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Histopathological effect of the pesticide imidacloprid on the muscles of *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae)

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Abstract
Histopathology is a helpful indicator for environmental pollution. Histopathological analysis of fish tissue can be used to detect disease-related early warning signals as well as long-term harm to cells, tissues, and organs. The use of pesticides disturbs the aquatic food cycle, which is particularly harmful to fish. Neonicotinoids are among the several pesticides that interact with the nicotinic acetylcholine receptors (nAChR) in pests central nervous systems. Neonicotinoids gained popularity due to their high-water solubility, which allows for thorough penetration of the plant by their soil application. The first generation of neonicotinoids, of which imidacloprid is a member, are extensively used worldwide. Because of its widespread usage in agriculture, insecticides are present in the environment and may be toxicologically hazardous to fish. Monitoring histopathological alterations can be useful for assessing the pathological consequences of water-borne pollution and the histopathological changes that organic trace contamination has on fish organs. In the present study, exposure of *Clarias gariepinus* to Imidacloprid in various sublethal concentrations resulted in structural alterations excessive increased in myofibrils spaces, degraded muscle fibres, separation of muscle bundles, necrosis, swelling between muscle fibres, gap formation in myofibrils, oedema leading to disintegration and congestion of dermal blood cells.

Keywords: *Clarias gariepinus*, imidacloprid, histopathological, muscles, neonicotinoids

Introduction

Since 1940, the use of synthetic organic pesticides led to the maximum production of high-quality crops. According to the World Health Organization (WHO), there are an estimated one million cases of acute poisoning from pesticide exposure each year, with a death rate between 0.4% and 1.9%. Pesticides pose substantial health risks to biological systems due to their quick lipid solubility and bioaccumulation in creatures other than their intended targets. Pesticides may have several negative impacts even at low concentrations, which could be observed at the biochemical, molecular, or behavioural levels (Agrawal *et al.*, 2010) [1].

Widely used pesticides called neonicotinoids interact with the nicotinic acetylcholine receptors (nAChR) in insect's central nervous systems. Neonicotinoids gained popularity due to their high-water solubility, which allows for thorough penetration of the plant by their soil application. Imidacloprid, also known as 1-(6-chloro-1, 3-thiazol- 5-ylmethyl)-1, 3, 5-oxadiazinan-4-ylidenene (nitro) amine, was the first generation of this pesticide class to be employed (Natalia & Robert, 2016) [10]. Since neonicotinoids can linger in the soil for many years, they may eventually poison unintended species of animals and plants. They infect the soil, the water, the fish, and other living things (Huseth & Groves, 2014) [4]. There have been reports of neonicotinoid pesticides in wetlands and other aquatic ecosystems, and they account for 27% of the insecticide market globally (Hrynyk *et al.*, 2018) [3]. A few non-EU nations and the European Union limited the use of several neonicotinoids in 2013. The three main neonicotinoids (Coltianidin, Imidacloprid, and Thiamethoxam) were outlawed by the EU in 2018 for all outdoor usage.

Assessing histopathological changes in fish as a result of organic trace pollution and evaluating the pathological impacts of waterborne pollution can both benefit from monitoring histopathological alterations. Information was given to a bio monitoring strategy created for various environmental risk assessment aspects (Kazempoor *et al.*, 2015) [8]. Histopathological studies can assess the short- and long-term impact of certain environmental stresses. In addition, Parikh *et al.*, (2010) [11] described mild to severe muscle changes in the dimethoate-treated freshwater fish *Oreocromis mossambicus*. Fish muscle is an essential and significant part of the human diet and has been demonstrated to have heart protective effects due to its high protein, mineral, and unsaturated fatty acid content as well as low fat level. The muscle in the control group had a spherical nucleus and the normal architecture of elongated muscle fibres connected by connective tissues (Sumi & Chitra, 2017) [15]. Using the fish Clarias gariepinus, several pesticides have been examined for their histopathological effects on various types of tissue (Burchell, 1822; Verma *et al.*, 2022a, b, c) [16]. The current study documents the histological alterations in the catfish *Clarias gariepinus* muscles (Burchell, 1822) caused by the pesticide Imidacloprid.

Material and Methods

Clarias gariepinus spawns weighing 12-13 gm and length of 10-11 cm were collected from local fish market and brought live to the laboratory and were acclimatized under laboratory conditions for 15 days and were fed with fish

food at every 24 h interval. After 15 days of acclimatization, the fishes were treated with Imidacloprid.

Chronic Toxicity Studies

Chronic Toxicity measures long-term effects of exposure (typically 21-28 days). Sub lethal or safe level concentrations were derived from 96h LC 50 (APHA, 1992). The sub lethal concentrations of Imidacloprid to *Clarias gariepinus* were calculated from the LC 50 value 95.09 mg/l are 9.5 mg/l (10%), 14.25 mg /l (15%) and 19 mg /l (20%). Ten fishes were exposed to each concentration for a period of 5, 10 and 15 days. A control batch was maintained simultaneously.

In the present study the 96 h LC_{50} value of Imidacloprid in *Clarias gariepinus*, was found to be 95.09mg/l with a 95% confidence limit ranging from 92.42mg/l (lower confidence limit) to 98.60mg/l (upper confidence limit) in the present study. LC_{50} values of 24, 48 and 72 h of Imidacloprid in *Clarias gariepinus* are 105.44, 102.64 and 99.41, respectively (Verma *et al*., 2022c). Chi-square test showed that the calculated values were less than the table values and is significant ($p<0.05$). Tissue from each group of fishes was dissected post- treatment, fixed in Bouin's and stained with Delafield's Haematoxylin – Eosin (Humason, 1962) [3].

Result & Discussion

In the present study indicated that the muscle tissue of *Clarias gariepinus* was affected by sub lethal concentrations of Imidacloprid. The muscle tissue of fish exposed to 4.75mg/l Imidacloprid for 5 days showed splitting of muscle fibre and broken myofibrils. The muscle tissue of fish exposed to 9.5mg/l Imidacloprid for 5 days showed, muscle degradation, increased intermyofibrils spaces and disintegration of muscle fibres. The muscle tissue of fishes exposed to 19mg/l Imidacloprid for 5 days showed excessive increased in myofibrils spaces, degeneration of muscle bundle, broken myofibrils and splitting of muscle bundle (Figs. 1- 4).

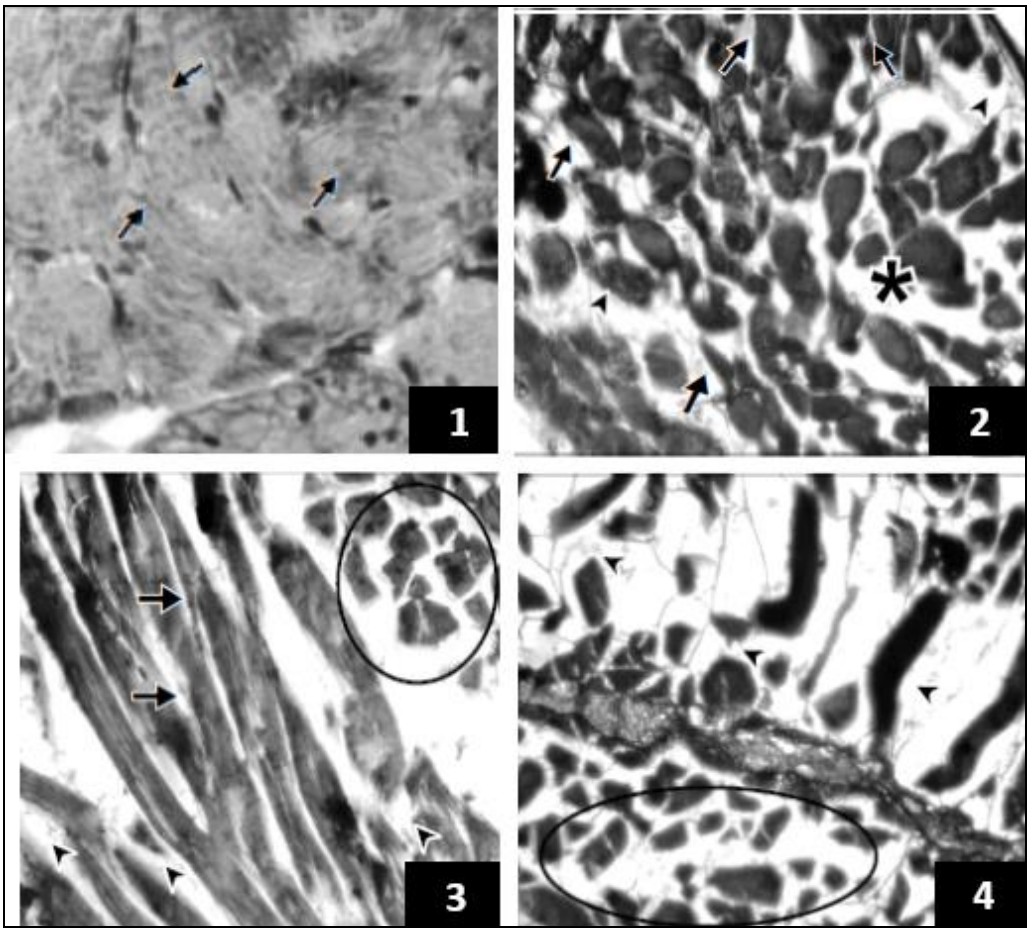

Fig 1-4: Section of muscle of control and treated fish, *Clarias gariepinus*.

Fig. 1. Section showing intact myofibrils of muscle tissue in the control fish (arrows) (HE x100). Fig. 2. Fish exposed to 9.5mg/l Imidacloprid for 5 days showing disintegrated myofibrils (arrows), increased inter myofibrils spaces (asterix) and degeneration of muscle fibres (arrowheads) (HE x100). Fig. 3. Fish exposed to 9.5mg/l Imidacloprid for 5 days showing muscle degradation (encircled) and increased inter myofibrils spaces (arrows) (HE x100). Fig. 4. Fish exposed to 19mg/l Imidacloprid for 5 days showing excessive increased distance in myofibrils spaces (arrowheads) and degeneration of muscle bundle (encircled) (HE x250).

The muscle tissue of fish exposed to 4.75mg/l Imidacloprid 10 days, exhibited oedema in between muscle fibres, disintegration of muscle fibre and increased inter myofibrils spaces. Fish exposed to 9.5mg/l Imidacloprid showed broken myofibrils and hyaline degeneration in the muscle tissue. Fishes exposed to 19mg/l Imidacloprid resulted in oedema between muscle fibres, degraded muscle fibres, separation of muscle bundles, disintegrating myofibrils, increased intermyofibrillar spaces and broken myofibril (Figs. 5-8).

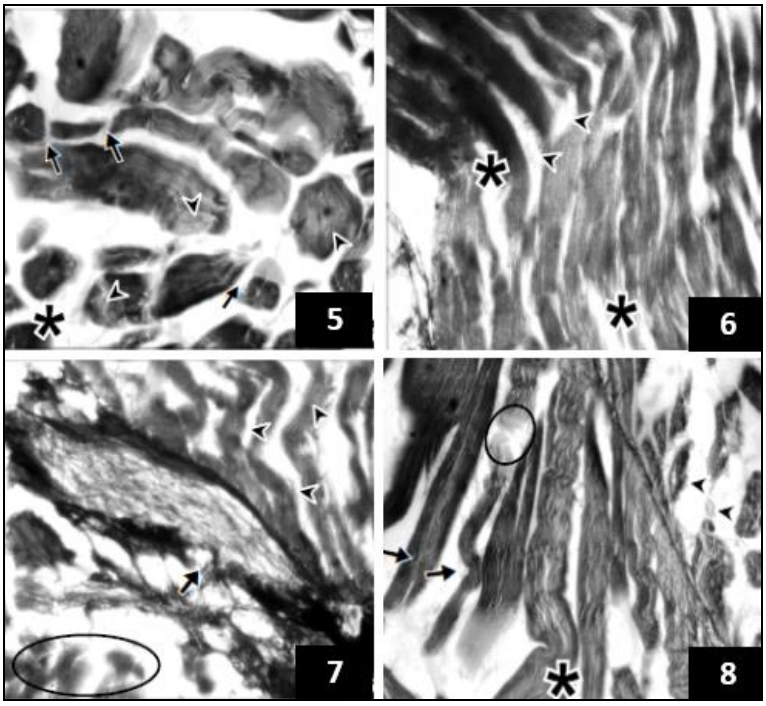

Fig 5-8: Section of muscle of treated fish, *Clarias gariepinus*.

Fig. 5. Fish exposed at 4.75mg/l for 10 days showing splitting of muscle bundle (arrows), oedema in between muscle fibres (arrowheads) (HE x400). Fig. 6. Fish exposed at 4.75mg/l Imidacloprid for 10 days showing disintegration of muscle fibre (arrowheads) and increased inter myofibrils spaces (asterix) (HE x400). Fig. 7. Fish exposed at 9.5mg/l Imidacloprid for 10 days showing increased inter myofibrils spaces (arrowheads), broken myofibrils (encircled) and hyaline degeneration (arrow) (HE x400). Fig. 8. Fish exposed at 19mg/l Imidacloprid for 10 days showing separation of muscle bundles (arrowheads), disintegrated myofibrils (asterix), increased inter myofibrils spaces (arrows) and muscle degradation (encircled) (HE x400).

The muscle tissue of fish exposed to 4.75mg/l Imidacloprid fish resulted in separation of muscle bundle and degenerated muscle bundle. At 9.5mg/l concentration of Imidacloprid the muscle tissue showed infiltration of blood cells, gap formation and broken myofibrils, degeneration of muscle bundle, degenerated muscle fibres and oedema and disintegrated myofibrils. Fishes exposed to 19mg/l Imidacloprid resulted in necrosis, swelling between muscle fibres, gap formation in myofibrils, oedema leading to disintegration, extremely damaged and disoriented muscle fibre and congestion of dermal blood cells (Figs.9-12).

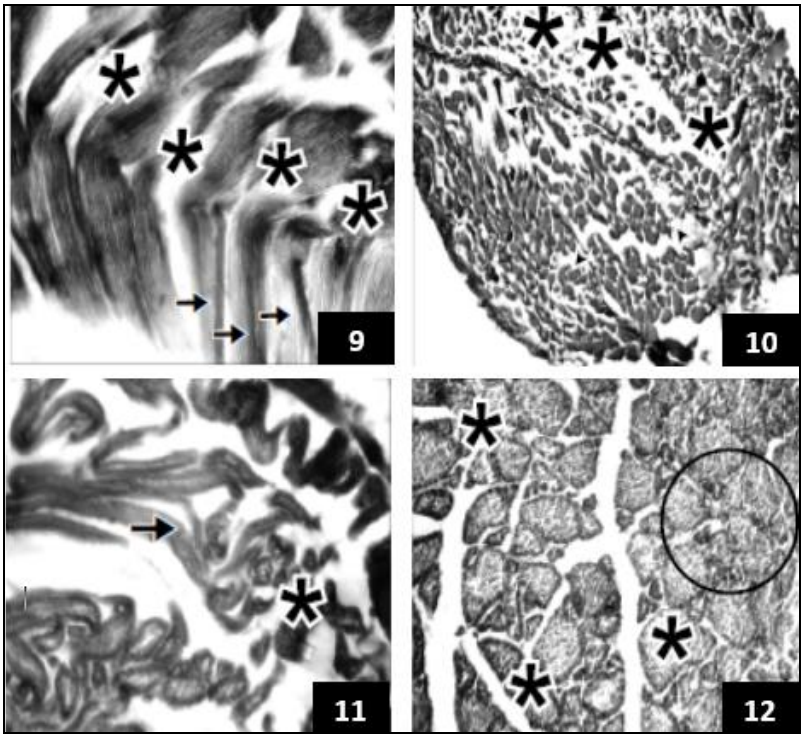

Fig 9-12: Section of muscle of treated fish, *Clarias gariepinus.*

Fig. 9. Fish exposed at 4.75mg/l Imid acloprid for 15 days showing disintegrated myofibrils (asterix) and increased inter myofibrils spaces (arrows) (HE x400). Fig. 10. Fish exposed at 9.5mg/l Imidacloprid for 15 days showing damaged muscle bundle (asterix) and disintegrated myofibrils (arrowheads) (HE x40). Fig. 11. Magnified view of Fig. 10 showing degenerated muscle fibres (arrows) broken myofibrils (asterix) (HE x400). Fig. 12. Fish exposed at 19.1mg/l Imidacloprid for 15 days showing oedema leading to disintegration (encircled), extremely damaged and disoriented muscle fibre (asterix) (HE x200).

These results indicate that the muscle tissue of *Clarias gariepinus* exposed to Imidacloprid exhibits histopathological changes like splitting of muscle fibre, dilation of dermal blood cells, infiltration of blood cells, congestion of dermal blood cells, congestion of dermal blood cells, muscle degradation, oedema between muscle fibres, swelling between muscle fibres necrosis, widening of inter myofibrillar spaces leading to disintegration of myofibrils, degeneration of muscle bundle, gap formation in myofibrils, inflammatory responses, vacuolar degeneration, degeneration of muscle fibre and atrophy. Results of the present study agree with those observed by many other investigators who have studied the effects of different pollutants on fish muscles. The changes like necrosis, oedema, destruction of muscle fibre, dilation of blood vessels are in accordance with results obtained by El-Serafy *et al*. (2005) [2] who worked on the histopathological changes induced on the muscle of *Oreochromis niloticus*. Mohamed (2009) observed several histological alterations caused by in the muscles of *Tilapia zillii* and *Solea vulgaris*, including degeneration in muscle bundles with focal areas of necrosis, atrophy of muscle bundles and oedema between muscle bundles. Parikh *et al*. (2010) [11] reported separation and degeneration of muscles, atrophy of muscle bundles and focal area, vacuolar degeneration and splitting of muscle fibre in freshwater fish *Oreocromis mossambicus* treated with Dimethoate. Ramesh & Nagarajan (2013) [12] reported markable changes in the histopathology of muscle tissue *Clarias batrachus* when exposed to untreated and treated sago effluent. Ibrahim *et al.* (2013) [5] studied bioaccumulation of non-essential heavy metals Cadmium and Lead and their histopathological impact on muscles of *Clarias gariepinus.* Jeheshadevi *et al*. (2014) observed infiltration and inflammatory responses in fishes exposed to sublethal concentrations of various pesticides. Histopathological changes like oedema, splitting of muscle fibre, separation of muscle fibre, necrosis and vacuolar degeneration were noticed during exposure of fish *Etroplus maculates* to sublethal concentrations of Fluben Diamide (Reethamma, 2014) [13]. Kazempoor *et al*. (2015) [8] also observed histopathological changes like necrosis and inflammation in muscle cells of *Acantopagrus latus* due to water soluble fraction of Iranian crude oil. Kaur *et al.* (2018) [7] studied histopathological effect of heavy metal contaminated water on muscle of *Clarias batrachus* with similar results as found in the present study.

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