Continental Veterinary Journal
ISSN: 3079-0212 (ONLINE)
www.cvetj.com; editor@cvetj.com

RESEARCH ARTICLE
DOI: 10.71081/cvj/2025.043

Physiological Dysfunction in *Hypophthalmichthys nobilis* (Freshwater carp) after Exposure to Two Insecticides Mixture

Adeeba Naseer*[1,2], Taskeen Shakir[2], Bushra Tariq[2], Sanya Naz[2], Nusheen Ahmad[2], Saud Iqbal[3], Azhar Ullah[3] and Muhammad Usman[3]

[1]College of Fisheries, Southwest University, Chongqing 400715, China; [2]Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, 63100, Pakistan; [3]Department of Zoology, Islamia College, Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan
*Correspondence: adeeba.iub22@gmail.com

ARTICLE INFO

ARTICLE HISTORY: CVJ-25-508

Received: 06 February 2025
Revised: 13 Apr 2025
Accepted: 16 Apr 2025
Published online: 19 Apr 2025

Key words:
Body weight,
Clinical signs,
Hypophthalmicthys nobilis,
Physiological dysfunctions,
Pyriproxyfen and
Acetamiprid

ABSTRACT

Pyriproxyfen and Acetamiprid are insecticides, extensively used to eradicate insects. The current investigation was carried out to ascertain the physiological dysfunctions as consequences of Acetamiprid and Pyriproxyfen exposure in fish Freshwater carp. For this purpose 27 Bighead carp were brought from Bahawalnagar hatchery and divided into three groups G0, G1 and G2, fish were acclimatized for 15 days before experiment. Acetamiprid and Pyriproxyfen were applied together with different concentrations, 0, 2 and 3 mg/L in group G0, G1 and G2 respectively. After administration of chemical, different clinical signs such as; bottom running, surface running, gasping, operculum movement, reversed tilting of pectoral fin, convulsions, jerking, surface breathing, unbalanced body movements, static position, and faintness were observed in fish at day 7 and 14. Dissection of fish was performed at equal intervals of day 7 and 14. Additionally, physiological dysfunctions such as reduction in body weight, reduced growth, and malfunctioning of the visceral organs were recorded during study. In group G2 in which high concentration was applied, fish showed perceptible physiological dysfunctions and other clinical signs. While the group in which low concentration was provided physiological dysfunctions comparatively was minor, but other clinical signs were recognizable in group G1. Results revealed that the toxicity of Pyriproxyfen and Acetamiprid disrupted the body weight of fish.

To Cite This Article: Naseer A, Shakir T, Tariq B, Naz S, Ahmad N, Iqbal S, Ullah A and Usman M, 2025. Physiological Dysfunction in *Hypophthalmichthys nobilis* (Freshwater carp) after Exposure to Two Insecticides Mixture. Continental Vet J, 5(1): 103-112 http://dx.doi.org/10.71081/cvj/2025.043

INTRODUCTION

Chemical substances known as pesticides are largely used to protect crops, prevent vector-borne diseases, control, eradicate, or alleviate pests (Richardson, 2019). With current agricultural techniques, the usage of pesticides is inevitable. Because pesticides suppress harmful weeds, fungus, and insects, agricultural production has been stabilized and altered. But only 1 percent of the pesticide used on crops really reaches the insect; the other 99 percent ends up in the environment, where it contaminates the water, soil, and biota (Mohiddin *et al.*, 2015a; Mohiddin *et al.*, 2015b). Pesticide use benefits include increased yield, labour savings, risk reduction in fertilizer use and crop output (Dasgupta *et al.*, 2001). Growing usage of pesticides since the 1960s has enabled farmers worldwide to significantly boost productivity without experiencing excessive crop losses due to insects, as reported by Oerke (2006). In order to increase effectiveness and lower the cost of crop pest treatment, pesticides are frequently combined in both agricultural and urban settings. The techniques used to apply pesticides (dusting and spraying) allow them to get into the aquatic environment (Elezovic *et al.*, 1994). Therefore, aquatic species certainly interact with a large variety of pesticides that are both structurally and toxicologically varied. Despite the fact that the toxicity of individual pesticides has been well investigated in fish (Joseph and Raj, 2011), nothing is known about the toxicological effects of their mixtures. One of the primary causes of freshwater diversity degradation worldwide is agrochemicals, which has led to growing worries about aquatic hazards from commonly used pesticides, such as neonicotinoids (Schulz *et al.*, 2021). Pesticides are among the most harmful types of pollutants that penetrate the aquatic environment because of their high lipid solubility, resistance to metabolism, and high chemical stability. Pesticides discharged by the agriculture industry have physiological effects on a variety of aquatic creatures, including fish, animals, algae, and shellfish.

Acetamiprid affects the synapses in the insect central nervous system by acting as an agonist on the nicotinic acetylcholine receptor (Wolf and Wheeler, 2018) to produce excitation, paralysis, and death. After being used on crops, this pesticide contaminates a variety of matrices, including soil, water, plants, and aquatic life (Raj and Baby 2015; Morrissey et al. 2015; Hoyle and Code, 2016). Acetamiprid has cytotoxic and genotoxic properties in mammals and aquatic organisms. According to reports, it results in chromosomal abnormalities, micronuclei forming in blood cells, and sister chromatid exchanges in cultures (Hladik *et al.,* 2018; Ma *et al.,* 2019). After being taken orally, acetamiprid travels via the circulation, crosses the blood-brain and blood-testes barriers, and is distributed to various bodily organs before being metabolized by the liver and eventually eliminated by the kidney (Ford and Casida, 2006a, b). As a target organ for detoxification, the liver is vulnerable to a number of illnesses brought on by exposure to environmental contaminants. Phase I enzymes, including cytochrome P450 (CYP) and aldehyde oxidase (AO) in mammals, quickly break down neonicotinoids like acetamiprid in the liver (Shi *et al.,* 2009).

A pyridine-based larvicide called pyriprofen is applied directly to water sources to prevent mosquito larvae from multiplying (Caixeta *et al.,* 2016; Dzieciolowska *et al.,* 2017; Peterson *et al.,* 2017; Maharajan *et al.,* 2018). This larvicide is an equivalent of a juvenile hormone that acts to inhibit the mosquito's metamorphosis and embryogenesis (Ohba *et al.,* 2013). Pyripoxyfen exhibits the potential to be ecologically persistent because to its low solubility, hydrophobicity and high partition coefficients. As a result, caution should be taken while using this pesticide on or near water bodies to prevent water contamination. Additionally, homeowners employed pyriproxyfen as a pesticide to control red fire ants, California red scale, and silver leaf whiteflies in citrus, cotton, vegetable, and peanut crops Maharajan et al. (2018) and Legrand et al. (2017). Pyriproxyfen has harmful deleterious effects on non-target species across the food chain and is extremely stable in a variety of habitats. Because pyriproxyfen is applied continuously and persistently, exposed animals have shown a number of negative consequences, such as neurodevelopmental toxicity, decreased reproduction, and disturbance of endocrine homeostasis (Maharajan *et al.,* 2018). Previous research has shown that PPF may cause fish and other aquatic creatures to perish while controlling mosquitoes (Caixeta *et al.,* 2016). Fish in particular may be negatively impacted by these pesticides' adverse effects on non-target creatures.

Aquatic ecosystems accumulate pesticides, which are then passed on to higher-ranking predators. Since fish are at the top of the aquatic food chain, bio-magnification of pesticides is a common occurrence among aquatic creatures (Henny *et al.,* 2003). Furthermore, fish exposure to pesticides is alarming and concerning since they are a representative group of aquatic animals that humans directly consume. Additionally, fish are indicators of environmental contaminants, with approximately half of pesticides remaining in fish for longer than 30 days (Guo *et al.,* 2008a; Guo *et al.,* 2008b). Chronic fish poisoning in an aquatic environment causes physiological and biochemical alterations in cells as well as morphological defects (Fernandes s, 2013). The most often used neonicotinoid in China is acetamiprid (Formula: C10H11ClN4), a first-generation neonicotinoid that produces 8,000 tons a year Shao, X *et al.,* (2013). The toxicity of pyriproxyfen to vertebrates, including fish, is not well documented. There have been reports of embryo abnormalities at very high concentrations of pyriproxyfen (Truong *et al.,* 2016) and erratic swimming (Caixeta *et al.,* 2016).

MATERIALS AND METHODS

This study was carried out in the Aquaculture, Genetic Toxicity, and Molecular Biology Laboratory of the Department of Zoology at the Islamia University of Bahawalpur in Pakistan. The institutional Bioethics Committee (IBC) of The Islamia University of Bahawalpur, Pakistan, mandated that animal handling ethics be strictly followed.

Fish Collection and Chemical

Healthy Bighead carp individuals weighing 100–120 grams were purchased from a nearby fisheries farm in Bahawalnagar, Pakistan. Fish were transported to the testing site in oxygen-filled polythene containers to guarantee their survival. Pyriproxyfen (19.80% w/w) and Acetamiprid (18.35% w/w) issued by orange protection pesticide company in dual formulation were acquired from commercial scientific store at Bahawalpur, Pakistan.

Acclimatization

Fish are acclimatized for one week in glass aquaria with a 150-liter water capacity and dimensions of 14" L × 10" W × 12" H. International water quality criteria that are appropriate for freshwater fish species were fulfilled by all water qualitywater quality. Aerators were used to control oxygen levels, and frequent fecal waste removal was done to prevent ammonia toxicity. Fish (n=27) were split evenly into three groups (G0-G2) based on their level of acclimation. In each tank, nine fish were kept in a 100-liter water space.

Experimental Setup

We arranged three aquariums and given them names like group G_0, group G_1 and group G_2. Group G_0 was control group which receive normal diet daily while the remaining groups G_1-G_2 were experimental groups treated with different concentration of Acetamiprid and Pyriproxyfen @0, 2 and 3 mg/L respectively. The fish were fed commercial feed that contained 30% protein every day at a rate of 2% of their body weight. Each tank was inspected daily for signs of clinical illness and death. Two samplings were conducted during the experiment: the first took place on day7, and the second took place on day 14.The temperature of the laboratory was kept 28-29^{O}C. The aerators were established in these aquariums for proper aeration. The feed was provided to the fishes regularly, and the aquariums are cleaned and siphoned regularly.

Physicochemical Parameters

After the distribution of the fish into different groups, physicochemical parameters had been observed at the day first to check the effect of toxicant on the fish and their environment. Following parameters are calculated:

Clinical Observations

Clinical signs which were observed are Bottom running, Surface running, Gasping, Operculum movement, Caudal fin movement, Convulsions, Unbalance body, Surface breathing, Jerking, Faintness and tilting of fin, Rapid gulping due to difficulty in breathing, Loss of appetite, low rate of feeding makes immune system weak and fish become susceptible to disease.

Statistical Analysis

The statistical analysis was conducted using SPSS V.26 software. One Way ANOVA was used for the intergroup comparison. The control and treated groups were compared using the t-test. For various variables, the mean and standard deviation had p values that were Significant 0.05. For creating graphs, the software GraphPad Prism (version 7) was used.

RESULTS

Behavioral Responses

Control group (G_0) fish were found without any mortality and clinical reactions. Fish of treated groups (G_1-G_2) with low to high doses of Acetamiprid and Pyriproxyfen showed moderate to severe clinical indications at day 7 and 14 such as gasping, jerking, convulsion, bottom running, surface running, surface breathing, body unbalancing, operculum movement, tin tilting, and static lurization were observed at higher concentration (2 and 3 mg/L). The maximum intensity of these clinical signs was observed in fish exposed to higher concentration of Acetamiprid and Pyriproxyfen in group G_1 and G_2 throughout the present experiment. Clinical signs were categorized as Absent (-), Mild to Moderate (+), Severe (++), very severe (+++). During first seven days normal clinical signs were observed in groups G_0 and G_1 G_2 but group G_2 indicates mild clinical abnormalities (Table 1). During 7-14 days normal clinical signs were observed in group G_0 but groups G_1 and G_2 indicate severe clinical abnormalities (Table 2)

Results at Day-7

At day 7, the gasping nearly remains same in the group G_1 and increased gasping at the surface was seen in group G2 in contrast to group G0, the control group as fish got stress due to lack of oxygen as a result of insecticide application. When compared to the control group G0, the jerking in group G1 is marginally higher, but it increases significantly in group G2. Fish lose control of their muscles and nerves, which causes them to begin migrating away from the area of application. While the convulsions remain generally slight in group G1 in contrast to group A, the control group but increased in the G_2, due to stress, compared to the G0 control group. The surface breathing at day 7 was increased in the G1 and G2 control groups when in contrast to the G0 control group as introduction of insecticide causes loss of oxygen in water. Surface running is higher in group G1 than in control group G0, although it mostly increases in group G2. Groups G1 and G2 experience higher levels of bottom running under stress than group G0, which is the control. The body unbalancing was observed in groups G1 and G2 in contrast to group G0, the control group. Normal operculum pumping was seen in control group G0, however rapid operculum movement was observed in groups G1 and G2, which is caused by an accelerated rate of breathing. The pectoral fins of the fishes were reversed. Since pesticides harm the nervous system, the strange behavior of the experimental fishes may be the result of neurotransmitters' ability to operate normally being impeded by the stress of the pesticide. Pectoral fin tilting was seen in groups G1 and G2, but not in group G0, which served as the control. The static position at day 7 is slight or sometimes occurs.

Table 1: Analysis of physicochemical parameters at the start of trial period

Sr. No	Parameters	G0	G1	G2
1	pH	7.8	7.8	7.8
2	Temperature (°C)	29°C	29.1°C	28.8°C
3	EC (mS/cm)	0.77	0.74	0.73
4	Salinity (ppt)	0.3	0.4	0.3
5	TDS (ppt)	0.41	0.	0.38

Table 2: shows clinical signs at day 7.

Clinical Signs	Treated Groups		
	G0	G1	G2
Gasping	+	+	++
Jerking		++	++
Convulsions	_	_	+
Faintness	_	_	+
Surface breathing	+	+	+
Surface Running	+	+	++
Bottom Running	+	+	++
Body unbalancing	_	_	+
Operculum movements	+	++	++
Tilting of Fin	+	+	++
Static position	+	+	+

Table 3: Clinical signs at day 14.

Clinical Signs	Treated Groups		
	G0	G1	G2
Gasping	+	+	_
Jerking	+	+	++
Convulsions	_	_	++
Faintness	_	_	+
Surface breathing	+	+	+
Surface Running	+	+	_
Bottom Running	+	+	++
Body unbalancing	_	_	+
Operculum movements	++	++	+++
Tilting of Fin	+	+	++
Static position	+	+	+

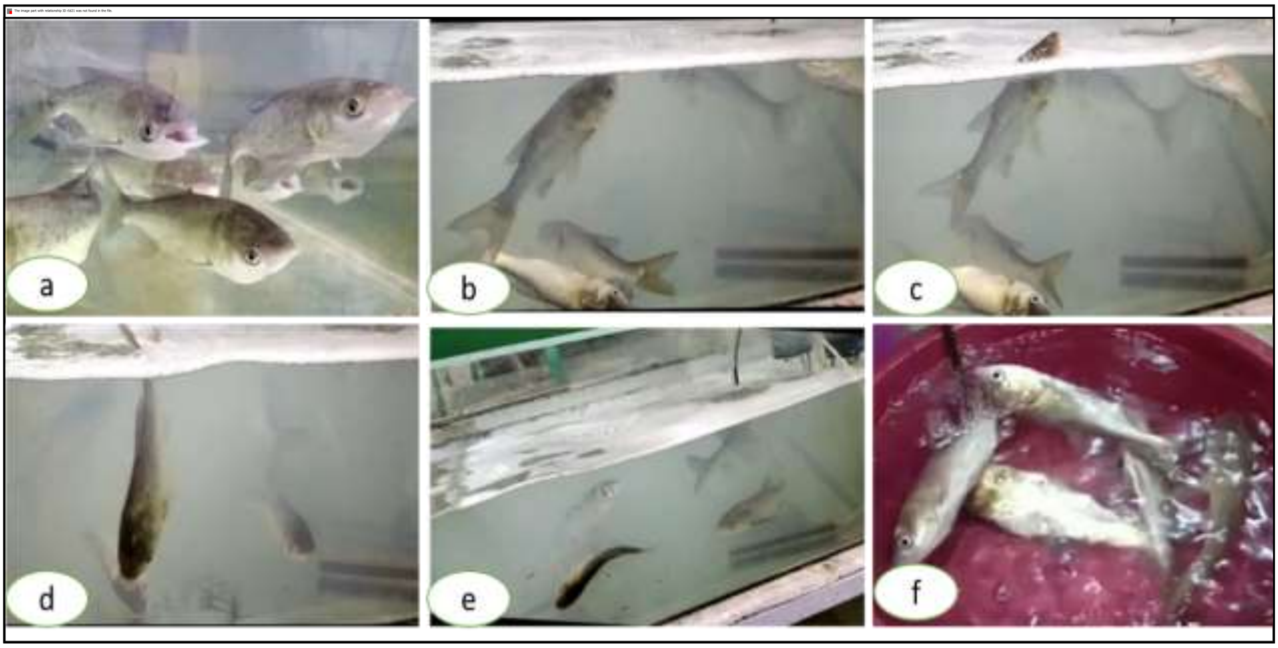

Fig. 1: Various physiological dysfunctions observed in *H.nobilis* (a) Rapid gulping (b) Body unbalancing (c) Surface breathing (d) Bottom swimming (e) Static movement (f) Faintness at 14 days of experimental period.

Results at Day 14

At day 14 following physiological dysfunctions were detected. The gasping at day 14 moderately increases in the group and in group G2 gasping was not detected, when compared to the control group G0. as fishes got stress due to lack of oxygen become slightly adapted to it. The jerking mainly increases in the group G1 largely severe at intervals in G2 in contrast to the G0 control group. Convulsions indicate that a fish has lost its ability to regulate its body and are symptoms rather than a single illness. It happens when a fish is under extreme stress. The convulsions at the day 14 remain generally slight in the G1 group in contrast to the G1 control group but increased in the G2, due to stress, in contrast to the G0 control group

The surface breathing was slightly increased in group G1 and group G2 control compared to group G0 control as introduction of insecticide causes loss of oxygen in water with increasing duration. Surface running is more in group G1 when compared the control group G0 but in group G2 it is detected at imperceptible rate. The bottom running with stress increases in groups G1 and G2 in contrast to group A, the control group due to loss of body control by dysfunction of neurotransmitter that carries out the effective movements. Groups G1 and G2 showed noticeable bodily un-balancing in comparison to group G0, which served as the control. While normal operculum pumping was seen in control group G0, rapid operculum movement was observed in groups G1 and G2, which is attributed to an accelerated rate of breathing. The pectoral fins of the fishes were reversed along with non-specified and non-oriented movements. Due to stress of the pesticide, the unusual behavior of the experimental fishes may be due to the obstructed function of neurotransmitters as pesticides cause damage to the nervous system. Tilting of pectoral fin was detectable in group G1 and G2 but not in control group G0. The static position at day 14 is slight or sometimes occurs in group G1 but can remain same in G2.

Physiological dysfunction in organ's weight

The results on the body weight of fish were presented in in Fig.2 (graph A). Before application of chemicals, during acclimatization period, the body weight of fish remain same in all the groups from G0-G2. After application of chemicals in experimental groups G1 and G2 body weight significantly reduced at day 14, when compared to control group G0. The results on absolute and relative weight of intestine are presented in graph B. When comparing experimental groups G1 and G2 to control group G0, the absolute weight of the fish's intestines stayed rather constant. Comparing groups G1 and G2 to control group G0, the relative weight of the intestines dropped considerably. The results on absolute and relative weight of liver are presented in graph C. In comparison to control group G0, the fish in experimental groups G1 and G2 had nearly identical liver absolute weights. Relative weight of the liver was substantially lower in groups G1 and G2 than in control group G0. The results on absolute and relative weight of Heart are presented in graph D. Heart absolute weight of fish remained almost same in experimental group G1 and G2, when compared with control group A. Heart relative weight significantly decreased in group G1 and G2, when compared to control group G0. The results on absolute and relative weight of Spleen are presented in graph E. Spleen absolute weight of the fish remained almost same in experimental group G1 and G2, in contrast to the G0 control group. When comparing groups G1 and G2 to control group G0, the relative weight of the spleens dropped dramatically. The results on absolute and relative weight of brain are presented in graph F. Fish in experimental groups G1 and G2 had nearly identical brain absolute weights when compared to control group G0. When comparing groups G1 and G2 to control group G0, the relative weight of the brains dropped considerably.

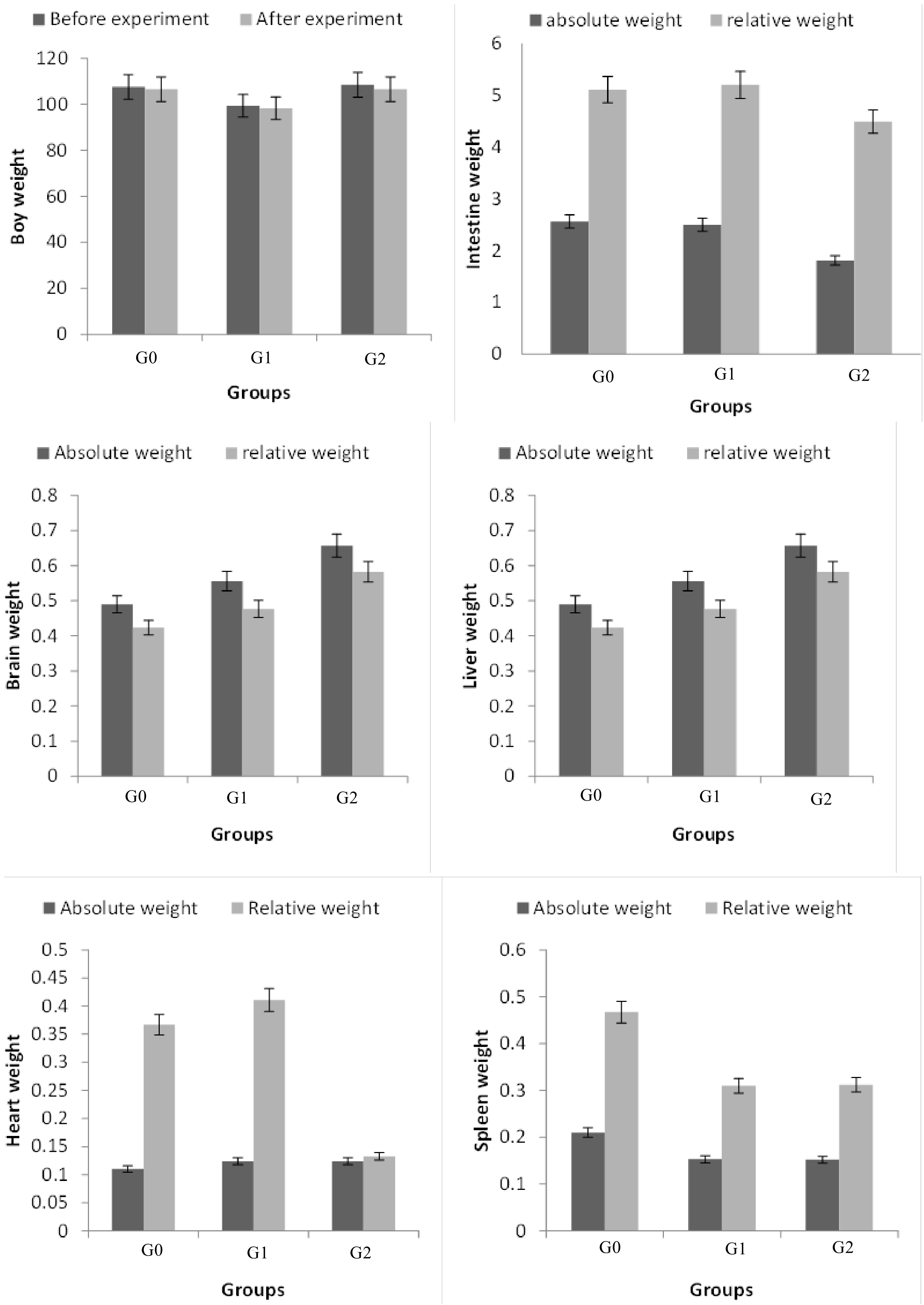

Fig. 2 (A) Body weight of fish (*Hypophathalmicthys nobilis)* before and after the experiment. (B) Relative and absolute weight of intestine (C) Relative and absolute weight of liver (D) Relative and absolute weight of heart (E) Relative and absolute weight of spleen (F) Relative and absolute weight of brain.

DISCUSSION

The purpose of this study was to evaluate the toxicity of two active insecticides on Bighead carp fish Acetamiprid and Pyriproxyfen under the controlled conditions. The juveniles of *Hypophathlmicthys niloticus*, after 15 days of acclimatization, were exposed for 14 days to low (2mg/L) and high (3mg/L) concentrations of (pyriproxyfen and acetamiprid). The tests were conducted in aquariums under carefully monitored circumstances. Fish may exhibit changed behavioral patterns in polluted environments, such as aggressiveness, avoidance, and movement activity. These behaviors may be an attempt by the fish to cope with the stressor or to flee it (Morgan, J. D., 2002).

Clinical signs that correspond with behavior include the series of measurable actions that are controlled by the central and peripheral nervous systems (Baatrup, E. 2009) as well as the cumulative expressions of physiological and biochemical processes that are vital to life, like feeding, reproduction, and avoiding predators. It differs greatly among fish species, exposure circumstances, and toxicant types and enables an organism to adapt to both internal and external stimuli in order to best face the challenge of surviving in a changing environment. The current study's findings show that the only medication that altered skin color was acetamiprid. Similar color changes in guppy fish (*Poecilia reticulate*) have been documented (Ylmaz, M., 2004). Adewoye (Adewoye, S. O. 2010) explained this discoloration of the skin observed with the fingerlings and the adults of African catfish Clarias gariepinus exposed to soap and detergent effluents to the weakening or inhibition of melanin production, due to the extent of the depletion of the oxygen content of the main melanin components. The discoloration that fish exposed to toxicants suffer, however, may be caused by the dispersion of melanin pigment in the chromatophores, according to Mishra and Shukla (Mishra, R., and Shukla, S. P.94).

In toxicity testing, pesticides often enter through the gills, which means that the respiratory system may be negatively impacted (Velmurugan, B., 2009). Our findings show that the combination of acetamiprid and pyriproxen in this research increased opercular activity via lowering dissolved oxygen. According to Kind (Kind, P. K., 2002), alterations in fish behavior may have been made to compensate for the chemicals' effects on aquatic hypoxia. In situations where escape from hypoxic stress is impossible, hyperventilation may be triggered in order to make up for the inadequate oxygen supply.

The hyperventilation seen in this study may potentially represent a respiratory distress, which is one of the first signs of pesticide poisoning and may result in modified gill histology. However, fish gills are essential organs for their respiratory and osmoregulatory processes and are the main entrance point for pesticides (Val, A. L., 2000). Indeed, research by Velmurugan et al. (Velmurugan, B., 2007) on the gill tissue of mrigal carp exposed to sub-lethal concentrations of lambda-cyhalothrin revealed changes in histopathology, including edema, epithelial lifting, aneurism, epithelial necrosis, desquamation, epithelial hyperplasia, lamellar fusion, and shortening of secondary lamellae. The effects of the active ingredients on the central nervous system could account for additional behavioral changes seen in this study, including jerking, depth activity, erratic swimming, loss of balance, lethargy, operculum movement, bottom running, surface running, static movement, loss of appetite, and convulsions.

There were other indications that the body had been exposed to a contaminant besides behavioral abnormalities. All of these behavioral alterations were only seen following the assessment of certain markers and are generally the consequence of modifications in physiological and biochemical processes at the individual level. It appeared that acetamiprid was not very harmful to fish. The key factors influencing fish mortality from pesticide exposure are their susceptibility to the toxicants, as well as the exposure's concentration and duration (Kamble, S. M., 2011). Prior to doing more research on animal physiological alterations, it is crucial to assess the LC50 concentration of contaminants.

Bighead carp juveniles exposed to the water/solvent control did not perish or have any less severe side effects, according to all confirmed acute toxicity investigations. A direct relationship between fish mortality and concentration was shown by the fact that fish mortality rose as the toxicant concentration rose. The first mortalities were recorded at the same time (2 h post-exposure). Our results show that Pyriproxyfen and Acetamiprid are extremely toxic to Bighead carp as they can cause disruption in its normal behavior and physiological dysfunctions to visceral organs should be avoided. Acetamiprid affects the synapses in the insect central nervous system by acting as an agonist on the nicotinic acetylcholine receptor (Wolf, J. C., and Wheeler, J. R. 2018) to produce excitation, paralysis, and death. Many matrices, including water, soil, plants, and aquatic animals, are contaminated by this pesticide once it is sprayed to crops (Raj and Baby 2015; Morrissey et al. 2015; Hoyle and Code 2016).

Conclusion

The purpose of this research was to examine the physiological dysfunctions in Bighead carp by applying the mixture of Acetamiprid and Pyriproxyfen. For this purpose 27 freshwater fishes were split into three groups at random (9 fishes in each group) named as G0, G1 and G2. Fish exposed to Acetamiprid and Pyriproxyfen in group G1 and group G2 were dissected after 7 days, their sampling and analysis indicated that fishes exhibited surface breathing, jerking, body tilting, erected fins, and lethargy, sluggishness, static movement, bottom breathing, faintness and irregular operculum movements. Other physiological dysfunctions are reduced body weight, decrease in relative weight of liver, kidney, brain, intestine, heart etc. conditions become severe when sampling was done on 14^{th} day. However, no mortality was detected during the period of experimental analysis. Hence, we concluded that Acetamiprid and Pyriproxyfen induce physiological dysfunctions in fish *Hypophathalmicthys nobilis.* This research unequivocally showed the negative physiological dysfunctions by pyriproxyfen and Acetamiprid pesticides on freshwater fish *Hypophthalmicthys nobilis*. It is concluded that in the realm of environmental bio-monitoring, the clinical and physiological characteristics examined in the current studies can be employed as possible biomarkers of pesticide toxicity to fish and other aquatic creatures.

DECLARATION

Funding: This study was not supported by national or international funding agencies.

Acknowledgement: None

Competing Interest: Authors declare no conflict of interests.

Availability of Data and Material: All the data is available and can be obtained on reasonable request from the corresponding author

Ethical Statement: This research was executed according to the guidelines of the ethical committee of the institution.

Author Contribution: Adeeba Naseer: Conceptualization, Investigation; Formal Analysis, Writing -original draft, Data Curation, Methodology, Visualization. Taskeen Shakir, Bushra Tariq and Sanya Naz, Review and editing. Nusheen Ahmad, Saud Iqbal Azhar Ullah and Muhammad Usman: Review final draft and editing.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Abdu U, Barki A, Karplus I, Barel S, Takac P, Yehezkel G, and Sagi A. (2001). Physiological effects of methyl farnesoate and pyriproxyfen on wintering female crayfish Cherax quadricarinatus. Aquaculture, 202(1-2), 163-175.

Alam A, Tabinda AB and Abdullah Y. (2014). Comparative toxicity of acetamiprid and Azadirachta indica leave extract on biochemical components of blood of Labeo rohita. Pakistan Journal of Zoology, 46(6).

Azevedo RD, Falcão KV, Assis CR, Martins RM, Araújo MC, Yogui GT and Bezerra R. S. (2021). Effects of pyriproxyfen on zebrafish brain mitochondria and acetylcholinesterase. Chemosphere, 263, 128029.

Azevedo-Linhares, M, Souza ATC, Lenz CA, Leite NF, Brito IA, Folle NT and Ribeiro CO. (2018). Microcystin and pyriproxyfen are toxic to early stages of development in Rhamdia quelen: An experimental and modelling study. Ecotoxicology and Environmental Safety, 166, 311-319.

Baatrup E (2009). Measuring complex behavior patterns in fish—effects of endocrine disruptors on the guppy reproductive behavior. Human and Ecological Risk Assessment, 15(1), 53-62.

Bunet JL, Badiou A and Belzunces LP. (2005). In vivo metabolic fate of [14C]-acetamiprid in six biological compartments of the honeybee, Apis mellifera L. Pest Management Science: formerly Pesticide Science, 61(8), 742-748.

Caixeta ES, Silva CF, Santos VSV, *et al.*, 2016. Ecotoxicological assessment of pyriproxyfen under environmentally realistic exposure conditions of integrated vector management for Aedes aegypti control in Brazil. J Toxicol Environ Health A 79:799–803.

Caixeta ES, Silva CF, Santos VSV, Olegário de Campos Júnior E and Pereira BB. (2016). Ecotoxicological assessment of pyriproxyfen under environmentally realistic exposure conditions of integrated vector management for Aedes aegypti control in Brazil. Journal of Toxicology and Environmental Health, Part A, 79(18), 799-803.

Caixeta ES, Silva CF, Santos VSV, Campos-Junior EO and Pereira BB. 2016. Ecotoxicological assessment of pyriproxyfen under environmentally realistic exposure conditions of integrated vector management for Aedes aegypti control in Brazil. Journal of Toxicology and Environmental Health, Part A, 79: 799-803.

Chakroun S, Ezzi L, Grissa I, Kerkeni E, Neffati F, Bhouri R and Ben Cheikh H. (2016). Hematological, biochemical, and toxicopathic effects of subchronic acetamiprid toxicity in Wistar rats. Environmental Science and Pollution Research, 23(24), 25191-25199.

Dasgupta S, Mamingi N and Meisner C. (2001). Pesticide use in Brazil in the era of agroindustrialization and globalization. Environment and Development Economics, 6(4), 459-482.

de Farias Araujo G, Soares LOS, Junior SFS, de Carvalho LVB, Rocha RCC, Saint'Pierre T and Saggioro EM. (2022). Oxidative stress and metal homeostasis alterations in Danio rerio (zebrafish) under single and combined carbamazepine, acetamiprid and cadmium exposures. Aquatic Toxicology, 245, 106122.

de Oliveira VS, Castro AJG, Marins K, Mendes AKB, Leite GAA, Zamoner A and Silva FRMB. (2021). Pyriproxyfen induces intracellular calcium overload and alters antioxidant defenses in Danio rerio testis that may influence ongoing spermatogenesis. Environmental pollution, 270, 116055.

Devillers J. (2020). Fate and ecotoxicological effects of pyriproxyfen in aquatic ecosystems. Environmental science and pollution research, 27(14), 16052-16068.

Dzieciolowska S, Larroque AL, Kranjec LA, Drapeau P and Samarut E. 2017. The larvicide pyriproxyfen blamed during the Zika virus outbreak does not cause microcephaly in zebrafish embryos. Scientific Reports, 7: 40067.

Dzieciolowska S, Larroque AL, Kranjec LA, Drapeau P and Samarut E. 2017. The larvicide pyriproxyfen blamed during the Zika virus outbreak does not cause microcephaly in zebrafish embryos. Scientific Reports, 7: 40067.

Elezovic I, Budimir M, Karan V, and Neskovic NK. (1994). Herbicides in water: Subacute toxic effects on fish.

European Food Safety Authority. (2011). Review of the existing maximum residue levels (MRLs) for dimethomorph according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal, 9(8), 2348.

Ghayyur S, Khan MF, Tabassum S, Ahmad MS, Sajid M, Badshah K and Qamer S. (2021). A comparative study on the effects of selected pesticides on hemato-biochemistry and tissue histology of freshwater fish Cirrhinus mrigala (Hamilton, 1822). Saudi Journal of Biological Sciences, 28(1), 603-611.

Ginjupalli GK and Baldwin WS, 2013. The time- and age-dependent effects of the juvenile hormone analog pesticide, pyriproxyfen on Daphnia magna reproduction. Chemosphere 92:1260-66.

Guedegba NL, Imorou Toko I, Agbohessi PT, Zoumenou BS, Douny C, Mandiki SN and Kestemont P. (2019). Comparative acute toxicity of two phytosanitary molecules, lambda-cyhalothrin and acetamiprid, on Nile Tilapia (Oreochromis Niloticus) juveniles. Journal of Environmental Science and Health, Part B, 54(7), 580-589.

Guedegba NL, Imorou Toko I, Agbohessi PT, Zoumenou BS, Douny C, Mandiki S N and Kestemont P. (2019). Comparative acute toxicity of two phytosanitary molecules, lambda-cyhalothrin and acetamiprid, on Nile Tilapia (Oreochromis Niloticus) juveniles. Journal of Environmental Science and Health, Part B, 54(7), 580-589.

Guo L, Qiu Y, Zhang G, Zheng GJ, Lam PK and Li X. (2008). Levels and bioaccumulation of organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) in fishes from the Pearl River estuary and Daya Bay, South China. Environmental Pollution, 152(3), 604-611.

Guo W, Yang Y, Zhou X, Ming R, Hu D and Lu P. (2022). Insight into the toxic effects, bioconcentration and oxidative stress of acetamiprid on Rana nigromaculata tadpoles. Chemosphere, 305, 135380.

Guo Y, Meng XZ, Tang HL and Zeng EY. (2008). Tissue distribution of organochlorine pesticides in fish collected from the Pearl River Delta, China: implications for fishery input source and bioaccumulation. Environmental Pollution, 155(1), 150-156.

Gusso D, Reolon GK, Gonzalez JB, Altenhofen S, Kist LW., Bogo MR and Bonan CD. (2020). Pyriproxyfen exposure impairs cognitive parameters and alters cortisol levels in zebrafish. Frontiers in Behavioral Neuroscience, 14, 103.

Hathout HM, Sobhy HM, Abou-Ghanima S and El-Garawani IM. (2021). Ameliorative role of ascorbic acid on the oxidative stress and genotoxicity induced by acetamiprid in Nile tilapia (Oreochromis niloticus). Environmental Science and Pollution Research, 28(39), 55089-55101.

Henny CJ, Kaiser JL, Grove RA, Bentley VR and Elliott JE. (2003). Biomagnification factors (fish to osprey eggs from Willamette River, Oregon, USA) for PCDDs, PCDFs, PCBs and OC pesticides. Environmental Monitoring and Assessment, 84(3), 275-315.

Hoyle S, Code A (2016) Neonicotinoids in California's surface waters: a preliminary review of potential risk to aquatic invertebrates. The Xerces Society for Invertebrate Conservation, pp 1–17.

Jaffer Mohiddin G, Srinivasulu M, Maddela NR, Manjunatha B, Rangaswamy V, Kaiser ARK and Darwin Rueda O. (2015). Influence of the insecticides acetamiprid and carbofuran on arylamidase and myrosinase activities in the tropical black and red clay soils. Environmental monitoring and assessment, 187(6), 1-9.

Jeschke P, Nauen R, Schindler M and Elbert A. (2011). Overview of the status and global strategy for neonicotinoids. Journal of agricultural and food chemistry, 59(7), 2897-2908.

Jian-chu MO, Tian-ci YANG, Jia-an CHENG and Xiao-gang SONG. (2002). Lethal and sublethal effects of acetamiprid on the larvae of Culex pipiens Pallens. Insect Science, 9(3), 45-49.

Joseph B, Raj SJ 2011. Impact of pesticide toxicity on selected biomarkers in fishes. Int. J. Zool. Res. 7 (2), 212–222.

Kamble SM, Bhagwan HK and Chinte DN. (2011). Acute toxicity of sevin concentration on mortality and behaviour of freshwater fish Barilius barila. International Refered research journal, 2(23), 15-17.

Kind PK, Grigg GC and Booth DT. (2002). Physiological responses to prolonged aquatic hypoxia in the Queensland lungfish Neoceratodus forsteri. Respiratory Physiology and Neurobiology, 132(2), 179-190.

Legrand E, Boulangé-Lecomte C, Restoux G, Trémolet G, Duflot A and Forget-Leray J. (2017). Individual and mixture acute toxicity of model pesticides chlordecone and pyriproxyfen in the estuarine copepod Eurytemora affinis. Environmental Science and Pollution Research, 24(6), 5976-5984.

Legrand E, Boulan-Gélecomte C, Restoux G, Trémolet G, Duflot A and Forget-Leray J. 2017. Individual and mixture acute toxicity of model pesticides chlordecone and pyriproxyfen in the estuarine copepod Eurytemora affinis. Environmental Science and Pollution Research, 24: 59-76.

Li X, Naseem S, Hussain R, Ghaffar A, Li K and Khan A. (2022). Evaluation of DNA Damage, Biomarkers of Oxidative Stress, and Status of Antioxidant Enzymes in Freshwater Fish (Labeo rohita) Exposed to Pyriproxyfen. Oxidative Medicine and Cellular Longevity, 2022.

Ma X, Li H, Xiong J, Mehler WT and You J. (2019). Developmental toxicity of a neonicotinoid insecticide, acetamiprid to zebrafish embryos. Journal of agricultural and food chemistry, 67(9), 2429-2436.

Ma X, Xiong J, Li H, Brooks BW and You J. (2022). Long-Term Exposure to Neonicotinoid Insecticide Acetamiprid at Environmentally Relevant Concentrations Impairs Endocrine Functions in Zebrafish: Bioaccumulation, Feminization, and

Transgenerational Effects. Environmental Science and Technology.

Maharajan K Sellamani M, Bojan N, *et al.*, 2018. Toxicity assessment of pyriproxyfen in vertebrate model zebrafish embryos (Danio rerio): A multi biomarker study. Aquatic Toxicol 196:132-45.

Maharajan K, Muthulakshmi S, Karthik C, Nataraj B, Nambirajan K, Hemalatha D, and Ramesh M. (2020). Pyriproxyfen induced impairment of reproductive endocrine homeostasis and gonadal histopathology in zebrafish (Danio rerio) by altered expression of hypothalamus-pituitary-gonadal (HPG) axis genes. Science of the Total Environment, 735, 139496.

Maharajan K, Muthulakshmi S, Nataraj B, Ramesh M, and Kadirvelu K. (2018). Toxicity assessment of pyriproxyfen in vertebrate model zebrafish embryos (Danio rerio): a multi biomarker study. Aquatic Toxicology, 196, 132-145.

Mishra R and Shukla SP. (1994). Effects of endosulfan on bioenergetic properties of liver-mitochondria from the freshwater catfish Clarias batrachus. Pesticide Biochemistry and Physiology, 50(3), 240-246.

Mohiddin GJ, Srinivasulu M, Subramanyam K, Madakka M, Meghana D and Rangaswamy V. (2015). Influence of insecticides flubendiamide and spinosad on biological activities in tropical black and red clay soils. 3 Biotech, 5(1), 13-21.

Moreira SM, Lima I, Ribeiro R and Guilhermino L. (2006). Effects of estuarine sediment contamination on feeding and on key physiological functions of the polychaete Hediste diversicolor: laboratory and in situ assays. Aquatic toxicology, 78(2), 186-201.

Morgan JD, Vigers GA, Farrell AP, Janz DM and Manville JF. (2002). Acute avoidance reactions and behavioral responses of juvenile rainbow trout (Oncorhynchus mykiss) to Garlon 4®, Garlon 3A® and Vision® herbicides. Environmental Toxicology and Chemistry: An International Journal, 10(1), 73-79.

Moura JA and Souza-Santos LP. (2020). Environmental risk assessment (ERA) of pyriproxyfen in non-target aquatic organisms. Aquatic Toxicology, 222, 105448.

Noaishi M, abdalhafez H, 2016. Hepatotoxicity and nephrotoxicity evaluation after repeated dose of acetamiprid in albino rats. Egypt. J. Chem. Environ. Health 2, 439–452.

Oerke EC. (2006). Crop losses to pests. The Journal of Agricultural Science, 144(1), 31-43.

Peterson EK, Buchwalter DB, Kerby JL, Lefauve MK, Varian-Ramos CW and Swaddle JP. 2017. Integrative behavioral ecotoxicology: bringing together fields to establish new insight to behavioral ecology, toxicology, and conservation. Current Zoology, 2: 185-194.

Qiu F, Qiong QF, Kai ZL, Feng CE and Tang DQ. (1981). The relationships between organic matter and enzymatic activities and soil fertility in black soils.

Raj SJ and Joseph B. (2015). Impact of acetamiprid toxicity on biochemical biomarkers (protein and carbohydrate) in some tissues of the fish Oreochromis mossambicus. Int J Zool Res, 11(5), 222-227.

Ranney TA and Bartlett RJ. (1972). Rapid field determination of nitrate in natural waters. Communications in Soil Science and Plant Analysis, 3(3), 183-186.

Richardson JR, Fitsanakis V, Westerink RH, Kanthasamy AG. Neurotoxicity of pesticides. Acta Neuropathol. 2019, 138, 343–362.

Schulz R, Bub S, Petschick LL, Stehle S, Wolfram J. Applied pesticide toxicity shifts toward plants and invertebrates, even in GM crops. Science 2021, 372, 81−84.

Shi H, Zhao G, Liu M, Fan L and Cao T. (2013). Aptamer-based colorimetric sensing of acetamiprid in soil samples: sensitivity, selectivity and mechanism. Journal of hazardous materials, 260, 754-761.

Shi X, Dick RA, Ford KA and Casida JE. (2009). Enzymes and inhibitors in neonicotinoid insecticide metabolism. Journal of agricultural and food chemistry, 57(11), 4861-4866.

Silva FFD, Silva JMD, Silva TDJD, Tenorio BM, Tenorio FDCAM, Santos EL and Soares EC. (2020). Evaluation of Nile tilapia (Oreochromis niloticus) fingerlings exposed to the pesticide pyriproxyfen. Latin american journal of aquatic research, 48(5), 826-835.

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C and Wiemers M. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environmental Science and Pollution Research, 22(1), 5-34.

Sullivan JJ and Goh KS. 2008. Environmental fate and properties of pyriproxyfen. Journal of Pesticide Science, 4: 339-350.

TISLER T, JEMEC A, MOZETIC B, TREBSE P. 2009. Hazard identification of imidacloprid to aquatic environment. Chemosphere, 76(7): 907–914.

Truong L, Gonnerman G, Simonich MT and Tanguay RL. (2016). Assessment of the developmental and neurotoxicity of the mosquito control larvicide, pyriproxyfen, using embryonic zebrafish. Environmental pollution, 218, 1089-1093.

Truong L, Gonnerman G, Simonich MT and Tanguay RL. 2016. Assessment of the developmental and neurotoxicity of the mosquito control larvicide, pyriproxyfen, using embryonic zebrafish. Environmental Pollution, 218: 1089-1093.

Turan F and Ergenler A. Investigation of the Genotoxic Effect of Acetamiprid in Cyprinus carpio using the Micronucleus Analysis and the Comet assay.

Val AL, Silva MNP and Almeida-Val VMF. (2000). Hypoxia adaptation in fish of the Amazon: a never-ending task. South African Journal of Zoology, 33(2), 107-114.

Velmurugan B, Selvanayagam M, Cengiz EI and Unlu E (2007). Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of Cirrhinus mrigala. Environmental Toxicology and Pharmacology, 24(3), 286-291.

Velmurugan B, Selvanayagam M, Cengiz EI and Unlu E. (2009). Histopathological changes in the gill and liver tissues of freshwater fish, Cirrhinus mrigala exposed to dichlorvos. Brazilian Archives of Biology and Technology, 52, 1291-1296.

Wei Y, Cui J, Zhai W, Liu X, Zhou Z, Wang P and Liu D. (2021). Toxicity and fate of chiral insecticide pyriproxyfen and its metabolites in zebrafish (Danio rerio). Environmental Pollution, 280, 116894.

Wolf JC and Wheeler JR. (2018). A critical review of histopathological findings associated with endocrine and non-endocrine hepatic toxicity in fish models. Aquatic Toxicology, 197, 60-78.

Ylmaz M, Gul A and Erbasl K. (2004). Acute toxicity of alpha-cypermethrin to guppy (Poecilia reticulata, Pallas, 1859). Chemosphere-Oxford-, 56(4), 381-385.

Zhang H, and Zhao L. (2017). Influence of sublethal doses of acetamiprid and halosulfuron-methyl on metabolites of zebra fish (Brachydanio rerio). Aquatic Toxicology, 191, 85-94.