Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(5): 265 – 279 (2024)
www.ejabf.journals.ekb.eg

Acute Toxicity of an Emerging Insecticide, Pymetrozine, to the Nile tilapia

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ARTICLE INFO
Article History:
Received: April 29, 2024
Accepted: Aug. 9, 2024
Online: Sep. 9, 2024

Keywords:
Pymetrozine,
Nile tilapia,
Histological alterations,
Behavior changes

ABSTRACT

The widespread use of pesticides in veterinary medicine, agriculture, public health, and aquatic environments has increasingly threatened aquatic life in recent years. This study aimed to evaluate the acute toxicity, behavioral changes, and histological alterations caused by pymetrozine (PY) in the Nile tilapia (*Oreochromis niloticus*). The 96-hour LC50 value for PY was found to be 0.28g/ L, indicating the concentration at which half of the test population succumbed within 96 hours. Behavioral changes such as hyperexcitation, irregular swimming, dark coloration, loss of balance, and lethargy were observed and varied with the concentration of PY. The study also demonstrated that the mortality rate, clinical lesions, behavioral changes, and histological alterations in the gills, liver, and kidneys were all influenced by both the dosage of PY and the duration of exposure, highlighting the toxicological impact of PY on the Nile tilapia.

INTRODUCTION

The extensive use of pesticides in veterinary medicine, agriculture, public health, and aquatic environments has increasingly become a serious risk in recent years **(Jabeen *et al.*, 2021, Naz *et al.*, 2021)**. In both aquatic and terrestrial animals, accidental pesticide contact including herbicides and fungicides kills and shortens the lifetime of a variety of target and non-target species **(Ghaffar *et al.*, 2020)**.

Aquatic animals are varied and incredibly delicate. These habitats may be severely impacted by pesticides and other pollutants. Furthermore, a range of environmental pressures commonly affect aquatic ecosystems **(Ahmad *et al.*, 2023)**. Hazardous effects are often caused by the interaction or synergy of chemical stressors. Fish are the aquatic species most vulnerable to pesticides, according to studies, and they are crucial biomarkers for assessing the health of the aquatic ecosystem **(Faheem & Lone, 2017)**. The tilapia passes through a variety of physiological and metabolic processes. Numerical value visualizations integrating all responses were produced using

an enhanced integrated biomarker response index (IBR) **(Huang *et al.*, 2020a, Hu *et al.*, 2023)**.

Pymetrozine (CGA 215′944) is a novel insecticide that is extremely effective and targeted at sucking insect pests. PY is the sole member of a new class of insecticides called pyridine azomethines, which is presently being developed globally to control brown planthoppers, *Nilaparvata lugens* (Staol), in rice, as well as the aphids and whiteflies in field crops, vegetables, ornamentals, cotton, hop, deciduous fruit, and citrus **(Flückiger *et al.*, 1992a, b)**. The compound's high degree of selectivity, minimal mammalian toxicity, and safety for fish, birds, and non-target arthropods make it seem to hold tremendous potential for use in integrated pest management (IPM) programs **(Fuog *et al.*, 1998)**. Toxic organophosphate insecticides have been replaced by PY **(Atabila *et al.*, 2019)**.

The United States Environmental Protection Agency (USEPA) lists pymetrozine as a potential human carcinogen due to its negative effects on reproduction and irritation of the respiratory tract, even though PY has greatly improved crop output by eliminating insects **(USEPA, 2010)**. Because of the significant effects on both occupational and environmental health, it is crucial to thoroughly study the environmental behavior of pymetrozine in aquaculture **(Tudi *et al.*, 2022)**.

Due to its limiting indication of inhalation toxicity, pymetrozine (PY) is associated with the second class of hazards **(Korshun, 2016)**. The World Health Organization (WHO) estimates that exposure to pesticides causes acute effects in close to one million people. According to **Eddleston (2020)**, pesticides cause between 0.4 and 1.9 percent of all fatalities annually. Aquatic animals are capable of absorbing PY, and skin contact with contaminated water can result in behavioral and physical changes such as reduced feeding, decreased movement, and altered swimming patterns **(Ahmad *et al.*, 2023)**.

There is limited information available regarding the environmental impact of pymetrozine (PY) and its metabolites, aside from their acute toxicity to aquatic life. In studies involving three freshwater fish species—*Oncorhynchus mykiss* (rainbow trout), *Lepomis macrochirus* (bluegill), and *Cyprinodon variegatus* (sheepshead minnow)—PY was categorized as nearly non-toxic, with 96-hour LC50 values exceeding 100mg/ L **(BAuA, 2017)**. However, larvae of the zebrafish (*Danio rerio*) have shown susceptibility to PY, necessitating further research **(Cho *et al.*, 2023; Könemann *et al.*, 2022)**. In contrast, PY exhibits severe toxicity to aquatic invertebrates. For example, the shrimp *Penaeus vannamei* is 100% lethally affected by PY even at a low concentration of 1.0mg/ L **(Ma *et al.*, 2012)**. Additionally, *Procambarus clarkii* (red swamp crayfish) has been studied to assess the acute toxicity and histological changes resulting from PY exposure, indicating significant detrimental effects. This highlights the need for more comprehensive studies on the environmental behavior of PY and its potential risks to various aquatic organisms. The 96-hour LC_{50} value was found to be 0.479mg/ L, and at a

concentration half from the 96-hour LC_{50} value, major lesions were found in all PY-treated tissues, including the crayfish's heart, stomach, gills, and abdominal muscle **(Yu *et al.*, 2018)**. Nevertheless, it is still unclear how hazardous PY metabolites are to the environment for aquatic species **(Cho *et al.*, 2023)**.

To evaluate the lethal (mortality) and sublethal (behaviour and histopathology in gills, liver, and kidney) effects of pymetrozine in the Nile tilapia, 96-hour toxicity test was conducted in this study. The behavioral and histological changes, as well as the LC_{50} values at 24, 48, 72, and 96 hours, were evaluated. The results will offer guidelines for the use of pymetrozine and a deeper comprehension of the toxicity in the Nile tilapia.

MATERIAL AND METHODS

1. Animal ethics and fish rearing conditions

The Institutional Animal Care and Use Committee of Zagazig University in Egypt accepted the experimental protocol (ZUIACUC–2-F–52–2024). Healthy Nile tilapia (*Oreochromis niloticus*) with an average body weight of 25± 0.5g were obtained from the Abassa private fish farm in Alsharqia province, Egypt. Fish were brought to the Aquatic Animal Medicine Department Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt. There were no indications of disease signs or outbreaks in the fish collected. They were randomly distributed among ten glass aquaria (150L) that were supplied with chlorine-free tap water; aquariums had a constant air supply, a daily partial water exchange of roughly 25%, and a weekly full water change for each aquarium. Before the experiment began, according to **Yu *et al.* (2017)**, fish were hand-fed a basic meal at 3% of the fish biomass twice a day (8:00 AM and 2:00 PM) after being acclimated to the experimental circumstances for 14 days. According to **CCoA (2005)**, a routine assessment of the fish's health state was carried out before the experiment. **APHA (2005)** recorded that water parameters (pH = 7.2± 0.5, ammonia = 0.02± 0.001mg/ L, nitrite = 0.017± 0.001mg/ L, water temperature = 24± 2°C, photoperiod 12:12 light: dark) were maintained within approved values during the observation period.

2. Monitoring behavior

Daily observation of the fish's behaviors, clinical signs, and postmortem lesions was conducted while they were exposed to PY. To examine the effects of concentration and duration of PY exposure on experimental fish, changes in swimming and reflexes, as well as any other obvious symptoms, were recorded for each group.

3. Determination of lethal concentration

Two hundred and ten Nile tilapia were divided into seven groups (G1 to G7), each containing three replicates, for a total of seven PY concentrations. The pre-range finding test was used to determine 0.25, 0.5, 0.75, 1, 1.25, and 1.5g L^{-1}. The control was set at 0g L^{-1}. For 96 hours, the fish were exposed to the various PY concentrations (with specification in Table 1) to calculate the LC_{50} values after 96 hours. Depending on the

probit analysis method, LC_{50} values and 95% lower and upper confidence limits were determined. The fish were not fed during the assay and were starved for twenty-four hours before being exposed. Dead fish were taken out of aquaria, and the mortality rate was noted for each group. To maintain the treatment concentration, a daily addition of a certain amount of pesticide was supplied to the aquaria, together with new fresh dechlorinated water **(Melika *et al.*, 2019)**.

Table 1. Specification of Pymetrozine

Pesticide	**CAS number**	**Supplier**	**Grade**	**Chemical name**	**Alternative name**
Pymetrozine	123312-89-0	Agrimar Commercial Agencies, Turkey	Commercial formulation (50% WG)	(E)-4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one.	Carbendazim

4. Histopathological technique

The samples were collected from the gills, liver, and kidney of different groups cleaned in xylene, embedded in melted paraffin wax, and fixed in 10% neutral buffer formalin. The samples were also dehydrated in escalating grades of alcohol. Using a microtome (Leica®), paraffin slices (5um) were cut, stained with hematoxylin and eosin, and prepared for microscopic inspection **(Suvarna, 2018)**.

5. Statistical analysis

The results were expressed as mean ± SE (Mean±Standard Error). The effect of the treatment groups on the various parameters was evaluated using the analysis of variance (ANOVA) with the Duncan multiple test serving as a post hoc test. The value of $P< 0.05$ was used to indicate statistical significance. Graph Pad Prism version 8.0.2 (GraphPad Software, Inc.) and SPSS version 28.0 (IBM Corp.: NY, USA) were used for all statistical analysis and charts **(Dawson *et al.*, 2004)**.

. RESULTS

1. Behavior changes and clinical lesions

Fish exposed to various PY concentrations displayed erratic behavior. In reaction to abrupt alterations in their surroundings, the fish started moving slowly. They swam quickly and with hyperexcitation to the water's surface to combat the toxic water, and it appears that this behavior may be caused by low oxygen levels. Additionally, the opercula were moving quickly; fish accumulated at the water surface gasping air. After a while, the fish started to swim erratically and slowly. The fish's color changed to a darker

shade, particularly on its tail, fins and then the whole body. The dark color of the body increased in the fish exposed to a higher concentration of PY. Fish suffered from low and erratic swimming and loss of equilibrium.

Fish showed haemorrhage at the tip and around the mouth, fins and fin rot. The post-mortem lesions were congestion of internal organs (liver, spleen and kidney), pale gills in addition to serosanguinus fluid in the abdomen. The Nile tilapia exposed to greater PY concentrations (1.25 and 1.5gL^{-1}) experienced these changes more quickly than those exposed to lower concentrations (0.25 and 0.5gL^{-1}).

2. Lethal concentration of PY

The acute toxicity is measured by lethal concentration (LC_{50}). The concentration of a toxicant chemical component, like PY, that kills 50% of the experimented fish in a given time (96 hours) is known as the lower lethal concentration or LC_{50}. A direct correlation between fish mortality and PY concentration is shown in Fig. (1), which shows that the concentration of PY increases the fish mortality rate. The LC_{50} of PY after 96 hours of exposure was 0.28gL^{1}.

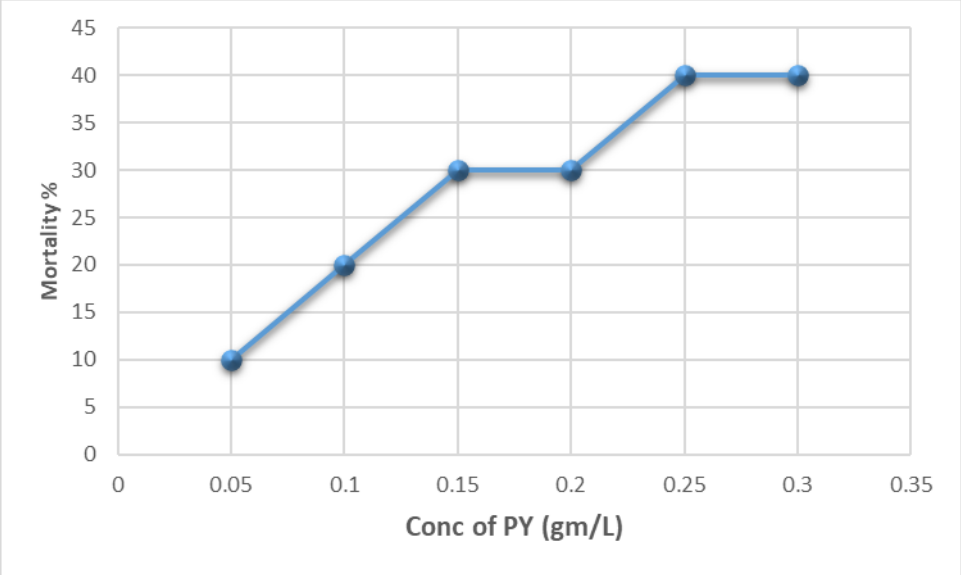

Fig. 1. Percentage mortality of the Nile tilapia after 96h exposure to different concentrations of PY

Table 1. Lethal concentration of PY

Duration (H)	**LC_{50} value (gL^{-1})**	**95% Confidence limit**		**Slope± SE**	**Intercept±SE**	**Significant**
		Lower	**Upper**			
96	0.28	0.09	0.36	0.19±0.02	0.23±0.14	0.001

3. Histological alteration

The gills of G1 exposed to PY at concentration of 0.25 gL^{-1} (Fig. 2A) revealed engorged capillaries of some gill filaments. Destructed and desquamated large number of secondary filaments were detected in G3 (PY at conc. 0.75 gL^{-1}) (Fig. 2B). On the other hand, the gills in G6 (PY at conc. 1.5 gL^{-1}) showed an extensive distortion of a large number of primary filaments and stacked secondary filaments with exudate (Fig. 2C).

The liver of G1 revealed vacuolations of hepatic parenchyma and congestion of the portal veins (Fig. 3A). In G3, increased vacuolated hepatocytes and vacuolated pancreatic acinar epithelium were detected with unicellular hepatic necrosis beside peri-pancreatic edema (Fig. 3B). For the G6, it showed vacuolated hepatic cells admixed with focal necrotic hepatocytes and dilated sinusoids. Additionally, necrosis of pancreatic acini that was replaced by inflammatory cells primarily lymphocytes was also seen in this group (Fig. 3C).

The kidney of G1 revealed degenerative changes within a few renal tubular epithelia and the presence of congested renal blood vessels (Fig. 4A). A moderate number of hydropic degenerated renal tubular epithelium were seen in G3 (Fig. 4B). On the other hand, the G6 showed necrotic in some renal tubular epithelium in addition to shrunk glomerular tufts (Fig. 4C).

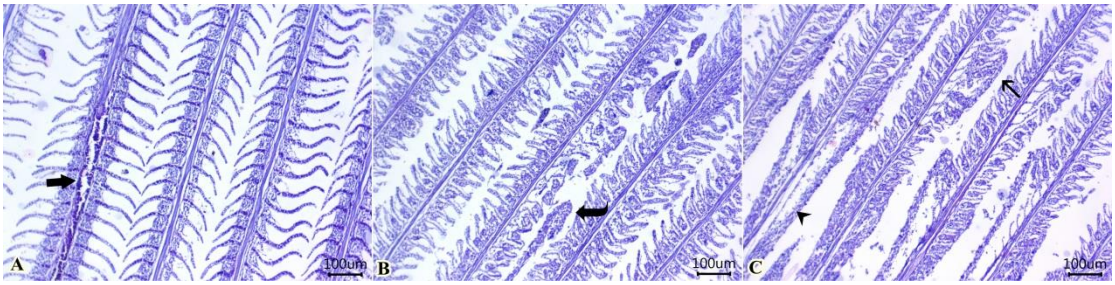

Fig. 2. Representative photomicrograph of H&E stained sections from gills (Scale bar 100µm) showing: **(A)** Engorged capillaries of some gill filaments (thick arrow) in Group 1 exposed to Pymetrozine at conc.0.25g/ 5L. **(B)** Destructed & desquamated a large number of secondary filaments (curved arrow) in pymetrozine at conc. 0.75g/ 5L. **(C)** Extensive distortion of the large number of primary filaments (arrowhead) and stuck secondary filaments with exudate (thin arrow) in pymetrozine at conc. 1.5g/ 5L

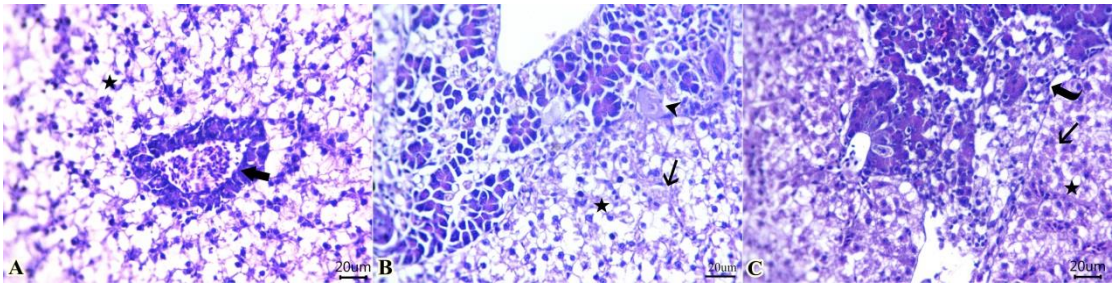

Fig. 3. Representative photomicrograph of H&E stained sections from liver (Scale bar 20µm) showing: **(A)** Vacuolations of hepatic parenchyma (star) and congestion of portal veins (thick arrow) in Group 1 exposed to pymetrozine at conc. 0.25g/ 5L. **(B)** Increase the number of vacuolated hepatocytes (star) and vacuolated pancreatic acinar epithelium, unicellular hepatic necrosis (thin arrow), and peri-pancreatic oedema (arrowhead) in Pymetrozine at conc.0.75 g/5L. **(C)** Vacuolated hepatic cells (star) are mixed with focal necrotic hepatocytes (thin arrow), dilated sinusoids, and necrosis of pancreatic acini replaced by lymphocytes (curved arrow) in pymetrozine at conc. 1.5g/ 5L

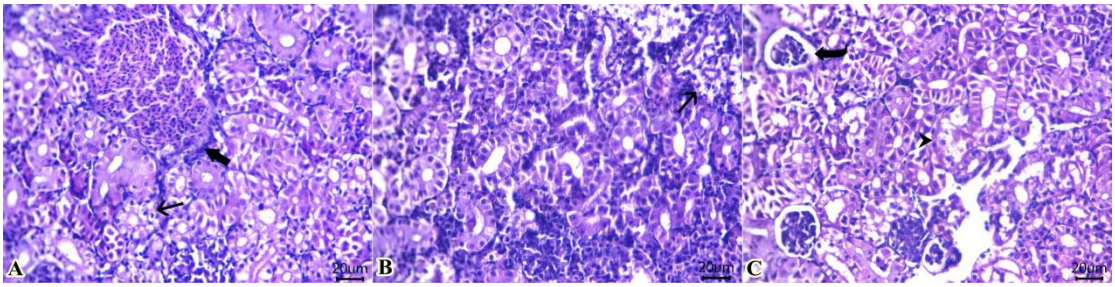

Fig. 4. Representative photomicrograph of H&E stained sections from kidney (Scale bar 20µm) showing: **(A)** Degenerative changes within a few renal tubular epithelium (thin arrow) beside congested renal blood vessels (thick arrow) in Group 1 exposed to pymetrozine at conc. 0.25g/ 5L. **(B)** A moderate number of hydropic degenerated renal tubular epithelium (thin arrow) in Pymetrozine at conc. 0.75g/ 5L. **(C)** Necrotic some renal tubular epithelium (arrowhead) and shrank glomerular tufts (curved arrow) in Pymetrozine at conc. 1.5g/ 5L

DISCUSSION

PY insecticide is widely used in the Middle East and other regions of the world. PY and other pesticides are widely used, which has led to ecological issues with soil, aquatic life, and public health. It has been demonstrated that organochemical pesticides, like PY, directly affect the agro-ecosystem by influencing the biological processes of both target and non-target animals, as well as the soil's organic matter breakdown and nutrient availability **(Andrén & Lagerlöf, 1983)**. PY decreases the richness and variety of non-target creatures and is extremely hazardous to humans and other mammals. Since aquatic ecosystems act as the final sink for all anthropogenic pollutants, aquatic species,

especially fish are regularly exposed to these chemical compounds **(Routledge *et al.*, 1998)**.

In the current study, the Nile tilapia treated with PY showed some noticeable behavioral changes, which were apparent about 30 minutes after exposure to the maximum concentration of PY ($1.5gL^{-1}$). The first signs of intoxication were swimming with a swinging motion due to sudden stress (this was evident at high PY concentrations), hyperactivity and erratic swimming, swimming near the water's surface for oxygen uptake, erratic swimming, body color darkening from pigment accumulation, and finally, lethargy from using up energy to fend off stress and death. Some of the intoxication symptoms and behavioral changes were seen in earlier research. In two species, *Hyphessobrycon bifasciatus* and *Brachydanio rerio*, exposed to acute endosulfan toxicity, **Jonsson and Toledo (1993)** reported excitation, erratic swimming with an increase in respiratory frequency, swimming toward the water's surface for oxygen uptake, convulsion, and nervous manifestation. Similar effects were also observed by **Joshi and Rege (1980)** in *Gambusia affinis* treated with a few inorganic salts and different organochlorine pesticides, including DDT and BHC. According to **Naqvi and Hawkins (1988)**, teleosts exposed to pyrethroids and certain organophosphates displayed altered behavior and signs of intoxication.

The symptoms associated with central nervous system issues include hyperactivity and erratic movement. PY may disrupt the association of certain neurotransmitters in particular receptors, similar to how they function in insects, even though the exact mechanism of action in fish is unknown **(Jonsson & Toledo, 1993)**. Fish exposed to pesticides may also exhibit hyperactivity and erratic swimming due to changes in the levels of intra- and extracellular potassium and sodium **(Swarup *et al.*, 1981)**.

Among the behavioral changes detected in *Oreochromis niloticus* and *Chrysichthys auratus* exposed to atrazine, *Channa punctatus* exposed to mercuric chloride and malathion, and *Heteropneutes fossilis* exposed to malathion are the abnormal swimming, jerks of the body, loss of balance, and anorexia **(Hussein *et al.*, 1996; Chandra, 2008)**. The intoxication symptoms observed in *Channa punctatus* exposed to carbosulfan, glyphosate, and atrazine included fast swimming and jumping, faster opercula activity, erratic swimming, vigorous jerks of the body, mucus elevation, increased body pigmentation, loss of balance and consciousness, rolling movement, and becoming exhausted and lethargic **(Nwani *et al.*, 2010)**.

The occurrence of dark coloration in fish exposed to pesticides has been documented in earlier studies **(Roy *et al.*, 2000; Taheri *et al.*, 2018)**. These studies included fish exposed to indoxacarb, *salmo salar*, and *Oncorhynchus mykiss* exposed to emamectin benzoate. The coloration of fish exposed to PY may be the consequence of

pesticide interference with melanophore aggregation due to elevated cortisol hormone levels during stressful conditions. **Nunes *et al.* (2008)** stated that a variety of circumstances, such as capture, environmental stress, and fish acting as a defence mechanism when they feel threatened, may have an impact on a fish's dark coloration. Fish that are exposed to stressful settings have higher pigmentation, which is correlated with their ability to respond to stress **(Backström *et al.*, 2014)**. Light coloration was observed in the common carp when exposed to 2,4-Dichlorophenoxyacetic acid **(Sarikaya & Yılmaz, 2003)**. Hypersecretion of mucus may be the cause of light coloring.

It is recorded that the gills are very useful and reliable tissues for early screening of adverse effects of various toxicants due to their direct contact with water. Gills are responsible for osmoregulatory mechanisms in aquatic animals and the maintainance of the ionic balance **(Ghaffar *et al.*, 2018)**.

As a general explanation of behavior changes, when an organism comes into contact with a contaminant, it either doesn't notice the change in its surroundings or its chemosensory perception triggers a series of behavioral reactions, including avoidance, movement, feeding, and mating **(Hebel *et al.*, 1997)**. Irritation at the commencement of the exposure is consistent with the universal behavioral responses **(Yu *et al.*, 2018)**. Hyperactivity may drive fish to death by boosting agonism **(Barbee & Stout, 2009)** and oxygen consumption **(Yu *et al.*, 2018)**. The ensuing modifications, which include sluggishness, loss of equilibrium, and lethargy, may make an organism more susceptible to predators having an impact on the survival rate, growth, and feeding of the organism **(Yu *et al.*, 2018)**.

Guidelines for water quality have been established using data on acute toxicity. At 96 hours, the LC_{50} value for PY in this investigation was 00 g L^{-1} (moderate toxicity). Moreover, PY appears to be a somewhat hazardous chemical for the Nile tilapia. The pesticides' toxicity was concentration and time-dependent. For many fish species, PY LC_{50} reports are somewhat little. Thus, additional research may yield different results, even with the Nile tilapia. It has been demonstrated that physiological characteristics such as temperature, pH, dissolved oxygen, turbidity, and species health, as well as chemical concentration and formulation, all influence an aquatic organism's toxicity to chemicals **(Farah *et al.*, 2004)**.

Previous studies on the histological changes in fish gills, liver, and kidney tissues revealed comparable results in fish treated with PY. Gills are the first organ to exhibit histological alterations, being a primary target of water-borne pollutants. Pymetrozine may impair fish's ability to breathe and regulate their osmoregulatory system, which could lead to their demise. The gill is the primary organ for breathing, osmotic, and ionic control. Studies on fenitrothion and trichlorfon's impacts on crustaceans serve as an

illustration of this **(Yu *et al.*, 2018)**. According to **Ahmad *et al.* (2023)**, fish treated with PY had necrosis and degeneration of primary and secondary lamellar epithelial cells in their gills, as well as abnormalities in the main and secondary lamellae's arrangements, necrosis of the lamellar epithelium, uplifting of the primary lamellae, and disarray in the cartilaginous core. Comparable pathological alterations in the gills caused by different chemicals have also been seen in the van fish **(Oguz *et al.*, 2018)**, *C. fluminea* **(Benjamin *et al.*, 2019)**, the bighead carp **(Akram *et al.*, 2020)**, and *Labeo rohita* **(Ghaffar *et al.*, 2021)**.

The liver tissue of *Ctenopharyngodon idella* **(Faheem & Lone, 2017)** and *Clarias gariepinus* **(Elias *et al.*, 2020)**, treated with various toxicants, was observed. The kidneys are the main organs exposed to various contaminants in water bodies, such as pesticides, herbicides, and insecticides according to various previous research **(Akram *et al.*, 2020)**. According to **Ahmad *et al.* (2023)**, many microscopic changes were noticed in the kidneys of fish treated with PY, including oedema, ceroid development, glomerular degeneration, enlarged Bowman's gap, congestion, tubule atrophy, and atrophy of the renal tubule lumen. Previous research has also documented comparable microscopic alterations in the kidneys of the tilapia **(Vinodhini & Narayanan, 2009)** and *Heteropneustes fossilis* **(Pal & Reddy, 2018)** treated with toxicants, such as congestion, necrosis, nuclear hypertrophy, melanomacrophage infiltrations, and degeneration of renal tubules.

CONCLUSION

The current experimental results indicate that pymetrozine has negative impacts on the Nile tilapias' clinical signs, behavior, mortality rate, and various tissues in a dose and time-dependent manner. The LC_{50} of PY after 96 hours of exposure was $0.28 gL^{-1}$ in the Nile tilapia.

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الملخص العربى

السمية الحادة للمبيد الحشري البيميتروزين على البلطي النيلي

زينب البوهي والشيماء خليل و محمد الهادي و فاطمة الهادي

قسم طب الأحياء المائية- كلية الطب البيطري- جامعة الزقازيق

كان الهدف من الدراسة الحالية هو تقييم السمية الحادة والتغيرات السلوكية والتغيرات النسيجية للبيميتروزين (PY) على البلطي النيلي "*اوريوكروميس نيلوتيكس*" وكانت قيم الجرعة نصف المميتة لمدة 96 ساعة هي 28.0 جم $^{-1}$. ولوحظت التغيرات السلوكية مثل فرط الحركة، والسباحة غير المنتظمة، و لون الجلد الداكن، وفقدان التوازن، والخمول اعتمادا على تركيزات " البيميتروزين " وبالتالي، فإن معدل النفوق، و الأعراض، والتغيرات السلوكية، والتغيرات النسيجية للخياشيم والكبد والكلى التي لوحظت استجابةً لـ البيميتروزين تعتمد على الجرعة و وقت التعرض.

الكلمات المفتاحية: البيميتروزين، التركيز القاتل، البلطي النيلي، التغيرات النسيجية، التغيرات السلوكية.