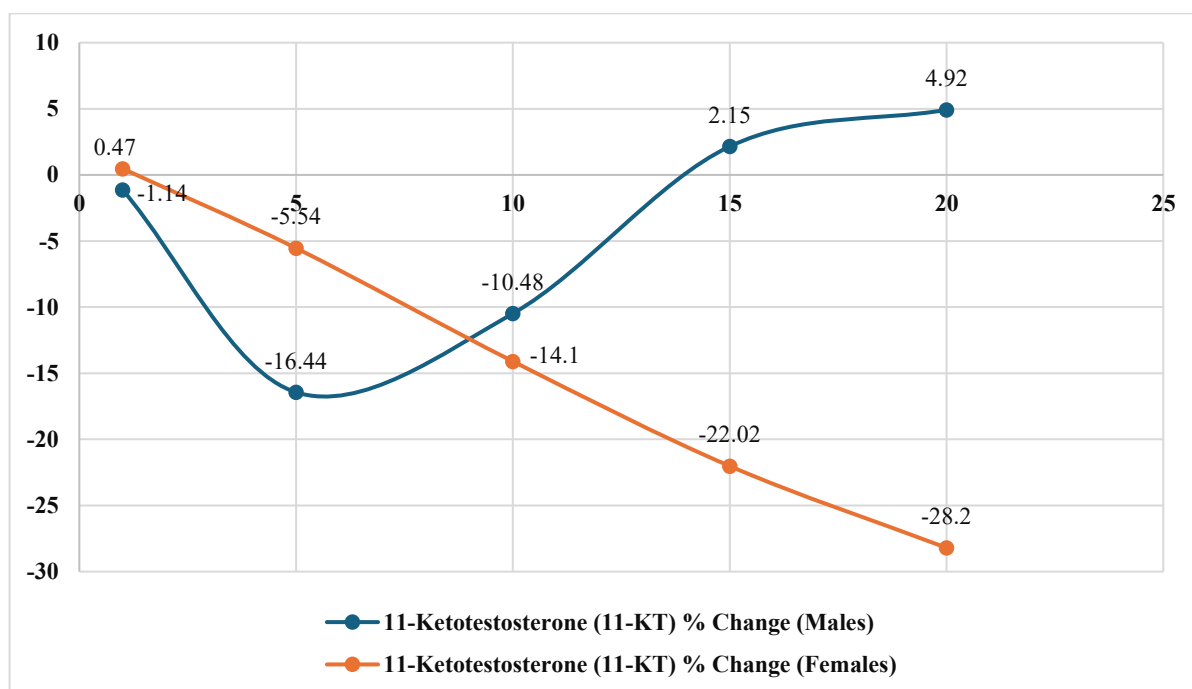


Figure 3. Effect of LCH Exposure on Serum 11-Ketotestosterone (11-KT) Levels in *Cyprinus carpio*

Overall, the results showed that chronic exposure to sub-lethal concentrations of Lambda-cyhalothrin significantly impaired gonadal structure, reduced GSI values, and altered hormonal profiles in *Cyprinus carpio*, indicating substantial reproductive toxicity.

Discussion

Reproduction is a critical biological function, highly sensitive to chemical disturbances, making it an effective endpoint to evaluate the toxicological impact of environmental pollutants. The exposure of *Cyprinus carpio* to sub-lethal concentrations of Lambda-cyhalothrin (LCH) in the study revealed a substantial decline in reproductive health, as evidenced by reductions in Gonadosomatic Index (GSI), histopathological deterioration of gonadal structures, and perturbations in sex steroid hormone profiles. Various methods are there to check the reproductive condition in the case of the fishes, different types of methods offered are microscopic gonadal staging, macroscopic gonadal staging, oocyte size–frequency distributions, sex steroid measurement, as well as gonadal indices (Lowerre-Barbieri, 2011). The findings align with the increasing concern over pesticide-induced endocrine disruption in aquatic organisms, posing potential risks to population dynamics and ecosystem stability. A study by Dawar et al. (2016) evaluated the influence of sub-lethal Cypermethrin (CYP) exposure (8.43 µg/L, 20% of LC₅₀) on early stages of rohu (*Labeo rohita*). Mortality increased progressively from 24 h to 96 h. Morphological deformities like elongated yolk sac, absent eyes, and paralysis appeared early (from blastula to fry stage). Antioxidant enzymes (CAT, POD, LPO) increased significantly under CYP stress, while Glutathione Reductase (GR) activity was also markedly higher in the treated group. Cortisol levels surged after 24 h of exposure but normalized by 96 h. Overall, even low concentrations of CYP severely disrupt development, survival, and stress physiology in rohu.

A static renewal bioassay was conducted by Marigoudar et al. (2009) to assess the toxicity of cypermethrin (92.25% purity) on *Labeo rohita*. Fish were exposed to varying concentrations for 96 hours, and the LC₅₀ value was determined as 4.0 µg/L. For sub-acute studies, a sub-lethal concentration of 0.57 µg/L (one-seventh of LC₅₀) was selected. Behavioural abnormalities were observed under both lethal (1, 2, 3, 4 days) and sub-lethal (1, 5, 10, 15 days) exposures, including erratic swimming, loss of balance, hyper/hypo-opercular movements, and excessive mucus secretion, likely due to neurotransmitter disruption.

In another study by Eni et al. (2019), *Clarias gariepinus* fish exposed to sublethal concentrations of pesticides such as cypermethrin and deltamethrin showed significant biochemical, hormonal, and gonadal disruptions. Plasma enzyme levels (AST, ALT, ALP) increased independently, while steroid hormones (E2 and T) were altered over time. Gonadal histology revealed intersex conditions and tissue degeneration, indicating strong endocrine-disrupting and reproductive toxic effects of these pesticides.

Sayed et al. (2022) examined the impact of 4-nonylphenol (4-NP) on juvenile *Clarias gariepinus* over 15 days of exposure at 0.1, 0.2, and 0.3 mg/L, followed by a 15-day recovery. Significant changes were observed in E2 and T hormone levels ($P < 0.05$), while FSH and LH showed no significant differences. Histopathological gonadal damage, haematological alterations (in RBCs, Hct, WBCs), and increased apoptosis in erythrocytes and brain cells occurred in a dose-dependent manner. Recovery improved but did not fully reverse the toxic effects.

A study by Raibeemol & Chitra. (2016) exposed *Pseudotroplus maculatus* to 0.661 µg/L chlorpyrifos for 24, 48, 72, and 96 hours. No major gill changes were seen at 24 h, but by 48 h, curling and fusion of lamellae appeared. Severe damage,

including destruction and vacuolization of lamellae, was observed at 72 h, and necrosis and aneurysm developed by 96 h. Gill damage intensified in a time-dependent manner, even at sublethal concentrations.

Synthesis of VTG, a lipophosphoprotein, is encouraged by estradiol present in the liver of female fish. The exposure of *Cyprinus carpio* (common carp) to sublethal concentrations of phenol and sulfide for 45 days resulted in a significant increase of nonesterified cholesterol in the ovary of the fish, thus demonstrating obstructed mobilization of this physiologically dynamic cholesterol molecule in the production of vitellogenin as well as steroid hormones (Mukherjee et al., 1992). But in our findings, the level of testosterone (T), 11 ketotestosterone (11-KT), and 17 β -estradiol (E2), significantly reduced in 5, 10, 15 and 20 days of exposure. Changes were observed in fish that were exposed for a longer duration. Therefore, it is clearly shown that LCH poses a threat to fish population dynamics by reducing fertility.

Conclusion and Future Scope

The present investigation demonstrates that exposure to sub-lethal concentrations of Lambda-cyhalothrin (LCH) exerts significant reproductive toxicity in *Cyprinus carpio*. The study revealed a consistent reduction in the Gonadosomatic Index (GSI) in male as well as female fish across all exposure durations, indicating impaired gonadal development. Histopathological evaluations confirmed extensive structural alterations, including disintegration of the germinal epithelium in testes and cytoplasmic clumping, follicular deformation, and atresia in ovaries. These structural damages corresponded closely with hormonal disruptions, as evidenced by significant reductions in serum testosterone, 17 β -estradiol, and 11-ketotestosterone levels. Collectively, the findings suggest that LCH exposure severely compromises the reproductive potential of *Cyprinus carpio*, posing a substantial threat to population sustainability. The study highlights the sensitivity of reproductive parameters as effective bioindicators of aquatic pollution and underscores the ecological risks associated with synthetic pyrethroid contamination in freshwater ecosystems. Building upon these outcomes, future research should focus on elucidating underlying biochemical mechanisms, particularly oxidative stress pathways, that contribute to LCH-induced gonadal toxicity. Studies examining the recovery potential of fish post-exposure would provide valuable insights into the reversibility of reproductive impairments. Additionally, multi-generational studies are recommended to assess the transgenerational impacts of pesticide exposure on reproductive fitness and population health. Field monitoring of natural water bodies contaminated with pyrethroids, using sensitive biomarkers such as GSI, histopathological assessments, and hormonal profiling, will be crucial for accurate ecological risk assessment. These findings emphasize the urgent need for regulatory measures to control pesticide runoff and protect aquatic biodiversity.

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