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Effect of Imidacloprid on Histopathological Alterations of Brain, Gills and Eyes in hatchling carp (*Cyprinus carpio* L.)

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Abstract

Imidacloprid, a neonicotinoid pesticide is used globally to control insect pest in rural and urban area where it enters into and contaminate the aquatic ecosystem. The present study was designed to study the toxic impacts of imidacloprid on developing hatchlings of *Cyprinus carpio*, for which fertilized eggs of *C. carpio* were exposed to different sublethal concentration of imidacloprid and 48 hrs LC_{50} was calculated as 78 ppm. Three different sublethal concentration of imidacloprid were selected for further experimentation (7.8 ppm, 15.6 ppm and 23.4 ppm) along with control. Hatchlings developed from treated and control eggs were fixed in Bouins fixative to analyze the histopathological effects of imidacloprid on brain, eyes and gills. The findings depicted several histopathological degenerations in brain, eyes and gills including neuronal degeneration, necrosis, vacuolation, Pyknotic nuclei, fragmented nuclei, mononuclear infiltration, spongiosis, reduced retinal pigmentation, ruptured lens tissue, uplifted sclerotic layer, ruptured arterial wall and degenerated lamellae.

Keywords: Brain, *Cyprinus carpio,* Eye, Gills, Histopathology, Imidacloprid

1. Introduction

Insect pests are one of the major limiting factors in agriculture and destroy large fraction of crops produced throughout world, which results in a great economic loss. In the process of eradication of these insect pests and to increase the food production, pesticides play an important role, and now their use has become an essential tool in the farming[1,2]. A large amount of used pesticides enter into nearby aquatic ecosystem via different routes mainly runoff, spray drift, erosion and leaching[3,4], leading to high levels of water contamination and ecological disturbance. Evidences are confirming that continuous exposure of pesticides causes toxicity risk to other non-targeted organisms including fishes which are extremely sensitive[5,6]. Inside aquatic ecosystem, pesticides enter and accumulate into fish body via three ways i.e. uptake through the gills, absorption through skin and by drinking or feeding[7]. This accumulation can cause acute and chronic poisoning, severe damage to vital organs and histopathological alterations in different target organs such as brain, gills and eyes of exposed fish[8–10]. In general, brain plays an important role in fish physiology and is an interesting tissue in toxicology, especially when the pesticides involved are neurotoxic.

Imidacloprid is one of the best and most commonly selling systemic neonicotinoid insecticide used in agriculture[11]. It is a colorless crystalline solid, with solubility of 0.51 to 0.61 gL^{-1} at 20°C [12]. It is recognized as an important systemic insecticide, since very effective on target pests (particularly sucking insects) which is due to irreversible binding to its respective neuronal receptors[13]. In the present study, attempts have been made to evaluate the sub lethal effects of imidacloprid on histopathological

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alterations in brain, eyes and gills of *Cyprinus carpio* hatchlings chosen as an experimental model because it is a valued food fish, commercially available throughout India and one of the best candidates for freshwater aquaculture.

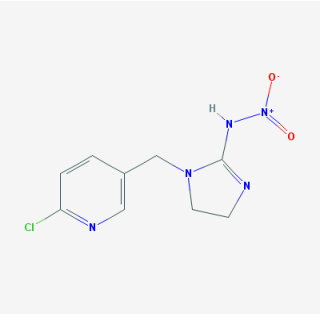
2. Materials and Methods

2.1 Selection of Fish

Fertilized eggs and hatchlings of freshwater teleost fish, *C. carpio* were procured from Fish Seed Farm Mandheri and Jyotisar (District Kurukshetra) used as test organism to evaluate the histopathological effects of pesticide imidacloprid on brain, eyes and gills.

2.2 Toxicant Used

Neonicotinoid pesticide imidacloprid (17.8% SL) purchased from local market of Kurukshetra was used as toxicant. Imidacloprid is one of the largest selling, water soluble and crystalline solid insecticide with molecular formula $C_9H_{10}ClN_5O_2$.



Chemical structure of Imidacloprid (https://pubchem.ncbi.nlm.nih.gov/)

2.3 Calculation of LC50

Semi-static method was used to calculate LC50 for fertilized eggs of *C. carpio*. Several groups of selected eggs were exposed to various concentration of imidacloprid with an initial dose of 5 ppm to higher concentration. The experiment was conducted with continuous increase in dose of test chemical to find the concentration causing 50% mortality of eggs. Mortality was recorded for each concentration and data was analyzed as per probit analysis by using software IBM SPSS Statics Version 20 (Windows 8). The calculated LC50 was found to be 78 ppm for 48 hrs (25°C).

2.4 Experimental Layout

Three different sublethal concentrations of imidacloprid (Table 1), along with control were selected in triplicate for each treatment and were coded as Control (C), T_1, T_2 and T_3.

Table 1. Concentrations of imidacloprid used in different treatment groups

	Treatment	Concentration
1	Control (C)	0.0 ppm
2	T_1	7.8 ppm (10% of LC50)
3	T_2	15.6 ppm (20% of LC50)
4	T_3	23.4 ppm (30% of LC50)

Equal numbers (100) of fertilized eggs of *C. carpio* were exposed to each selected concentration of imidacloprid. The eggs were placed in small happa fitted in 50 L plastic tubs having 30 liter dechlorinated water. Water was kept circulating and proper aeration was maintained with the help of aerators.

2.5 Histopathological Analysis

For histopathological analysis, the entire hatchlings, developed from control and pesticide exposed eggs, were fixed immediately for 24 hrs in Bouins fixative to avoid post mortal changes. Then samples were washed in running tap water and processed for histopathological studies using standard method given by Pearse[14].

The samples were washed in running tap water (2-3 hrs) and dehydrated in a graded series of ethanol (30, 50, 70, 90 and 100%) for specific time periods. Later on samples were embedded in paraffin wax blocks and after cooling, the embedded samples were sectioned (5µm thick) using a microtome. The sample sections were mounted on glass microscopic slides and air dried sections were stained using Haemotoxylin and Eosin (H&E) stains. Stained sections were then mounted with cover slips using DPX. The tissue sections were observed microscopically and photographs were taken to examine the histopathological changes.

3. Results

The calculated 48 hrs LC50 value of imidacloprid for fertilized eggs of *Cyprinus carpio* was 78 ppm. The results of present investigation showed that sublethal concentrations of imidacloprid cause considerable degree of alteration in the brain, eyes and gills of carp hatchlings.

Brain is a vital organ and proper brain functioning is important to maintain control and coordination. The brain histopathology of control hatchlings showed normal histological structure without any deformities (Figures 1A, B). However, like other vertebrates, the exposed hatchlings exhibited a series of severe histopathological responses. Hatchlings exposed to lowest concentration of pesticide (7.8 ppm) showed neuronal degeneration, necrosis, vacuolation, pyknotic nuclei, enlarged neurocytes and spongiosis (Figures 1C, D).

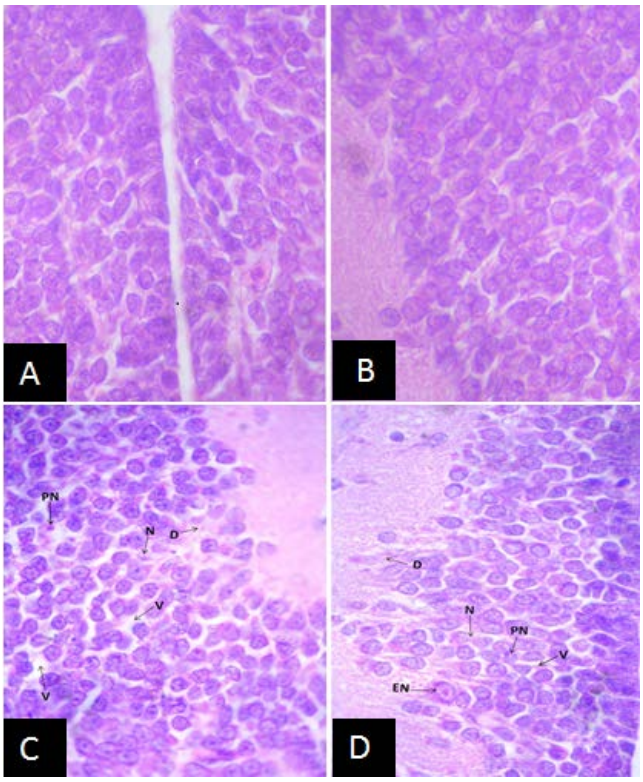


Figure 1. Brain section of *C. carpio* hatchling (**A&B**) control with no sign of necrosis, (**C&D**) exposed to 7.8 ppm concentration of imidacloprid showing neuronal degeneration (D), Necrosis (N), Vacuolation (V), Pyknotic nuclei (PN), Enlarged neurocyte (EN) and Spongiosis. (H and E, X400).

At concentration of 15.6 ppm, mononuclear infiltration, spongiosis, neuronal degeneration, necrosis, vacuolation, and fragmented nuclei were observed more frequently (Figures 2A, B). Severe degeneration of neuronal tissue of cerebrum, mononuclear infiltration, spongiosis, severe necrosis, vacuolation and fragmented nuclei at higher dose (23.4 ppm) indicated neurotoxic nature of imidacloprid (Figures 2C, D). The discoloration in the brain tissue of stressed fish was the most common response observed at 23.4 ppm concentration of imidacloprid.

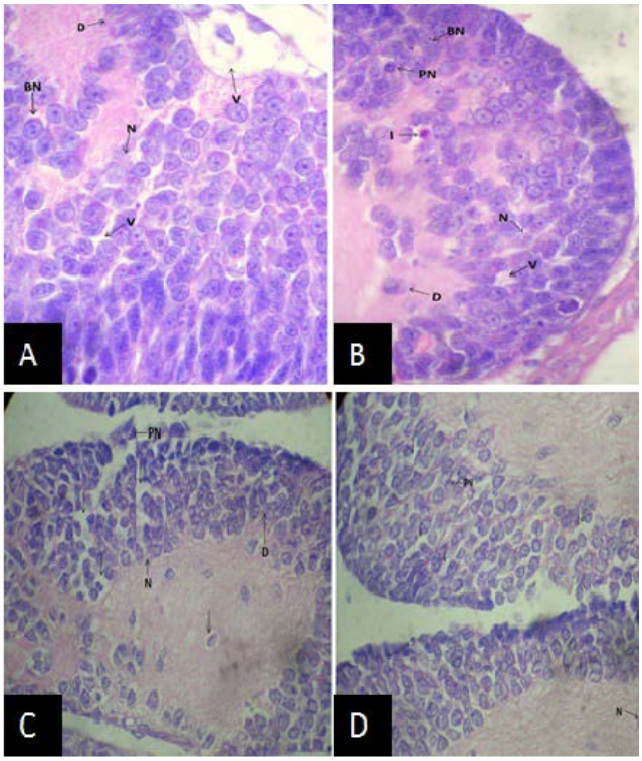


Figure 2. Brain section of *C. carpio* hatchling (**A&B**) exposed to 15.6 ppm and (**C&D**) exposed to 23.4 ppm concentration of imidacloprid showing neuronal degeneration (D), Necrosis (N), Vacuolation (V), Pyknotic nuclei (PN), mononuclear infiltration (I), binucleated (BN) and spongiosis. (H and E, X400).

The Eyes balls of carp are rounded consisted of two chamber, an outer aqueous chamber bounded by transparent cornea and iris, and inner vitreous chamber surrounded by iris and sclera. The lens is completely rounded, surrounded by a capsule and suspended between two chambers with the help of muscles and ligaments. Eye ball is composed of three concentric

layers i.e. outermost sclero-corneal, middle uveal and innermost retinal layer. The innermost retinal layer is further made up of 10 distinct layers of varied thickness (Figure 3 B). In the present investigation, the eye ball of control hatchlings showed normal histological architect under light microscope, without any pathological signs (Figures 3 A, B). Their lens was completely rounded with its smooth epithelium and retina was properly developed with correct number of chromatophores.

In contrast, eye section of pesticide stressed hatchlings showed some anomalies in eyeball structure. At concentration of 7.8 ppm, less pigmentation and uplifted sclerotic layer, were commonly reported (Figures 3 C, D).

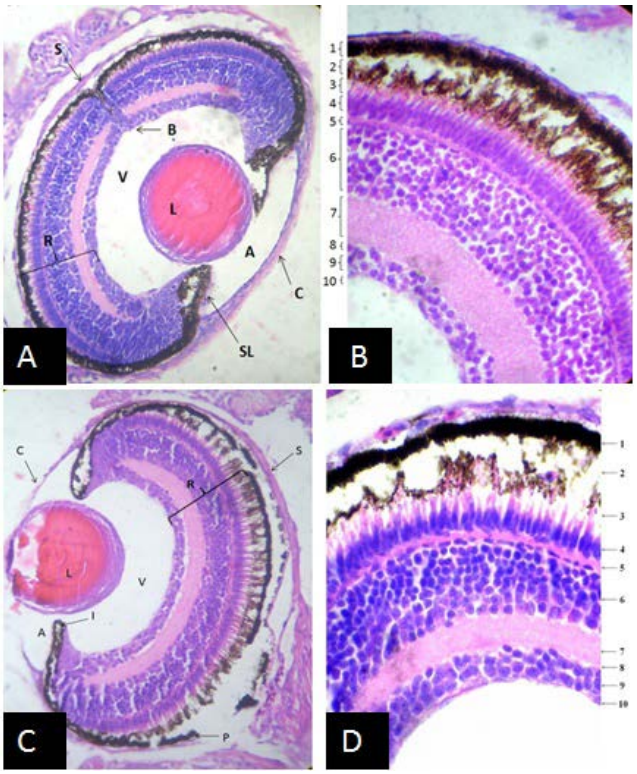


Figure 3. Eye sections of *C. carpio* hatchling of control (**A&B**) and (**C&D**) exposed to 7.8 ppm of imidacloprid. Control eye section depicting large Vitreous chamber (V), small Aqueous chamber (A), rounded Lens (L), Blind spot (B), Retinal layers (R), Sclera (S), Suspensory ligaments (SL), Pigmented epithelium (P). Eye sections of treated larvae (C&D) showing reduced retinal pigmentation, less number of cells in inner nuclear layer, reduced inner plexiform layer, ruptured lens tissue, ruptured pigment epithelium, space in between cells of retinal issue and uplifted sclerotic layer. (H&E), (X400).

Moreover, the severity of alterations was increased with increase in pesticide doses. At concentration of 15.6 ppm, the observed eye section showing ruptured lens tissue, pigment epithelium, space in between cells of retinal issue and uplifted sclerotic layer. Still the lens was rounded but ruptured (Figures 4A, B). Degenerative changes in rods and cone cells, lacking of limiting membrane, loosening and vacuolization in different layers and reduced number of chromatophores was also observed at concentration of 23.4 ppm (Figures 4C, D).

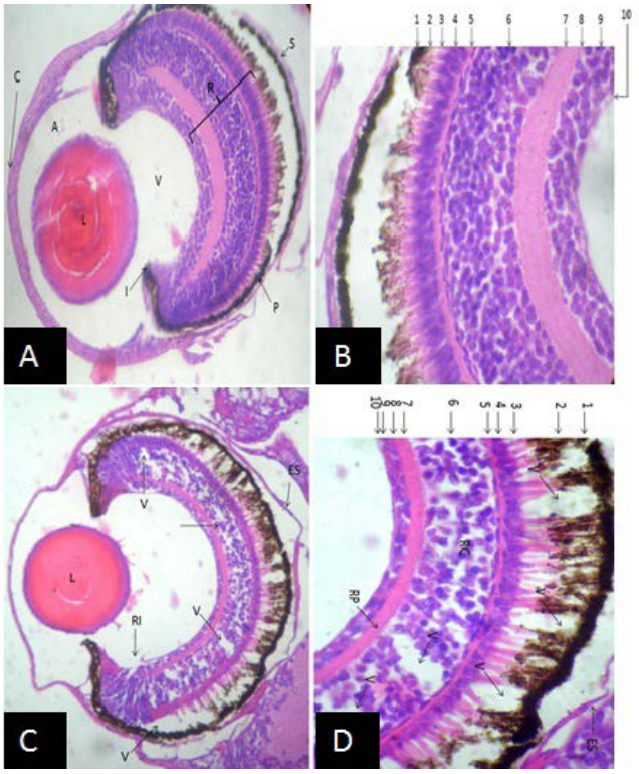


Figure 4. Eye sections of eye of *C. carpio* hatchling (**A&B**) 15.6 ppm and (**C&D**) 23.4 ppm of imidacloprid showing reduced retinal pigmentation, less number of cells in inner nuclear layer, reduced inner plexiform layer, ruptured lens tissue, ruptured pigment epithelium, space in between cells of retinal issue and uplifted sclerotic layer. (H&E, X400).

Histological architect of normal gills revealed that each gill arch bear a number of gill filaments supported by central cartilage along with afferent and efferent arterioles. Each filament is covered by a thin squamous epithelium containing mucous secreting cells supported by pillar cells. No recognizable changes were observed in the gills of the control fish larvae. Their epithelium was connected

with gill arches surrounded by pillar cells (Figures 5A, B). Branchial arteries were regular in shape and filled with numerous erythrocytes. However, in the gill section of treated larvae, severe histomorphological alterations were reported. Various histopathological alterations observed in the gills section of hatchlings exposed to 7.8 ppm imidacloprid including dilatation of branchial arteries as well as capillaries, ruptured arterial wall, Infiltration, lysis of erythrocytes and vacuolization were more common (Figures 5C, D).

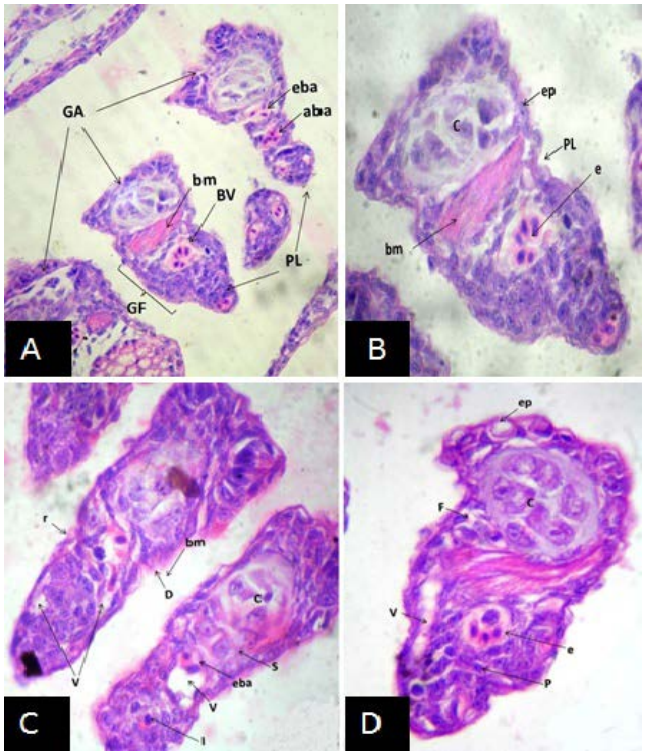


Figure 5. Longitudinal section of gills of carp hatchling. (**A&B**) Control showing normal gill architect i.e. arch (GA), gill filament (GF), gill arch cartilage (C), branchial muscle (bm), primary lamellae (PL) and epithelial cells (ep). Each filament is supplied by afferent (aba) and efferent arteries (eba). (**C&D**) exposed to 7.8 ppm of Imidacloprid indicating dilated branchial arteries (eba), lysis of erythrocytes (e), Infiltration (I), vacuolization (V), degenerated primary lamellae (D), cloudy swelling (S), fragmented nuclei (F), pyknotic nuclei (P). (H&E), (X400).

Severe histopathological changes were observed at concentration of 15.6 ppm including degenerated lamellae with ruptured epithelium, hyperplasia, vacuolization, dilation as well as rupturing of arterial wall and lysis of erythrocytes (Figures 6A, B). Infiltration, necrosis, cloudy swelling and few blood cells (RBC) were observed in the inter-branchial space due to rupturing of blood vessels. Hyperplasia as well as cell proliferation in the epithelial layers decrease the gaseous exchanges by reducing the respiratory area.

The degenerative changes were further more severe at concentration of 23.4 ppm (Figures 6C, D). Degeneration and fragmentation at distal end of primary lamellae was clearly visible. Throughout rupturing in epithelium and frequent vacuolization were also observed. Detachment of gill epithelium, ruptured blood vessels, lysis of erythrocytes, cloudy swelling and hypertrophy were more prominent. Pyknotic nuclei and severe necrosis were clearly visible at some places. The detachment of the gill epithelium increases the diffusion distance for oxygen, adversely affect the respiration mechanism and even cause the death of the treated fish.

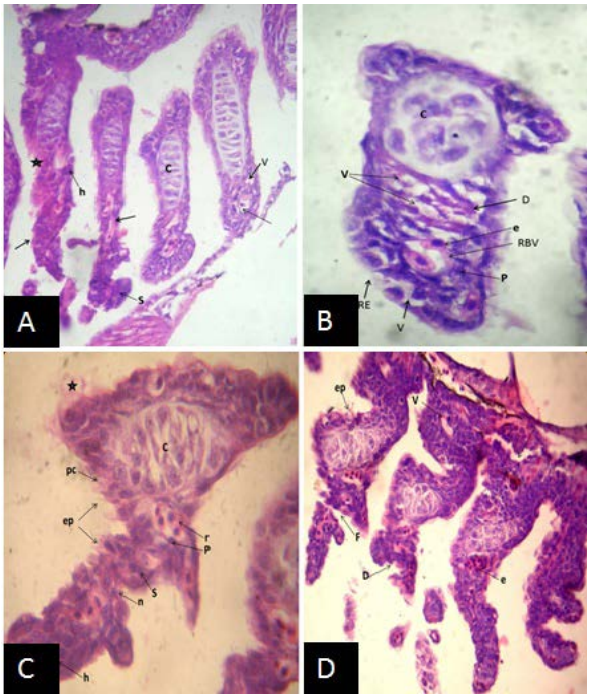


Figure 6. Longitudinal section of gills of carp hatchling exposed to (**A&B**) 15.6 ppm and (**C&D**) 23.4 ppm of imidacloprid indicating dilated branchial arteries, lysis of erythrocytes (e), ruptured arterial wall (rbv), ruptured epithelial (ep) and vacuolization (V), degenerated primary lamellae (D), cloudy swelling (S), fragmented nuclei (F), pyknotic nuclei (P), degeneration at distal end of primary lamellae, dilation (d), necrosis (n) and hyalinization (h). (H&E), (X400)

4. Discussion

Pesticides are deliberately used in agricultural land to control insect pests and subsequently persist in the soil, water and food, with toxicity related outcomes to both humans and other animals including fish[15]. Highly toxicity and biomagnification of organochlorines and organophosphates pesticides resulted in increased practice of various Neonicotinoid pesticides all around the world. Pesticides are the key factors that affect the structural integrity and physiology of the vital organs[16]. Therefore it becomes necessary to identify early detectable warning signs of their toxicity which can be easily achieved through histopathological analysis[17], as it is a very powerful, sensitive and cost effective technique to detect direct effects of toxic compounds within different organs of fish both in the field and laboratory studies[9,18]. Therefore, our aim was to detect the remarkable histopathological alterations in brain, eyes and gills of hatchlings of *C. carpio* exposed to imidacloprid.

Most of neurotoxic pesticides inhibit the enzyme acetylcholinesterase and disrupt the neurotransmission vital for proper functioning of brain. Pesticides also interfere with ion channels in the nerve membrane and causes severe alterations in neural tissue[19]. In present work, brain tissue of control hatchlings was normal without any type of histological deformities. However, a number of alterations such as necrosis, neuronal degeneration, pyknotic nuclei, vacuolation, enlarged neurocytes and spongiosis were found in the neural tissue of hatchlings exposed to various concentrations of imidacloprid. It was also reported that extent of damage was more at high concentration of imidacloprid.

Vacuolation and necrosis are evidence of fatty degeneration as well as glycolysis leading to microsomal and mitochondrial dysfunction[20,21]. Similar types of degenerations have been previously observed in *Labeo rohita* exposed to various sublethal concentration of endosulfan[22]. Spongiosis of neural tissue indicate severe brain damage and is agreed with the findings of Omitoyin *et al.*[16] Das and Mukherje[20] have also recorded hexachlorocyclohexane induced severe necrosis of neuronal cells and vacuolar changes in the parenchymal tissue of *L. rohita*. The above findings were also in support to the observation of Altinok and Capkin[23] in the molecular and granular layers of the cerebellum when rainbow trout (*Oncorhynchus mykiss*) was exposed to increasing concentration of methiocarb. Santhakumar *et al.*[24] also reported histopathological effects on the nervous tissue of fish *Anabas testudineus* exposed to sublethal concentrations of monocrotophos.

Vision is the important sensory system for the fish and other organisms due to their role in collection, focusing and transformation of the visual information into neural signals[25]. As the visual systems of fish and mammals are similar in many ways, the present finding may be useful to establish a correlation between toxicological and ophthalmological studies with reference to humans[26]. Fish eyes has a wide surface area which is in direct contact with external medium that makes them more susceptible to uptake the pesticide even at sublethal concentration resulting into severe histopathological alterations in the fish eyes[27]. However, due to lack of toxicological investigation on eyes, we have poor understanding of toxicological response of this system.

In the present investigation, eye ball of control hatchling showed normal histological architect without any pathological signs. However, a number of histological alterations have been reported in the eye ball of hatchlings exposed to different sublethal concentrations of imidacloprid. The decreased pigmentation and uplifted sclerotic layer were the most common anomalies reported in all the treatments. Uppal *et al.*[26] also reported pesticide (monocrotophos) induced irreversible alterations in the cornea epithelium of *Cyprinus carpio* when exposed to monocrotophos for 30 days. In addition, Hejduk and Svobodova[28] also reported haemorrhage in the eyes of the carp exposed to carbamates based pesticides. In the previous investigation of Pain[29], it has been reported that pesticide Nuvan causes cataracts in fish eye by increasing calcium ions concentration in the fiber cells of the lens. They hypothesized that pesticide might have disturbed the permeability of the lens for calcium, sodium and potassium by opening some cation channels.

Recently, Jindal *et al.*[25] has reported lead induced histopathological alterations in the eyes of *Ctenopharyngodon idella* which were characterized by degeneration of stromal layer, erosion and severe necrosis of epithelial cells. In addition to toxicants, change in water quality (pH) also causes a number of anomalies in the fish eyeball such as necrosis of lens epithelium, anaplasia, peeling of epithelial cells, undifferentiated retina, lack of external limiting membrane and reduced number of chromatophores[30]. Thus, toxicological studies of fish eye ball provide valuable information about the toxic effect of

pollutants on histological structure of eye tissues which may leads to severe vision impairment.

Now a day, histological alterations in the fish gills has been used more frequently to understand the extent of damage in their structural integrity caused by environmental pollution[31]. Any aquatic contamination damages the gills, break the gas exchange process resulting into hypoxic and other pathological conditions[32]. During the present investigation, gills of control hatchling were normal without any recognizable changes. However, a number of severe alterations were reported in the gill section of treated hatchlings. Their branchial arteries and capillaries were dilated with ruptured wall, vacuolization and infiltration of erythrocytes were also common. Along with this, hyalinization, fragmentation, pyknotic nuclei, necrosis and cloudy swelling were also observed frequently.

Similar results have been also observed in gills of fishes exposed to water containing copper[33] and organophosphorus compounds[18]. Similarly, Babatunde *et al.*[34] has also reported severe congestion, lamellar hyperplasia, disruption and distortion in the gill lamellae of *O. niloticus* exposed to paraquat.

However, according to Dutta *et al.*[35] epithelial detachment play a defensive role against the entrance of water pollutants as it increases the distance for the toxic substances to reach the blood stream. Similar reports on gill alterations in *Rivulus marmoratus* exposed to diethyl nitrosamine were documented by Thigargarajah and Grizzle[36]. Recently, Ahmadivand *et al.*[37] also tested histopathological alterations in the gill section of pesticide treated fish and revealed pathological symptoms such as hyperplasia of lamellar epithelium and fusion of gill lamellae. Similar type of gill degenerations has been also reported on exposure of *Cyprinus carpio* to Dipterex[38] and *C. gariepinus* to inorganic fertilizers[39]. Further, fusion of secondary lamellae is another severe alteration in the gill of pesticide treated fish (*P. mesopotamicus*) that has been reported by Ojha *et al.*[40] and Karan *et al.*[41] which subsequently reduce the respiratory area and capacity to capture the oxygen resulting into a condition known as hypoxia.

Throughout the investigation, at higher concentration of imidacloprid, histopathological and degenerative changes were enhanced in a dose dependent manner. Thus, exposure of imidacloprid and other neonicotinoid pesticides are one of the major health issues for aquatic organisms. The findings of present study also help to understand the toxicity of imidacloprid as aquatic pollutant on fishes and also to assess the safer level of this pesticide in the aquatic medium for the protection of aquatic biodiversity. The agricultural use of these pesticides must be replaced by some other means of pest control such as Integrated Pest Management (IPM) because of its effectiveness, cost beneficial and harmless for other non-target organisms.

5. References

1. Mathur SC. Future of Indian pesticides industry in next millennium. Pesticide Information. 1999; 24:9–23.
2. Abdou KA, Hend MAE. Epidemiology of pesticides in developing countries. Advances in Clinical Toxicology. 2018; 3:128–33. https://doi.org/10.23880/ACT-16000128
3. Edwards CA. Pesticide residues in soil and water. In: Edwards CA (ed) Environmental pollution by pesticides. London: Plenum Press; 1973. https://doi.org/10.1007/978-1-4615-8942-6_12. PMid: 4124892
4. Murty AS. Toxicity of Pesticides to fish. Boca Raton:CRC Press Inc; 1986.
5. Hayes WJ, Laws ER. Handbook of pesticide toxicology. San Diego: Academic Press; 1991.
6. Koprucu K, Aydin R. The toxic effects of pyrethroid deltamethrin on the common carp (*Cyprinus carpio* L.) embryos and larvae. Pesticide Biochemistry and Physiology. 2004; 80:47–53. https://doi.org/10.1016/j.pestbp.2004.05.004
7. Sweilum MA. Effect of sublethal toxicity of some pesticides on growth parameters, haematological properties and total production of Nile tilapia (*Oreochromis niloticus* L.) and water quality of ponds. Aquaculture Research. 2006; 37:1079–89. https://doi.org/10.1111/j.1365-2109.2006.01531.x
8. Dutta HM, Arends D. Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish. Environmental Research. 2003; 91:157–62. https://doi.org/10.1016/S0013-9351(02)00062-2
9. Sakr SA, Jamal ALSM. Fenvalerate Induced Histopathological and Histochemical Changes in the Liver of the Catfish *Clarias gariepinus*. Journal of Applied Sciences Research. 2005; 1:263–67.
10. Perez MR, Rossi AS, Bacchetta C, et al. In situ evaluation of the toxicological impact of a waste water effluent on the fish *Prochilodus lineatus*: Biochemical and histological assessment. Ecological Indicators. 2018; 84:345–53. https://doi.org/10.1016/j.ecolind.2017.09.004
11. Hovda LR, Hooser SB. Toxicology of newer pesticides for use in dogs and cats. Veterinary Clinics of North America: Small Animal Practice. 2002; 32:455–67. https://doi.org/10.1016/S0195-5616(01)00013-4

12. Tomlin CDS. The Pesticide Manual, 12th Edition. Bracknel: British Crop Protection Council; 2000.
13. Tennekes HA, Sanchez-Bayo F. Time-dependent toxicity of neonicotinoids and other toxicants: Implications for a new approach to risk assessment. Journal of Environmental & Analytical Toxicology. 2011; 4:1–8. https://doi.org/10.4172/2161-0525.S4-001
14. Pearse AGE. Histochemistry: Theoretical and Applied (revised edition of A.G.E. Pearse). Awnsham and John Churchill, London; 1968.
15. Moraes BS, Loro VL, Glusczak L, Pretto A, Menezes C, Marchezan E, Machado SO. Effects of four rice herbicides on some metabolic and toxicology parameters of teleost fish (*Leporinus obtusidens*). Chemosphere. 2007; 68:1597–1601. https://doi.org/10.1016/j.chemosphere.2007.03.006. PMid:17451783
16. Omitoyin BO, Ajani EK, Fajimi AO. Toxicity Gramoxone (paraquat) to juvenile African catfish, *Clarias gariepinus* (Burchell, 1822). American-Eurasian Journal of Agricultural & Environmental Sciences. 2006; 1:26–30.
17. Penrith ML, Robinson JTR. Selenium toxicosis with focal symmetrical poliomielomalacia in postweaning pigs in South Africa. Onderstepoort Journal of Veterinary Research. 1996; 63:171–9.
18. Machado MR, Fanta E. Effects of the organophosphorous methyl parathion on the branchial epithelium of a freshwater fish *Metynnis roosevelti*. Brazilian Archives of Biology and Technology. 2003; 46:361–72. https://doi.org/10.1590/S1516-89132003000300008
19. Sharbidide AA, Metkari V, Patode P. Effect of Diazinon on acetylcholinesterase activity and lipid peroxidation of Poecilia reticulata. Research Journal of Environmental Toxicology. 2011; 5:152–61. https://doi.org/10.3923/rjet.2011.152.161
20. Das BK, Mukherjee SC. A histological study of carp (*Labeo rohita*) exposed to hexachlorocyclohexane. Vet Arh. 2000; 70:169–80.
21. Rahman MZ, Hossain Z, Mellah MFA, Ahmed GU. Effect of diazinon 60EC on *Anabus testudineus*, *Channa punctatus* and *Barbades gomonotus*. Naga: The ICLARM Quarterly. 2002; 25:8–11.
22. Das BK. Studies on the effect of some pesticides and common used chemicals on Indian major carp and their ecosystem. PhD Thesis, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India; 1997.
23. Altinok I, Capkin E. Histopathology of rainbow trout exposed to sublethal concentrations of Methiocarb or Endosulfan. Toxicologic Pathology. 2007; 35:405–10. https://doi.org/10.1080/01926230701230353. PMid:17455089
24. Santhakumar M, Balaji M, Ramudu K. Histopathological lesions in the brain of ateleost *Anabas testudineus* exposed to monocrotophos. Journal of Environmental Biology. 2000; 21:297–9.
25. Jindal R, Kaur M, Kaur J. Lead induced toxicopathic corneal lesions *in Ctenopharyngodon idellus* (Cuvier and Valenciennes). International Journal of Zoology Studies. 2017; 2:29–33.
26. Uppal RK, Johal MS, Sharma ML. Toxicological effects and recovery of the corneal epithelium in *Cyprinus carpio communis* Linn. exposed to monocrotophos: An scanning electron microscope study. Veterinary Ophthalmology. 2013; 18:214–20. https://doi.org/10.1111/vop.12134. PMid:24373492
27. Chang SW, Chi RF, Wu CC, Su MJ. Benzalkonium chloride and gentamicin cause a leak in corneal epithelial cell membrane. Experimental Eye Research. 2000; 71:3–10. PMid:10880271
28. Hejduk J, Svobodova Z. Acute toxicity of Carbamate-based Pesticides for fish. Acta Veterinaria Brno. 1980; 49:251–7. https://doi.org/10.2754/avb198049030251
29. Pain S. Pesticide causes cataracts in salmon. New Science. 1989; 123:30.
30. Ostaszewska T, Kossakowski MK, Czarnogorski M, Resiak G. Histopathological changes in Juvenile Carp, *Cyprinus carpio* (L.) continuously Exposed to Alkaline levels of pH, from Hatching. Archives of Polish Fisheries. 1999; 7:329–42.
31. Selvanathan J, Vincent S, Nirmala A. Histopathological changes in freshwater fish *Clarias batrachus* (Linn.) exposed to mercury and cadmium. International Journal of Life science and Pharma Research. 2012; 3:1–11.
32. Natarajan GM. Effect of sublethal concentration of metasystox on selective oxidative enzymes, tissue respiration and haematology of the freshwater air-breathing fish, *Channa striatus* (Bleeker). Pesticide Biochemistry and Physiology. 1985; 21:194–8. https://doi.org/10.1016/0048-3575(84)90054-3
33. Arellano JM, Storch V, Sarasquete C. Histological changes and copper accumulation in liver and gills of the *Senegales sole*, *Solea senegalensis*. Ecotoxicology and Environmental Safety. 1999; 44:62–72. https://doi.org/10.1006/eesa.1999.1801. PMid:10499990
34. Babatunde MM, Oladimeji AA, Rafindadi AH. Histopathological changes in the gills, livers and brains of *O. niloticus* (Trewavas) exposed to paraquat in chronic bioassay. International Journal of Science and Technology. 2014; 4:1–10.
35. Dutta HM, Richmonds CR, Zeno T. Effects of diazinon on the gills of bluegill sunfish *Lepomis macrochirus*. Journal

of Environmental Pathology, Toxicology, and Oncology. 1993; 12:219–27.

36. Thigargarajah A, Grizzle JM. Pathology of diethyl nitrosamine toxicity to the fish (*Rivulus marmoratus*). Journal of Environmental Pathology, Toxicology, and Oncology. 1985; 2:219–32.
37. Ahmadivand S, Farahmand H, Mirvaghefi AR, Eagderi S, Shokrpoor S, Rahmati-Holasoo H. Histopathological and haematological response of male rainbow trout (*Oncorhynchus mykiss*) subjected to butachlor. Veterinary Medicine – Czech. 2014; 59:433–9. https://doi.org/10.17221/7683-VETMED
38. Chinabul S, Limsuwan S, Kanchana K. Toxic and sublethal effects of dipterex on fresh water fishes. NACA, WP; 1987
39. Ufodike EBC, Omoregie E. Toxicity of Gammalin 20 and Actellic 25 EC to *O niloticus*. Acta Hydrobiol. 1990; 32:447-55.
40. Ojha J, Rooj NC, Mittal AK, Munshi Datta JS. Light and scanning electron microscopic studies on the effect of biocidal plant sap on the gills of a hill stream fish, *Garra lanta* (Ham.). Journal of Fish Biology. 1989; 34:165–70. https://doi.org/10.1111/j.1095-8649.1989.tb03299.x
41. Karan V, Vitorovic S, Tutundzic V, Poleksic V. Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicology and Environmental Safety. 1998; 40:49–55. https://doi.org/10.1006/eesa.1998.1641. PMid:9626535