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Mohammad Sohidul Islam
Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh

MD. Mansurul Haque
Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh

MD. Nazim Uddin
Directorate of Secondary and Higher Education, Dhaka, Bangladesh

MD. Hasanuzzaman
Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Ganakbari, Savar, GPO Box- 3787, Dhaka- 1000, Bangladesh

Corresponding Author:
Mohammad Sohidul Islam
Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh

Histopathology in the fish *Channa punctatus*, *Heteropneustes fossilis* and *Anabas testudineus* exposed to diazinon

Mohammad Sohidul Islam, MD. Mansurul Haque, MD. Nazim Uddin and MD. Hasanuzzaman

Abstract
Pesticides deteriorate the normal function of vital organs of fishes and in case of high concentrations, it totally damaged those organs. Histological changes in gills, liver, heart, intestine and kidney of three common fish species (*Channa punctatus*, *Heteropneustes fossilis* and *Anabas testudineus*) have been studies to observe the effect of Diazinon as it has become a very common pollutant in the aquatic environment. Six different concentrations (2.5. 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l) of pesticide had been applied in the test aquaria (Chari) to experience the major alterations of the vital organs of the three treated species of fish. Lower concentrations (2.5 to 10.0 mg/l) had no remarkable effect on the treated organs, whereas, Curley gill lamellae, irregular blood vessels in the liver, muscle fiber destruction in heart, blended Submucosa and glomerular necrosis were found in *C. punctatus* from 15.0 to 25.0 mg/l doses. Lamellar fusion, sinusoid vacuoles, fused villi and hemorrhagic renal tubules were observed in *H. fossilis* and bent in secondary lamellae, irregular blood vessels in liver, fragmented muscle fiber in heart, ruptured villi as well as necrotic glomerular were noticed in *A. testudineus* in the same doses of Diazinon. The present study demonstrated that pesticide (Diazinon) disrupted the normal function of the sensible organs of fishes by damaging them in different ways.

Keywords: Histological alteration, pesticide, ecosystems, freshwater fishes

1. Introduction
Pesticides are regarded as important tools for pest control in agro-farm and forestry though it has become the contributor to gradual aquatic ecosystem degradation that recommended as a greater part of the natural environment [1, 2]. It boosts the crop yields in one hand, contaminates the water bodies through spray drift and leaching from the soil surface that induces the ecological balance and hazardous health effects on a diversity of non-target creatures including fish on the other hand [3]. In recent years, Pesticides have become a growing concern of bioaccumulation as well as the persistence of various pesticides in the aquatic environment comprehended a great threat to the overall ecosystem including human beings [4]. Since 50 years ago pesticides present in surface waters were reported in Canada, North America and Europe. After that a large number of reports had been documented revealing the toxic effects of these pollutants to the aquatic ecosystem [5, 6]. *C. punctatus*, *H. fossilis*, *A. testudineus*, *Barbodes gonionotus* and some other indigenous small fishes use paddy fields and adjacent aquatic ground (canals, lakes, ponds, swamps and inland water bodies) as breeding and nursing of their fries, hence they played as biological indicators of ecotoxicological studies. Pesticides with high concentration attenuated the survival, growth and reproduction of these indigenous fish species [7] and exposed some visible effects on fish. This is because, fishes are very sensitive to contaminated water occurring by pesticides, and certain physiological and biochemical processes may damage when pesticides enter into different organs of fish [8].
Organophosphates are extensively used pesticides in the agriculture sector and it accounts for approximately 50% of global pesticide use (John, 2007). Diazinon (0,0-diethyl 0-[6-methyl-2(1-methylethyl)-4-pyrimidinyl] is a widely used organophosphate insecticide for agriculture and domestic pest control. It is also used to restrain a variety of Hymenopterans and Hemipteran insects including aphids, beetles, scales and pill bugs [10, 11]. After application in the agro-farm, Diazinon easily washes away into the surface water and ultimately brings into

adjacent water bodies, ponds, rivers and lakes where they contaminate the aquatic ecosystems and affects the aquatic organisms. The recent work that has been done on evaluating the effects of Diazinon on different species of fishes [12-23] and so on. Due to their low cumulative ability and short persistence in the environment organophosphorus (Diazinon) insecticides have fully replaced the chlorinated insecticide in 1970's and at the beginning of 1980's [24]. Though organophosphorus pesticides are extensively used in fish culture in order to suppress some parasitic diseases i.e. monogeneoses and arthropodoses [25, 26], some important organs *i.e.* kidney, gills, stomach, brain, muscles and genital organs are damaged by its residual effects.

Fishes are the main source of protein in human food among other aquatic organisms. So it is necessary to find out the adverse effects of pesticide pollutants on fish as they have a direct link with the food chain as well as contamination of water bodies occurring by pesticides imbalanced the aquatic system [27]. Fishes are severely affected due to insecticides in different ways, mostly affected to crucial organs *i.e.* gills, liver, heart, kidney, intestine etc. Among them, gills are primarily attracted because they are the first organs to be exposed by pollutants [28]. The water-born toxic particles infected kidney as it regulates the extracellular and composition of fluid volume as well as the acid-base balance of fish. Insecticides disrupt the functions of the kidney and most cases it causes temporary or permanently derangement of homeostasis [5]. Through these backdrops, the present study has been conducted to investigate the toxicity effects of Diazinon on some histopathological indices of the three indigenous freshwater fish species *i.e. C. punctatus*, *H. fossilis* and *A. testudineus* that would facilitate knowledge for the management of freshwater reservoirs regarding Diazinon application in agriculture fields.

2. Materials and Methods

2.1 Collection and maintenance of experimental fish species: Three experimented fish species- *C. punctatus* with 12 to 16 cm length and 24 to 30 gm weight, *H. fossilis* with 13 to 18 cm length and 25 to 32 gm weight and *A. testudineus* with 4.3 to 7.1 cm length and 10.6 to 28.3 gm weight were collected from the local water bodies (beels) in Dhamrai area located in Savar district, Bangladesh. Selected fishes were transported in plastic containers and reared in 5 clay pots locally called Chari of 18×5×10 inches in size with 20 liters of water receptivity of each. All samples were cleaned with 0.5% $KMnO_4$ solution for five minutes to set free external infections and acclimatized them under tap water in a large glass tank with 100 liters capacity for a week. Water temperature 26±0.17º C with pH 6.5±0.02 and 7.3±0.22 mg/l dissolved oxygen were maintained in the test Chari. The experiments were conducted in the Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh with the photoperiod of 12D:12L. Fishes were fed twice daily with earthworm and dead fishes were immediately removed to avoid possible water quality deterioration. The water of the experimented Chari was changed regularly that maintained the better possible effects of Diazinon.

2.2 Insecticide formulation

Insecticide was purchased from the local authorized dealer in Savar market. 200 ml stock solution was prepared following the EC% active ingredient (mg/l) with the formula of (200×60)/1000= X (X= amount of Diazinon 60EC), 200-X= DW (distil water). Finally, 200 ml stock solution = (X+DW) ml. The desired dose concentrations for 20L tap water were formulated with the aphorism of $S_1V_1 = S_2V_2$ (Here, S_1= 200mg/l, S_2= 1 mg/l, V_1= dose concentration, V_2 =20L). The selected concentrations of chemicals were poured into the 20L tap water in the Chari with a micropipette and stirred the solution gently with a glass rod for mixing completely.

2.3 Histological study (Dissection and Slide preparation)

For the histological investigation, the fishes (20×3) were exposed to various concentrations of Diazinon (2.5. 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l) with three replications for each group. The control group fishes (20) had not treated with insecticide. After 04 days (96 hours), both the control and insecticide treated fishes (randomly selected three fishes in each concentration) were dissected and examined for histological study by the use of dissecting tray and surgical tools (scalpels, forceps, needle etc.) following the methods of Keneko [29] and Schalm *et al.* [30]. Gill, Liver, Heart, Intestine and Kidney were dissected sophisticatedly and kept in plastic vial with 10% formaldehyde. After fixation for 24 hours, the sections of tissues were dehydrated by ethyl alcohol for removed water fromthe tissue block and then embedded within a small cube of paraffin. The paraffin embedded tissue blocks were sectioning accomplished by using a microtome. The microtome drives a knife across the surface of the paraffin cubes and produces a series of thinsections (5µm) with acontinuous 'ribbon'. Then the sections were mounted on individual microscope slides and stained with hematoxylin andeosin. In the end, the sections of tissue were permanently mounted under a coverslip. After preparation, slides were viewed under Olympus CX41microscope (X10) and photographs were taken for further analysis.

3. Results

Histopathological observation of different concentrations of Diazinon was done on gills, liver, heart, intestine and kidney of three treated fish species are presented in Table 1. The specific changes of the different organ of the insecticide exposed fishes are described herein-

Table 1: Alterations appeared in the selected organs of the experimented fishes due to insecticide (Diazinon) treated with different concentrations

Name of Fish species	Insecticide concentrations (mg/l)	Effects on organs of fish				
		Gills	Liver	Heart	Intestine	Kidney
Channa punctatus	2.5	✓	✓	✓	✓	✓
	5.0	✓	✓	✓	✓	✓
	10.0	✓	✓	✓	✓	✓
	15.0	Curly and bent lamellae	Blood vessels irregular	Necrosis and fragmentation muscle fibers	Submucosa disintegrated	Hemorrhage renal tubules
	20.0	Lamellar fusion	Rupture sinusoids	Destruction blood vessels	Submucosa blended	Necrotic changes in glomerulus
	25.0	Blending and	Vacuoles in	Fragmentation muscle fibers	Villi fused/	Necrotic changes

		destructiongill arches	sinusoids		ruptured	in glomerulus
Heteropneustes fossilis	2.5	✓	✓	✓	✓	✓
	5.0	✓	✓	✓	✓	✓
	10.0	✓	✓	✓	✓	✓
	15.0	✓	Destruction blood vessels	Necrosis muscle fibers	✓	Hemorrhage renal tubules
	20.0	Lamellar fusion	Hemorrhage blood vessels	Destruction blood vessels	Serosa slightly damaged	Necrotic changes in glomerulus
	25.0	Destruction gill filaments and gill arches	Vacuoles in sinusoids	Fragmentation muscle fibers	Villi fused	Necrotic changes in glomerulus
Anabas testudineus	2.5	✓	✓	✓	✓	✓
	5.0	✓	✓	✓	✓	✓
	10.0	✓	✓	✓	✓	✓
	15.0	Curly and bent secondary lamellae	Blood vessels irregular	Fragmentation muscle fibers	✓	Hemorrhage renal tubules
	20.0	Curly and bent secondary lamellae	Hemorrhage central vessels.	Destruction blood vessels	Submucosa disintegrated and vacuolated	Changes in glomerulus
	25.0	Blending and destruction lamellae	Vacuoles in sinusoids	Fragmentation muscle fibers	Villi ruptured	Necrotic changes in glomerulus

✓ = No changes apparently

3.1. Channa punctatus

3.1.1 Gills: Fusion of secondary lamellae, epithelial lifting, epithelial hyperplasia and degeneration of secondary lamellae were observed in the gills of fishes exposed to different concentrations of Diazinon. Apparently, there were no changes of the desired organs were noticed in lower concentrations (2.5-10.0 mg/l) of the treated fishes. Abnormalities were found with comparatively high concentrations. Fusions of secondary lamellae were noticed in 15.0 to 20.0 mg/l concentrations. In 25.0 mg/l concentration level showed bending and destructions of gill arches (Fig. 1, A-D).

3.1.2 Liver: Normal structures of liver tissues were found upto 15.0 mg/l concentrations of insecticide treated fishes. Abnormalities started from 20.0 to 25.0 mg/l concentrations level. Sinusoids and bile duct of liver were ruptured in those levels, irregular blood also found in the treated fishes (Fig. 2, A-C).

3.1.3. Heart: Some significant changes were found after treated with the 20.0 mg/l concentrations ofthe insecticide for 96 hours exposure period in the fishes. In 25.0 mg/l the sections of the tissue showed congestion of blood vessels and fragmentations of muscle fibers (Fig. 3, A-C).

3.1.4 Intestine: The intestine of *C. punctatus* has a mucosa, submucosa, muscularis and serous membrane among them serosa was the outermost protective cover of intestine. Lower concentrations level (2.5- 10.0 mg/l) had shown no changes in the membrane of intestine but from apparently higher concentrations submucosa was disintegrated in 15.0 mg/l dose and in 20-25mg/l of concentrations blended mucosawith rupture villi were found (Fig. 4, A-C).

3.1.5 Kidney: Kidney tubules and haematopoietic cells were normal forms the concentration limits of 10.0 mg/l. From 15.0 to 25.0 mg/l level, degeneration of renal tubules, Bowman's capsule with atrophying glomeruli and severe hemorrhage were observed (Fig. 5, A-C) in the insecticide treated fishes.

3.2 *Heteropneustes fossilis*

3.2.1 Gills: No changes of gills were found in lower concentrations upto 15.0 mg/l unit of insecticide treated fishes for 96 hours**.** Lamellar fusion was first noticed **i**n 20.0 mg/l concentration and in 25.0 mg/l level gill filaments and gill arches were found destructed condition (Fig. 1, E-G).

3.2.2 Liver: Histological observation of liver of both control fishes and upto 10.0 mg/l concentrations of insecticide exposed fishes in the present study showed a normal homogenous mass of hepatocytes with no abnormalities. The destruction of blood vessels was started from 15 mg/l dose. Hemorrhage in blood vessels was found in 20.0 mg/l concentration level and Vacuoles were seen in sinusoids in the highest concentration level (25.0 mg/l) in the present study (Fig. 2, D-F).

3.2.3 Heart: Treated fish's heart tissues showed minor changes at low concentrations 2.5 to 10.0 mg/l. In higher concentrations from 15.0 to 25.0 mg/l, the tissue exhibited remarkable changes including necrosis, destruction of blood vessels and fragmentation of muscle fibers of heart (Fig. 3, D-G).

3.2.4 Intestine: Intestine of *H. fossilis* was not significantly infected in lower concentrations of insecticide (2.5-15.0 mg/l). From 20.0 to 25.0 mg/l unit of concentrations muscularis swollen, disintegrated sub-mucosa, slightly damaged serosa and fused or ruptured villi were found at 96 hours observation period (Fig. 4, D&E).

3.2.5 Kidney: Kidney tubules and haematopoietic cells were found normal both control and insecticide treated concentration limits upto15.0 mg/l. From 20.0 to 25.0 mg/l Diazinon 60 EC promoted necrosis of tubular and haematopoietic cells of the kidney. Hemorrhage of renal tubules and necrotic changes of glomerulus were also observed in these concentrations level (Fig. 5, D&E).

3.3 *Anabas testudineus*

3.3.1 Gills: Lower concentrations of Diazinon had not remarkably affected to gills of *A. testudineus* and it has gone upto 10.0 mg/l. Curly form and bent of secondary lamellae were noticed from 15.0 to 25.0 mg/l concentrations. Lastly, the gill lamellae were found destructed in the present highest treated dose (25.0 mg/l) (Fig. 1, H-J).

3.3.2 Liver: Liver tissues were normal upto 10.0 mg/l concentrations of both control and insecticide treated fishes. Abnormal structure of blood vessels and sinusoids started from 15.0 to 25.0 mg/l concentrations level. Irregular blood vessels were found in 15.0 mg/l concentration unit and it turns to hemorrhagic in 20.0 mg/l level. Severe necrosis in liver tissues was observed in this concentration. Some vacuoles were seen in sinusoids in the highest exposed concentration (25.0 mg/l) of insecticide at 96 hours observation period (Fig. 2, G-I).

3.3.3 Heart: Apparently no changes were found in lower concentrations (2.5 to 10.0 mg/l) of Diazinon treated fishes compare with the control fishes while two major changes were noticed in the higher concentrations (15.0 to 25.0 mg/l) of insecticide treated fishes. In these concentrations level fragmented muscle fibers and destructed blood vessels were found in the heart tissues of the experimented fish samples (Fig. 3, H-J).

3.3.4 Intestine: Diazinon concentrations upto 15.0 mg/l had not affected to the intestine of *A. testudineus* alike to the control group samples. In 20.0 mg/l concentration, the submucosa layer was found disintegrated and vacuolated and in the highest treated dose of 25.0 mg/l level intestinal villi were attained in ruptured and fused condition (Fig. 4, F&G).

3.3.5 Kidney: Kidneys of the experimented fishes became infected by Diazinon at 15.0 mg/l concentration. At that stage, renal tubules were hemorrhagic while glomeruli were changed in 20.0 mg/l concentration level. Severe necrosis in tissues was observed in 25.0 mg/l dose at 96 hours of the observation period (Fig. 5, F&G).

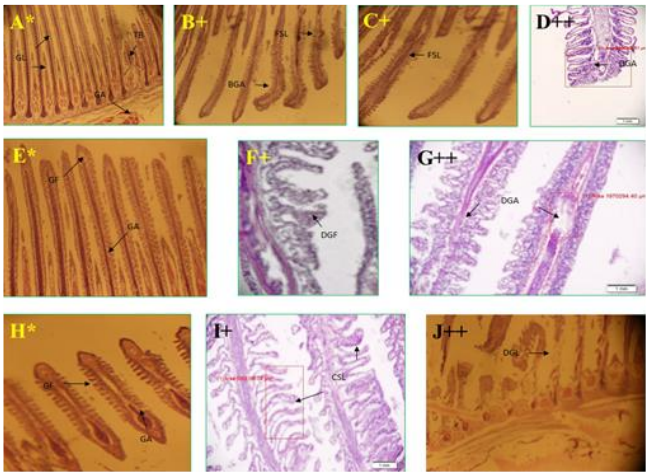

Fig 1: Histological photomicrograph of control and diazinon affected gill tissues of three fish species; A-D = *C. punctatus*; E-G = *H. fossilis*; H-J = *A. testudineus*; * = control, + = 20 mg/l; ++ = 25 mg/l concentration; GL= gill lamellae; GA= gill arch; TB= test buds; FSL= fusion of secondary lamellae; BGA= bend of gill arch; DGA= damage of gill arch; GF= gill filament; DGF= destruction of gill filament; CSL= curly of secondary lamellae; DGL= destruction of gill lamellae.

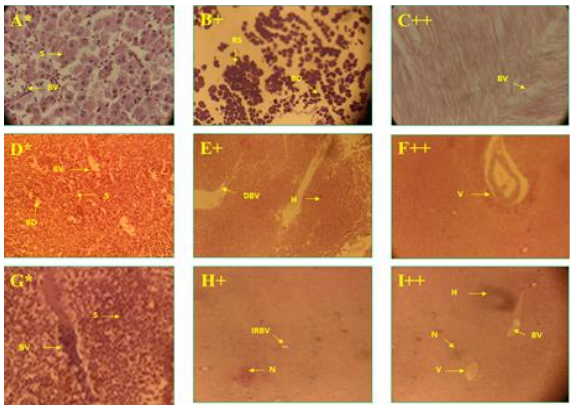

Fig 2: Histological photomicrograph of control and diazinon affected liver tissues of three fish species; A-C = *C. punctatus*; D-F = *H. fossilis*; G-I = *A. testudineus*; * = control; + = 20 mg/l; ++ = 25 mg/l concentration; S= sinusoids; BV= blood vessel; RS= rupture of sinusoids; BD= bile duct; DBV= destruction of bile duct; H= hemorrhage; V= vacuole; IRBV= irregular blood vessel; N= necrosis.

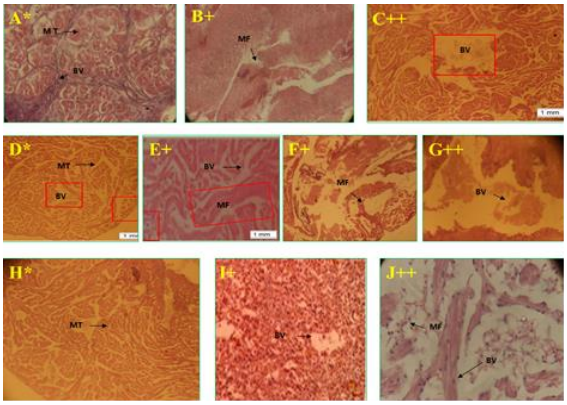

Fig 3: Histological photomicrograph of control and diazinon affected heart tissues of three fish species; A-C = *C. punctatus*; D-G = *H. fossilis*; H-J = *A. testudineus*; * = control; + = 20 mg/l; ++ = 25 mg/l concentration; MT= muscle tissue; BV= blood vessel; MF= muscle fiber fragmentation.

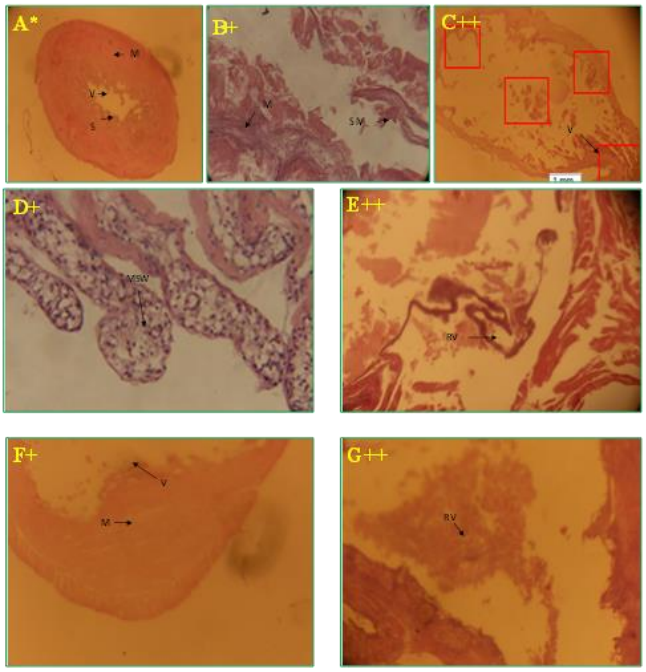

Fig 4: Histological photomicrograph of control and diazinon affected intestine tissues of three fish species; A-C = *C. punctatus*; D&E = *H. fossilis*; F&G = *A. testudineus*; * = control; + = 20 mg/l; ++ = 25 mg/l concentration; M= muscular tissue; S= sub mucosa; V= villi; MSW= muscularies swollen; RV= rupture of villi

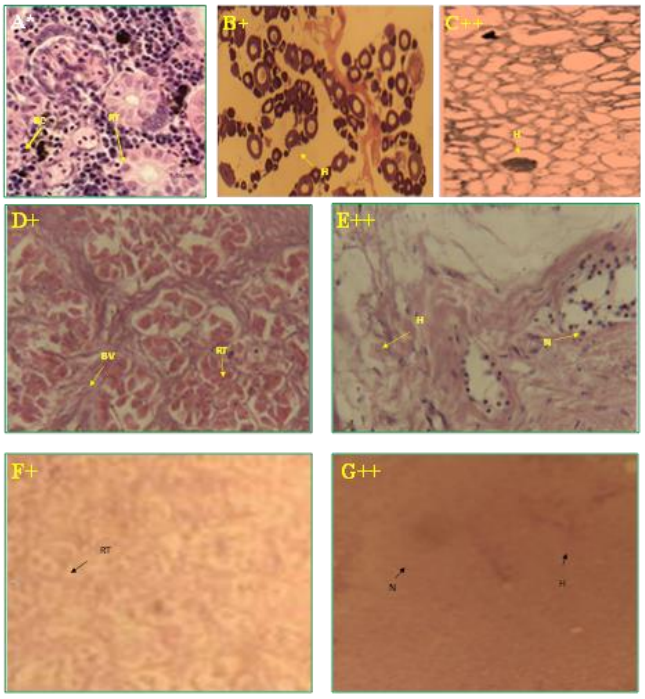

Fig 5: Histological photomicrograph of control and diazinon affected kidney tissues of three fish species; A-C = *C. punctatus;* D&E = *H. fossilis*; F&G = *A. testudineus*; * = control; + = 20 mg/l; ++ = 25 mg/l concentration; BC= bauman's capsule; RT= renal tubule; BV= blood vessel; G= glomerulus; H= hemorrhage; N= necrosis.

4. Discussion

Histopathological observation of different tissues of treated fish species is work as a catalyst for toxicological studies and monitoring of water quality of the aquatic ecosystem. Structural changes of tissues in the experimented fishes exposed to different concentrations of insecticides play a significance role as of active response to the organisms that facilitate knowledge of the nature of toxicants [31]. Major and rapid changes of tissues depend on the concentrations of insecticides and the time duration that fishes are exposed to the toxicants [32]. Some histopathological studies evidences such as, liver tissue lesions were found in freshwater fish *Cirrhinus mrigala* [33], common carp *Cyprinus carpio* [34] after 10 and 30 days treated to sub-lethal concentrations of Dichlorvos and Diazinon respectively. Histopathological alterations of different tissues in Diazinon treated fishes have also observed from the study of Deltamethrin treated fishes by Cengiz [35], Cengiz and Unlu [36], Fenitrothion treated studies by Benli and Ozkul [37, 38]. The lesions of different sophisticated organs *i.e.* gills, liver, heart, kidney and digestive truck of various insecticides treated fishes disturbed homeostasis that leads to physiological disorders and consequently dead of these fishes. The results of the present study revealed that Diazinon severely affected the gills of the three treated (*C. punctatus*, *H. fossilis* and *A. testudineus*) fish species where curl and bent of secondary lamellae, lamellar fusion and gill filaments and gill arches were destructed in different concentrations. Similar results reported from other studies. Mallatt [39] and McKim and Erickson [40] showed that gills were the main target tissue induced by Diazinon that used as the main route of arrival pesticides. In fish, gills are regarded as the vital organ, work as their respiratory osmoregulatory and secretory functions. Irregular and decrease of respiration are the early symptoms of insecticide toxicity [35] that dominate the physiological functions and may cause the death of the fish. Khosrava-Katuli *et al.* [41] studied the Diazinon effects on Caspian roach *Rutilus rutilus*. After 96 hours of exposure, they found shortening of secondary lamellae, oedema, destruction of epithelial lamella, epithelial lifting, curling of secondary lamellae, epithelial hyperplasia and lamellar fusion. It fully supported the result of the present study. Some other studies also noticed the similar effects of insecticides on gills of different species of fish. For example, histopathological effects of Deltamethrin on gills of Nile tilapia *Oreochromis niloticus* were studied by Yeldrim *et al.* [42], Atrazine induced of degenerative effects in the gill epithelium of *Gnathonemus petersii* by Alazemi *et al.* [43], Lambda-cyhalothrin effects on gills of *Cirrhinus mrigala* by Velmurugan *et al.* [44]. Dutta *et al.* [45] showed that due to Diazinon several changes occurred in Atlantic salmon *Salmosalar*. Epithelial layer lifting, hyperplasia and necrosis, shortening of the lamellae, frequent epithelial rupture, lamellar fusion, mucous cells hypertrophy, extensive fusion and clavate lamellae were found in the experimented fishes. Oruc and Usta [46] reported that when Diazinon exposed to common carp *C. carpio* lamellar fusion and epithelial membrane lifting of gills are being observed supporting the findings of the present study.

Histopathological analysis of the three species of Diazinon treated fish samples revealed of the liver tissue alterations, including irregular and destruction of blood vessels, hemorrhagic in the blood vessel and rapture and vacuoles in sinusoids in the present study. These results are incorporated into Cattaneo *et al.* [47] who reported that disorder in hepatocyte's cods, rupture in cell membrane, vacuoles in sinusoids of the liver tissues of silver catfish, *Rhamdi aquelen* for the effect of 2,4-dichlorophenoxiacetic acid. It also followed the results of Cengiz and Unlu [36], Mishra and Mohanty [48] and Vinodhini and Narayanan [49]. They reported that hypertrophy of hepatocytes, circulatory disturbances, focal necrosis, narrowing of sinusoids were seen in *Gambusia affinis* and *C. punctatus* and *C. carpio* exposed to

deltamethrin and heavy metal. The present study also followed the finding of Matos *et al*. [50] and Sepici-Dincel *et al*. [51]. They notice the similar histopathological changes in liver tissues of *O. niloticus* and *C. carpio* exposed to sub-lethal concentrations of carbaryl and cyfluthrinto the experimented fishes.

Different concentrations of Diazinon severely affected the kidney of the three species of treated fish samples. Hemorrhage in renal tubules, glomerular changes and necrosis in glomerulus were observed in the treated fishes. Similar results were found from the studies of Glover *et al*. [52]. They exposed endosulfan on Atlantic salmon (*Salmo salar*) at a concentration range from 4 to 710 µg kg^{-1} for 35 days duration, where they found irregular structure and malfunction of the kidney from low concentration to high degree of insecticide formulation. Banaee [31] reviewed in fish exposed to 0.1 mg/l diazinon and noticed that glomerular disorientation, urinary tubular dilation and cloudy swelling of kidney tissues while in case of 0.2 mg/l concentration level histopathological damaged *i.e.* degeneration in the epithelial cells, necrosis in the hematopoietic tissue, glomerular degeneration, vacuoles in epithelial cell's cytoplasm were described. In support with the result of the present study, Boran *et al*. [53] evaluated the acute toxicity of maneb and carbaryls on juvenile rainbow trout, *Oncorhynchus mykiss*. They found lamellar edema, deviation of epithelium from lamellae, the fusion of lamellae, necrosis in epithelial cell of the treated fishes were infected by the insecticide. They noticed both insecticides were almost similar in histopathological tissue lesions in the treated fish species and the major affected organs were gills, trunk kidney and liver.

Rahman *et al*. [54] experimented with the effect of Diazinon 60 EC on *A. testudineus*, *C. punctatus* and *Barbodes gonionotus* where they found degenerated kidney tubules, necrosis and hemorrhage in kidney tissues. Rand and Petrocelli [55] noticed necrotic tubules, pyknosis and karyorrhexis in kidney tissues when 100 ppm Amitrole was exposed to Salmon for 144 hours. Similar findings were reported by Mishra and Srivastava [56] exposed to Trichlorophon on Indian catfish. Kabir and Begum [57] found cytoplasmic degeneration, pyknosis in liver tissues, vacuoles in hepatic cells, rupture in hepatic blood vessels of *H. fossilis* when Diazinon 5, 10 and 20 ppm concentrations exposed for 25 days, supporting the findings of present experiment. Dutta *et al*. [58] reported that Diazinon severely affected the kidney and spleen of bluegill sunfish, *Lepomis macrochirus*. In the experiment, Anees [59] showed that gut wall of freshwater teleost *C. punctatus* was seriously damaged by the sub-lethal toxicity of Diazinon at different exposure periods followed the present findings.

Al-Otaibe *et al*. [27] reported necrosis in hepatocytes, bleeding in hepatic blood vessels, hypertrophy in glomerulus, bleeding in kidneys, fusion and degenerated secondary lamellae and epithelial hyperplasia in gills were obtained from the Diazinon treated fishes. It supported the present results. Ayoola and Ajani [60] also reported that cypermethrin occurred pathological changes on gills, liver and kidneys of catfish, founding with tubular fusion, glomerular condensation in the experimented fishes. Ikele *et al*. [61] registered pyknotic nucleus, fusion or destruction of hepatic tubules, nuclear condensation of the kidney of *Clarias gariepinus* treated with diethyl phthalate in various concentrations supporting the present results.

5. Conclusions

The results of the study concluded that even a small amount of pesticide (Diazinon) presence in fresh water reservoirs causes harmful effects on fish physiology and subsequently make death of the fish species. So, from the pesticides standpoint, it should take necessary precautions to applied pesticides in natural environment or use of pesticide in minimum quantity to protect the aquatic creatures. Therefore, further investigations required to select the optimum concentrations of pesticides as well as histopathological changes in other organs of the experimented fish species.

6. Acknowledgement

This research work is a part of first author's PhD thesis.

7. Conflict of Interest

There is no conflict of interest between authors.

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