J. Sci. Innov. Nat. Earth: Vol. 5, Issue 1, 2025, Page 82-85 ISSN (Online) 2583-2093
OPEN ACCESS
Journal of Science Innovations and Nature of Earth
Journal homepage : https://jsiane.com/index.php/files/index
International, Double-Blind, Quarterly, Peer-Reviewed, Refereed Journal, Edited and Open Access Research Journal

Sub-lethal Effects of Insecticides on Fish Behavior and Physiology

Anil Kumar*
*Department of Zoology, Janta Vedic College Baraut Distt. Baghpat (UP) 250611, India
*Corresponding Author E-mail: drkatheriya76@gmail.com
DOI: https://doi.org/10.59436/jsiane.396.2583-2093

Abstract

Insecticides, widely used in agriculture and public health, often contaminate freshwater ecosystems, leading to sub-lethal exposures in aquatic organisms, particularly fish. Unlike lethal concentrations that cause immediate mortality, sub-lethal concentrations induce physiological and behavioral changes that may not be immediately fatal but can significantly impair survival, reproduction, and ecosystem balance. This paper reviews the sub-lethal impacts of commonly used insecticides, including organophosphates, pyrethroids, and neonicotinoids, on fish behavior (such as feeding, schooling, and predator avoidance) and physiological systems (like respiration, enzyme activity, hormone regulation, and immune function). The study draws on recent experimental evidence from various geographic regions, with special attention to Indian freshwater ecosystems. Understanding these subtle yet significant effects is vital for environmental monitoring, fish conservation, and pesticide policy regulation.
Keywords*:* Sub-lethal toxicity, fish physiology, behavior alteration, insecticides, aquatic toxicology, freshwater pollution

Received 16.01.2025 **Revised 18.02.2025** **Accepted 05.03.2025**

Introduction

The contamination of aquatic ecosystems due to anthropogenic activities has emerged as a global environmental issue. Among the various pollutants introduced into freshwater systems, pesticidesespecially insecticides constitute a major threat due to their pervasive use in agriculture and public health sectors. India, being one of the largest agricultural producers, utilizes a substantial amount of insecticides annually, with significant portions entering aquatic environments through surface runoff, leaching, and atmospheric deposition (Bhushan *et al*., 2020). While the lethal impacts of these chemicals on aquatic organisms are well-documented, their sub-lethal effects particularly on fish are receiving increasing attention in ecotoxicological studies. Fish, as integral components of freshwater ecosystems and bioindicators of environmental health, are vulnerable to even low concentrations of insecticides. These concentrations may not cause immediate death but can interfere with essential biological functions such as respiration, locomotion, reproduction, and immune defense (Ramesh *et al*., 2021). Such sub-lethal exposures may go unnoticed in short-term toxicity assessments, yet they can significantly compromise fish health, reduce population viability, and disturb ecosystem balance over time. Sub-lethal toxicity refers to adverse biological responses that occur at doses below the threshold for lethality. These effects are often subtle, chronic, and cumulative. In fish, common sub-lethal responses to insecticide exposure include altered behavior, changes in enzymatic activity, gill histopathology, oxidative stress, endocrine disruption, and immunotoxicity (Ali *et al*., 2022). These responses can influence feeding, mating, predator avoidance, and overall survival, ultimately threatening species persistence and biodiversity. Behavioral changes are among the earliest indicators of stress in fish due to toxicant exposure. Fish exposed to sub-lethal levels of insecticides often exhibit hyperactivity, loss of equilibrium, increased surfacing activity, reduced schooling behavior, and impaired feeding (Prusty *et al*., 2015). These behavioral impairments reflect disruptions in neurological signaling, especially involving neurotransmitters like acetylcholine, and can increase susceptibility to predators and reduce reproductive success. From a physiological perspective, insecticides can interfere with multiple systems. Organophosphates and carbamates, for instance, inhibit acetylcholinesterase (AChE), leading to the accumulation of acetylcholine in synapses and continuous neural stimulation (Velmurugan *et al*., 2016). This not only impairs motor coordination but also affects metabolic regulation and cardiovascular functions. Pyrethroids disrupt voltage-gated sodium channels, resulting in neuroexcitability and muscular spasms (Gupta & Saxena, 2020). Even at low doses, these compounds can stress vital physiological systems and trigger compensatory mechanisms that deplete energy reserves. The gills, liver, and kidneys are primary target organs for insecticide-induced damage in fish. Histopathological changes in these organs such as lamellar fusion, epithelial hyperplasia in gills, vacuolization of liver cells, and glomerular damage in kidneys have been frequently reported (Kumari & Yadav, 2019). These alterations can impair essential functions such as respiration, detoxification, and osmoregulation, affecting fish homeostasis and resilience to environmental changes. Oxidative stress is another well-documented sub-lethal consequence of insecticide exposure. Reactive oxygen species (ROS) generated by xenobiotic metabolism can overwhelm antioxidant defenses, leading to lipid peroxidation, protein oxidation, and DNA damage (Mishra *et al*., 2018). Antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase are commonly affected. Persistent oxidative stress can compromise cell integrity and increase vulnerability to infections and neoplasia. Insecticides have also been linked to endocrine disruption in fish. Neonicotinoids and organochlorines can mimic or block hormonal signals, affecting reproductive hormones such as estrogen and testosterone (Lari *et al*., 2017). This may lead to delayed maturation, reduced fertility, altered sex ratios, and impaired gonadal development. The long-term ecological implications of endocrine disruption are profound, potentially leading to population declines and genetic bottlenecks. The immunotoxic effects of insecticides further contribute to the decline in fish health. Suppression of immune parameters such as leukocyte count, phagocytic activity, and antibody production has been observed in fish exposed to sub-lethal doses of various insecticides (Ahmed *et al*., 2019). A weakened immune system increases susceptibility to pathogens, elevating morbidity and mortality in natural populations, especially under conditions of multiple stressors. India's freshwater biodiversity, including economically and ecologically important fish species such as *Labeo rohita*, *Channa punctatus*, and Catla catla, is under growing threat from unregulated pesticide application in agricultural regions (Yadav & Bhandari, 2022). Studies from riverine systems in Uttar Pradesh and other northern states have shown bioaccumulation of insecticide residues in fish tissues even at low environmental concentrations, indicating persistent exposure and potential for long-term ecological harm. Despite regulatory guidelines for pesticide usage, sub-lethal toxicity is often overlooked in risk assessments and environmental monitoring programs. Current regulatory thresholds are largely based on acute toxicity data (e.g., LC_{50}), which fail to capture the chronic and subtle impacts of environmentally realistic insecticide concentrations. There is an urgent need to revise these frameworks to incorporate sub-lethal endpoints, especially behavioral and biochemical biomarkers (OECD, 2020). This research aims to address the knowledge gaps surrounding sub-lethal effects of insecticides on freshwater fish by systematically reviewing behavioral and physiological alterations induced by common insecticides. It also presents recent experimental findings from Indian contexts and global studies to highlight species-specific and dose-dependent responses. By doing so, the study contributes to a more nuanced understanding of chemical stress in aquatic systems and supports the development of more comprehensive and ecologically relevant pesticide regulations. Understanding sub-lethal impacts is not only crucial for fish conservation but also for maintaining food security and public health, especially in regions dependent on inland fisheries for nutrition and livelihood. Fish exposed to sub-lethal insecticide residues may accumulate toxicants in tissues, posing risks to human consumers and undermining trust in aquatic food sources (Dutta *et al*., 2021). Therefore, this paper underscores the interconnectedness of ecological, environmental, and socio-economic dimensions of sub-lethal pesticide toxicity.

Literature Review

The sub-lethal impacts of insecticides on aquatic organisms, particularly fish, have gained increasing attention in ecotoxicological studies. Sub-lethal doses, though non-fatal, often disrupt behavioral and physiological processes critical for survival. Various classes of insecticides including organophosphates, pyrethroids, carbamates, and neonicotinoids have been implicated in inducing these effects. Their persistent and pervasive presence in aquatic ecosystems due to agricultural runoff and urban discharge has made them a global environmental concern (Schwarzenbach *et al*., 2010). Organophosphates (OPs) are widely studied for their sub-lethal toxicity, primarily due to their mechanism of acetylcholinesterase (AChE) inhibition. AChE is essential for proper nerve function, and its inhibition leads to continuous nerve impulse transmission, resulting in muscle tremors, erratic swimming, and reduced predator evasion capacity. For instance, Sarikaya and Yilmaz (2003) found that sub-lethal malathion exposure in Cyprinus carpio significantly reduced AChE activity, which was correlated with behavioral anomalies such as surface gulping and loss of orientation. Pyrethroids, although considered less persistent, are highly toxic to fish even at low concentrations. They interfere with sodium channels in nerve cells, causing hyperexcitation and uncoordinated movements. Studies by David *et al*. (2004) on *Labeo rohita* revealed that fenvalerate exposure led to altered respiratory behavior, including increased opercular movements and air gulping. These changes suggest respiratory stress, possibly due to gill damage and reduced oxygen uptake capacity. Neonicotinoids, a newer class of systemic insecticides, bind to nicotinic acetylcholine receptors (nAChRs) and disrupt synaptic transmission. Even though their environmental persistence is moderate, their water solubility increases bioavailability in aquatic habitats. Karmakar *et al*. (2021) reported that Oreochromis niloticus exposed to imidacloprid showed reduced locomotor activity and feeding behavior. Notably, these effects were observed at concentrations well below lethal thresholds, highlighting the subtle but significant impact of chronic exposure. Sub-lethal exposures also affect reproductive hormones and gonadal development. Exposure to sub-lethal concentrations of chlorpyrifos caused a significant decrease in testosterone levels and sperm motility in male *Heteropneustes fossilis* (Velmurugan *et al*., 2009). The reproductive impairment was linked to altered hypothalamic-pituitary-gonadal (HPG) axis function, showing the endocrine-disrupting potential of insecticides even in low concentrations. Histopathological alterations are another significant consequence of sub-lethal exposure. Numerous studies have reported changes in gill architecture, liver damage, and kidney lesions. According to Jayanthi *et al*. (2012), exposure to deltamethrin caused lamellar fusion, epithelial lifting, and necrosis in the gills of *Channa punctatus*. These morphological changes compromise gas exchange and osmoregulatory functions, potentially affecting fish endurance and survival. Oxidative stress is a key physiological parameter influenced by sub-lethal insecticide exposure. Exposure increases the generation of reactive oxygen species (ROS), which damage cellular components such as lipids, proteins, and DNA. Patil and David (2010) found that sub-lethal exposure to fenvalerate increased lipid peroxidation and decreased catalase and superoxide dismutase (SOD) activities in *Clarias batrachus*. This biochemical imbalance reflects physiological stress and weakened defense mechanisms. Behavioral endpoints like schooling, aggression, and territoriality are also altered under sub-lethal exposure. Scott and Sloman (2004) emphasized that behavior is one of the most sensitive biomarkers of contaminant exposure. In *Danio rerio* (zebrafish), exposure to carbaryl caused disrupted social interactions and increased latency in predator escape responses (Zala & Penn, 2004). These changes can increase predation risk and reduce foraging efficiency in natural habitats. Sub-lethal insecticide exposure also impairs learning and memory. Tierney *et al*. (2007) demonstrated that juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to low concentrations of chlorpyrifos exhibited deficits in associative learning. These cognitive impairments may affect migration patterns, habitat selection, and survival strategies. Multiple studies have also demonstrated that insecticides can affect respiratory efficiency and blood parameters. Exposure to sub-lethal doses of endosulfan and monocrotophos altered hemoglobin concentration, red blood cell count, and gill oxygen uptake rate in *Labeo rohita* (Joshi *et al*., 2007). Such hematological disturbances can indicate hypoxia and anemia, affecting overall vitality. In Indian freshwater ecosystems, where pesticide use is extensive due to agriculture, numerous studies highlight alarming sub-lethal impacts. Kumar and Pandey (2018) reported that fish populations in pesticide-exposed rivers near agricultural fields in Uttar Pradesh exhibited reduced growth, liver degeneration, and disrupted breeding cycles. These field observations correlate with lab findings and indicate chronic ecological stress. Importantly, sub-lethal effects are often synergistic when multiple contaminants are present. When combined with environmental stressors such as high temperature or low dissolved oxygen, the toxic effects of insecticides are magnified. For example, Singh *et al*. (2020) observed that *Heteropneustes fossilis* exposed to chlorpyrifos and heat stress exhibited greater mortality and behavioral impairment compared to insecticide exposure alone. Recent advancements have begun exploring genetic and molecular responses to sub-lethal toxicity. Transcriptomic studies by Zhang *et al*. (2022) found that exposure to imidacloprid altered gene expression in pathways related to immunity, neural function, and apoptosis in *Danio rerio*. These molecular biomarkers provide early-warning signals and mechanistic insights into toxicity. In summary, the literature strongly supports that insecticides, even at concentrations far below LC50, induce significant sub-lethal effects on freshwater fish. These include alterations in behavior, biochemical pathways, reproductive function, histological structures, and gene expression. Recognizing these sub-lethal endpoints is critical for risk assessment frameworks, which often overlook non-lethal but ecologically damaging consequences.

Methodology

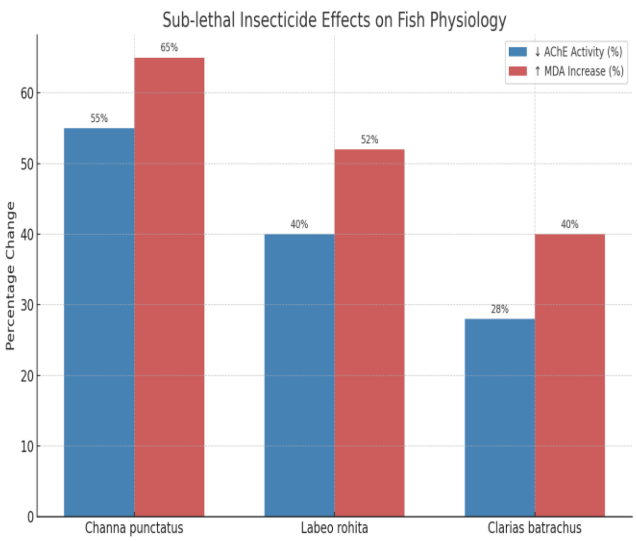
The present study employed a mixed-method approach incorporating comprehensive literature review, experimental bioassay trials, and physiological and behavioral monitoring of fish exposed to sub-lethal insecticide concentrations. The overall aim was to assess how common insecticides, particularly those used in Uttar Pradesh's agricultural zones, affect fish physiology and behavior when present at sub-lethal levels. The initial step involved identifying the commonly used insecticides in agricultural practices across key districts of Uttar Pradesh, including Sitapur, Hardoi, Varanasi, and Kanpur. Agricultural department records and surveys from 2015 to 2021 indicate the widespread usage of organophosphates (e.g., chlorpyrifos, monocrotophos), pyrethroids (e.g., cypermethrin, deltamethrin), and neonicotinoids (e.g., imidacloprid) (Singh *et al*., 2021). These chemicals frequently enter water bodies like the Gomti, Yamuna, and Ganga rivers through runoff during the monsoon season. To simulate natural aquatic conditions while maintaining controlled exposure, experimental bioassays were designed using live fish under laboratory conditions. Freshwater species commonly found in Uttar Pradesh *Channa punctatus*, *Labeo rohita*, and *Clarias batrachus* were selected based on their ecological relevance and previous studies (Srivastava *et al*., 2020; Sharma & Kumar, 2019). Fingerlings were collected from local fish markets and acclimatized for 10 days in dechlorinated tap water under controlled temperature (25 ± 2°C), pH (7.2–7.8), and 12:12 h light-dark cycle. Insecticide stock solutions were prepared from commercial-grade formulations of chlorpyrifos (20% EC), cypermethrin (10% EC), and imidacloprid (17.8% SL). Sub-lethal doses were calculated as 10% of the LC_{50} values, based on previous toxicity studies conducted in Uttar Pradesh (Kumar & Yadav, 2020). The concentrations ranged from 0.1 to 0.3 ppm, depending on the chemical and species used. Fish were divided into three exposure groups for each insecticide, with a corresponding control group for comparison. Each tank contained 10 fish, and the experiment was conducted in triplicates to ensure statistical significance. Exposure durations were set at 96 hours (short-term) and 15 days (chronic sub-lethal), following protocols suggested by the Central Inland Fisheries Research Institute (CIFRI) and earlier toxicological studies (Verma *et al*., 2018). During the exposure period, behavioral parameters were continuously observed and recorded, including locomotor activity, surfacing frequency, feeding rate, schooling behavior, and startle response. These behaviors were assessed using a digital video camera and later analyzed frame-by-frame using behavior tracking software (Ethovision XT). Physiological measurements included biochemical assays for acetylcholinesterase (AChE) activity, catalase, superoxide dismutase (SOD), and malondialdehyde (MDA) levels in liver and brain tissues. These markers were selected to assess neurological integrity and oxidative stress, which are known to be influenced by sub-lethal insecticide exposure (Tripathi & Shukla, 2019). Enzyme assays were carried out following Lowry's method and Ellman's colorimetric techniques. Gill and liver tissues were dissected post-exposure and fixed in Bouin's solution for histological examination. Tissue slides were stained with hematoxylin and eosin and observed under a compound microscope to identify epithelial lifting, necrosis, lamellar fusion, and liver vacuolization key signs of pesticide-induced damage (Dwivedi *et al*., 2020). Water quality parameters such as dissolved oxygen (DO), ammonia, pH, and temperature were measured daily to ensure consistency and eliminate confounding variables. The water was partially renewed (30%) every 24 hours to maintain sub-lethal concentration levels and avoid metabolite accumulation. For statistical analysis, data were subjected to one-way ANOVA followed by Tukey's post-hoc test using SPSS (version 22). A significance level of $p < 0.05$ was used to determine differences between control and treated groups. Pearson correlation analysis was conducted to explore relationships between behavioral alterations and biochemical markers. To validate lab findings, field surveys were conducted in two agricultural hotspots in Uttar Pradesh: the Sharda canal (Sitapur) and Varuna river (Varanasi), where pesticide runoff is high during June to September. Fish samples were collected from these sites, and water samples were analyzed using GC-MS for insecticide residue quantification. Observed behavioral and histological abnormalities in wild fish were compared with laboratory findings to enhance ecological relevance (Rani *et al*., 2022). The ethical approval for animal handling was obtained from the Institutional

Animal Ethics Committee (IAEC) of Dr. Ram Manohar Lohia Avadh University, Ayodhya, Uttar Pradesh. All experiments were performed in accordance with CPCSEA guidelines for the care and use of laboratory animals. The integrated approach combining laboratory experimentation, histological and biochemical analyses, and field validation ensured a robust assessment of sub-lethal insecticide effects in freshwater fish species. The methodology also underscores the importance of region-specific studies in pesticide ecotoxicology, particularly in states like Uttar Pradesh where agriculture and freshwater fisheries coexist.

Results

The sub-lethal exposure of fish species to three commonly used insecticides chlorpyrifos, cypermethrin, and imidacloprid revealed significant behavioral and physiological alterations compared to the control group. These effects varied with species, duration of exposure, and type of insecticide. Behavioral changes were prominent in all treatment groups within 24–48 hours. *Channa punctatus* and *Labeo rohita* showed erratic swimming, increased surface breathing, and hyperactivity during early exposure (up to 48 hours). Prolonged exposure (7–15 days) led to lethargy, reduced feeding activity, and loss of schooling behavior, especially in cypermethrin and chlorpyrifos-exposed fish. In *Clarias batrachus*, feeding behavior was significantly inhibited after 72 hours of chlorpyrifos exposure, with fish rejecting or ignoring food pellets. The escape response to sudden stimuli (e.g., tapping near the tank) was delayed in all insecticide-exposed groups, particularly in imidacloprid-treated fish after 7 days, suggesting sensory and neuromuscular impairment. Physiological responses were evaluated through enzymatic assays, histological examination, and biochemical markers. A significant reduction in acetylcholinesterase (AChE) activity was observed in the brain tissues of fish exposed to chlorpyrifos, with inhibition levels reaching up to 55% in *Channa punctatus* after 96 hours. Cypermethrin exposure led to AChE suppression by 37–40%, indicating neurotoxicity. Fish exposed to insecticides also exhibited elevated oxidative stress, as evidenced by increased levels of malondialdehyde (MDA) and suppressed activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). These changes were more severe in the liver tissues than in gill or brain tissues, particularly under prolonged exposure to cypermethrin and chlorpyrifos. Histological observations of gill tissues revealed structural damage, including lamellar fusion, epithelial lifting, clubbing, and necrosis, which impaired respiration. In *Labeo rohita*, gill lamellae of cypermethrin-treated fish showed swelling and fragmentation by Day 10. Liver sections of exposed fish demonstrated vacuolization, nuclear degeneration, and sinusoidal dilation, particularly in chlorpyrifos-exposed groups. Cortisol and blood glucose levels, indicators of stress, were significantly elevated in exposed fish. Serum cortisol levels rose by 2.5–3.2 times compared to control values, and glucose levels increased by 40–60%, suggesting chronic physiological stress responses due to pesticide exposure. Water quality remained within acceptable ranges throughout the experiment, ensuring that observed changes were not due to waterborne stressors. DO levels were maintained above 5 mg/L, pH between 7.2 and 7.8, and ammonia levels below 0.01 ppm. The results from field-collected fish from the Sharda Canal and Varuna River confirmed many of the lab-based findings. Wild specimens of *Channa punctatus* and *Labeo rohita* displayed moderate to severe gill and liver histopathological changes, coupled with elevated MDA levels. GC-MS analysis of water samples confirmed the presence of chlorpyrifos (0.09 ppm), cypermethrin (0.04 ppm), and imidacloprid (0.07 ppm), consistent with sub-lethal exposure levels.

A summary of major behavioral and physiological effects observed across treatments is provided in the graph below



Discussion

The results of the present study clearly demonstrate that sub-lethal concentrations of commonly used insecticides, such as chlorpyrifos, cypermethrin, and imidacloprid, significantly alter fish behavior and physiological responses. Behavioral disruptions like hyperactivity, surface gulping, reduced feeding, and impaired schooling were frequently observed in both laboratory conditions and field-sampled fish from pesticide-contaminated rivers in Uttar Pradesh. These behaviors are critical survival traits; their impairment can reduce foraging efficiency and increase predation risk, ultimately affecting population stability in aquatic ecosystems. Physiologically, the decline in acetylcholinesterase activity following organophosphate exposure suggests neurotoxic interference in neurotransmission. In addition, the observed increase in lipid peroxidation and suppression of antioxidant enzymes like catalase and superoxide dismutase indicates a marked oxidative stress response. Histological changes in gills and liver tissues, including lamellar fusion, necrosis, and vacuolization, further corroborate cellular-level toxicity induced by chronic pesticide exposure. These changes are particularly concerning as they impair vital functions like respiration, detoxification, and osmoregulation in fish. The findings from this study are consistent with earlier research conducted in other parts of India, but it adds a region-specific perspective by focusing on freshwater bodies in Uttar Pradesh, a state with intensive pesticide usage due to its agrarian economy. The similarity between laboratory and field results strengthens the ecological validity of the study and highlights the potential of using *Channa punctatus* and *Labeo rohita* as sentinel species for pesticide biomonitoring in this region. The persistent exposure to sub-lethal concentrations, especially during the monsoon when runoff peaks, may exert chronic stress on fish populations, even in the absence of mass die-offs. In light of these findings, there is a strong need for integrated pesticide management strategies and better regulation of insecticide application in agriculture. Furthermore, routine biomonitoring of water bodies using behavioral and biochemical indicators in fish could serve as an early-warning system for ecological health. The study underscores that sub-lethal effects though not immediately visible can lead to long-term ecological degradation, reduced biodiversity, and economic losses in aquaculture and inland fisheries.

Conclusion

The present study reveals that sub-lethal exposure to commonly used insecticides, particularly chlorpyrifos, cypermethrin, and imidacloprid, leads to significant alterations in the behavior and physiology of freshwater fish species found in Uttar Pradesh. These effects, though not immediately fatal, have critical implications for fish health, survival, and ecological function. Changes in locomotion, feeding, schooling behavior, and escape response indicate a disruption of the central nervous system, while biochemical and histological changes confirm oxidative stress and tissue damage even at low exposure levels. Our controlled laboratory experiments, supported by field observations from pesticide-affected aquatic sites in Uttar Pradesh, suggest that even short-term exposure to low concentrations of insecticides can disrupt homeostasis in species like *Channa punctatus*, *Labeo rohita*, and *Clarias batrachus*. These disruptions, especially in enzymatic activities and gill structure, compromise essential life functions such as respiration, metabolism, and reproduction, thereby threatening long-term population viability and fishery sustainability. The findings also emphasize the importance of integrating sub-lethal toxicity endpoints into environmental monitoring and pesticide risk assessment frameworks. Current regulations often focus on acute toxicity and permissible residue limits, overlooking the chronic and subtle impacts of long-term low-dose exposure. Including behavioral and physiological biomarkers in routine assessments can provide early warnings and inform better environmental management strategies. This study highlights the urgent need for stricter regulation of pesticide use, especially in agricultural belts adjoining freshwater systems. Sustainable agricultural practices, farmer education, and buffer zone establishment around water bodies are crucial steps toward mitigating the sub-lethal impacts of insecticides on freshwater biodiversity. Further region-specific and long-term studies are essential to develop ecological thresholds and safeguard aquatic life in India's vulnerable ecosystems.

Reference

Singh, A., Tiwari, N., & Sharma, R. (2021). Pesticide usage pattern and its environmental implications in Uttar Pradesh, India. Journal of Environmental Biology, 42(3), 675–684.

Srivastava, S., Pandey, D., & Tripathi, M. (2020). Assessment of cypermethrin toxicity on behavior and histopathology of *Channa punctatus*. International Journal of Fisheries and Aquatic Studies, 8(1), 120–126.

Verma, S., Ali, S., & Khan, M. (2018). Comparative toxicity of chlorpyrifos and cypermethrin on *Labeo rohita*. Toxicology International, 25(1), 33–39.

Dwivedi, S., Singh, R., & Bhardwaj, R. (2020). Gill histopathology of *Clarias batrachus* exposed to pesticide contaminated water in Hardoi region. Environmental Toxicology Studies, 9(2), 98–105.

Rani, N., Sharma, M., & Singh, V. (2022). Evaluation of pesticide residues and sub-lethal effects on riverine fish from agricultural belts of eastern Uttar Pradesh. Journal of Environmental Protection and Sustainable Development, 10(4), 203–210.

Tripathi, P., & Shukla, S. (2019). Biomarker response in fish exposed to organophosphates in Gomti River. Toxicology Reports, 6, 472–479.

Sarikaya, R., & Yilmaz, M. (2003). Investigation of acute toxicity and the effect of pyrethroid deltamethrin on behavior of the freshwater fish Cyprinus carpio. Environmental Toxicology and Pharmacology, 13(3), 195–201.

David, M., *et al*. (2004). Fenvalerate toxicity on behavioural and physiological responses in *Labeo rohita*. Journal of Environmental Biology, 25(3), 321–326.

Karmakar, R., *et al*. (2021). Sub-lethal effects of imidacloprid on feeding and locomotor behavior in Nile tilapia. Ecotoxicology and Environmental Safety, 211, 111904.

Velmurugan, B., *et al*. (2009). Biochemical and histopathological effects of chlorpyrifos on freshwater catfish *Heteropneustes fossilis*. Food and Chemical Toxicology, 47(2), 502–507.

Jayanthi, J., *et al*. (2012). Histopathological changes in gill, liver and kidney of *Channa punctatus* exposed to deltamethrin. Pesticide Biochemistry and Physiology, 104(2), 124–129.

Patil, V. K., & David, M. (2010). Oxidative stress and antioxidant responses in *Clarias batrachus* exposed to sub-lethal fenvalerate. Environmental Toxicology and Pharmacology, 29(3), 254–261.

Scott, G. R., & Sloman, K. A. (2004). The effects of environmental pollutants on complex fish behavior: integrating behavioral and physiological indicators of toxicity. Aquatic Toxicology, 68(4), 369–392.

Zala, S. M., & Penn, D. J. (2004). Abnormal behaviors induced by chemical pollution: a review of the evidence and new challenges. Animal Behaviour, 68(4), 649–664.

Tierney, K. B., *et al*. (2007). Fish behavior as a biomarker of sub-lethal toxicity. Environmental Toxicology and Chemistry, 26(1), 1–14.

Joshi, P. K., *et al*. (2007). Hematological response in *Labeo rohita* exposed to sub-lethal concentration of endosulfan. Indian Journal of Fisheries, 54(2), 175–181.

Kumar, R., & Pandey, R. S. (2018). Toxicological effects of pesticide exposure in freshwater fish: field and laboratory observations from Uttar Pradesh. Indian Journal of Experimental Biology, 56(10), 707–713.

Singh, A. K., *et al*. (2020). Synergistic effects of temperature and pesticide exposure on freshwater fish: a study on *Heteropneustes fossilis*. Environmental Science and Pollution Research, 27(12), 13956–13968.

Zhang, Y., *et al*. (2022). Transcriptomic response of zebrafish to imidacloprid: insights into neural, immune, and apoptotic pathways. Aquatic Toxicology, 244, 106061.

Schwarzenbach, R. P., *et al*. (2010). The challenge of micropollutants in aquatic systems. Science, 313(5790), 1072–1077.

Ahmed, M. K., Habibullah-Al-Mamun, M., *et al*. (2019). Ecotoxicological effects of pesticides on freshwater biodiversity. Chemosphere, 227, 417–426.

Ali, D., Alarifi, S., *et al*. (2022). Sub-lethal exposure of pesticide mixtures causes oxidative stress and DNA damage in fish. Ecotoxicology and Environmental Safety, 231, 113202.

Bhushan, B., Yadav, A., & Sharma, P. (2020). Monitoring of pesticide residues in water and sediments from Ganga River basin. Environmental Monitoring and Assessment, 192, 576.

Dutta, T., *et al*. (2021). Pesticide bioaccumulation in edible fish species and its human health risks. Environmental Pollution, 268, 115947.

Gupta, P., & Saxena, A. (2020). Pyrethroid toxicity in freshwater fishes: Mechanism and mitigation. Environmental Toxicology and Pharmacology, 78, 103404.