Fenitrothion mediated acute histopathological changes in gill and liver of *Oreochromis niloticus* and *Ctenopharyngodon idella*

Ayesha Riaz, Aroosa Shah and Nabila Farah

Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan

ABSTRACT

The present study was conducted to find the histopathological variations in fingerlings of *Oreochromis niloticus* and *Ctenopharyngodon idella* after exposure to different test concentrations of fenitrothion for a period of 96 hour. The whole acute toxicity trials was carried out at constant water temperature 25°C, hardness (230 mg/L)and pH (7.5). Fishes were divided into four groups control group I, treated group II, III and IV. The treated groups were induced with sub-lethal concentrations of 3mg/L (3/10), 4mg/L (5/10) and 5mg/L (9/10) of fenitrothion concentrations for 96 hours. Sampling of liver and gills were done at intervals of 24, 48, 72 and 96 for histopathological examination. Histological examination of the liver of *Oreochromis niloticus* and *Ctenopharyngodon idella* revealed congested sinusoids and central veins. The hepatocytes showed vacuolar degeneration. No evidence of granuloma or malignancy was seen. In addition histological examination of the gills revealed presence of lamellar epithelium hyperplasia, lamellar fusion, telangiectasis, vacuolations and lamellar blood congestion. Inflammatory cells infiltration with a minimal congestion in primary lamellae was observed. After the exposure, morphological changes appear including fusion of fins, necrosis and lesion on skin. In conclusion Fenitrothion is highly toxic and cause histopathological changes in liver and gills of both fishes i.e *Oreochromis niloticus* and *Ctenopharyngodon idella*.

Key words: Fenitrothion, *Oreochromis niloticus*, *Ctenopharyngodon idella*, Gills, Liver, Histopathology.

INTRODUCTION

Environmental contamination has always been a source of harmful (Ozkara and Bagozzi, 2021). Toxic organic pollutants include a wide range of agro-chemicals such as insecticides or pesticides. As a result of being exposed to pesticide contaminated water, fish and other aquatic biota have suffered, and face a substantially greater risk of death (Reza et al. 2022). Fish are extensively employed as efficient models of water pollution because they absorb toxins and exhibit biochemically, cellularly, physiologically, and histologically diverse responses, making it a great biomarker with broad applicability in environmental monitoring (Guedes et al. 2020; Marinowic et al. 2021). Fish mortality have been connected to unsuitable environmental factors such as insufficient oxygen availability, low water quality, Pesticides dissolved in water or ingested in feed can cause acute or chronic exposure (Goessens et al. 2022).

Toxicological effects of insecticides on fish varies according to pesticide category, with insecticides being the most harmful (Sabra and Mehana, 2015). Pesticides' ability to remain in the environment and accumulate in fish organs may cause pathological changes in these tissues, resulting in stress and fish mortality (Arcury et al.2016). Several effects of

organophosphate pesticides on the tissues of various fish species, such as *Oreochromis niloticus* (Abdul et al. 2020). Farmers faced a challenge while using this insecticide since the pesticide is particularly hazardous to freshwater fish in terms of behavioural impacts and hispathological results. As farmers expanded their output, the potential consequences of pesticide on freshwater fish and the environment may become significant, because the amount of water polluted is directly proportional to the amount of insecticide utilised (Reitsma et al. 2017). Organophosphate insecticides alter the histoarchitecture of the liver, brain, eyes and gills among other things, causing malfunction in several fish species (Topal et al. 2014; Lakshmaiah, 2017; Zahran et al. 2018).

Fenitrothion is extensively used insecticide (Itoh et al. 2018). Fenitrothion when used agriculture to increase crop yield agriculture reaches direct spray, runoff, leaching, dumping and washing of containers and equipment in water all have an impact on aquatic habitats. When fenitrothion enters water bodies and exceeds the threshold level, it may damage non target aquatic animals of various trophic levels (Sumon, et al. 2018). Because of the potential for toxicity, compositions containing fenitrothion as an active component should be labelled with the term "caution" (Shahjahan et al. 2021).

Histopathological biomarkers are considered reliable in toxicological studies that are aimed to assess the relationship between exposure of a toxicant such as pesticide and damage to variety of tissues playing important roles in excretory, respiratory and metabolic activities (Franzellitti et al. 2014). Alterations caused in their shape, limiting the performance and endangering fish's survival, Hence assessing the gill morphology is critical for the monitoring quality of the aquatic environment (Strzyzewska et al. 2016).

Histopathological investigations of liver and gills tissues have been discovered as valuable methods for understanding the acute toxic effects of OP pesticides on grass carp, and have been recognised as key indicators of physiological stress generated by any anthropogenic stressor (Kamal et al. 2016; Awual et al. 2019). Evaluate the behavioural changes and histological tests of the liver and gills of sublethal doses of acute fenitrothion exposure (Coward et al. 2019). There is a comprehensive grasp of the organophosphate fenitrothion's affect on Nile tilapia, as well as its harmful effects on liver and gills tissues. Organophosphate insecticides are widely used and have been shown to have toxic effects on fish (Pucher et al. 2013).

Keeping in view the above mentioned facts this study is planned to investigate the fenitrothion mediated acute histopathological alterations in liver and gills of *Oreochromis niloticus* and *Ctenopharyngodon idella*. Despite the fact that *Ctenopharyngodon idella* is commercially significant, precise selective breeding procedures are still lacking. Most fish farms use fish from many sources to produce progeny without keeping extensive records of genetic and performance data. Further genetic improvement of *Ctenopharyngodon idella* necessitates extensive understanding of its genetic status, as this information is critical for the effective design of breeding programes and the successful management of these populations (Jaramillo et al. 2013).

MATERIALS AND METHODS

STUDY DESIGN

Juveniles of *Oreochromis niloticus* and *Ctenopharyngodon idella* were obtained from the fish seed hatchery Manawan Lahore and tranported to the fish laboratory at Department of the Zoology, The University of Lahore in plastic containers well equipped with oxygen. Fishes were initially acclimatized for one week in open tanks under laboratory conditions with proper

aerations system and flourescent light. Healthy juveniles of both fishes species having uniform length (12.7-15.24 cm) and average weight (45-50g) mesurements were screened out and Fishes were randomly divided into batches of ten and glass aquaria were washed prior to the commencement of toxicity trials. A known quantity of liquid fenitrothion were added to volumetric flask and mixed well with organic solvent like acetone to prepare the stock solution.

PREPARATION STOCK SOLUTION OF FENITROTHION

The stock solution of fenitrothion was prepared by dissolving 1mg of the fenitrothion in appropriate 100mL of acetone. Fish were randomly selected from stock and exposed to different concentrations of fenitrothion (3mgL^{-1}, 4mgL^{-1}, 5mgL^{-1}) and mortality of fish was recorded at 24, 48, 72, 96 hours of test fish (Ehsan et al. 2014).

ACUTE TOXICITY EXAMINATION

Glass aquaria were filled with clean water approximately 3/4 of their whole carrying capacity and each batch of ten fishes (in triplicates) were released in separate aquarium and exposed to different test concentrations of fenitrothion. Each group consists of two replicas, each having ten fishes per aquarium.

Group I was treated as control and was raised in pesticide-free tap water. Fish from group II, III and IV were exposed to the mentioned acute sub lethal concentrations of fenitrothion respectively for 96 hrs. Renewal of pesticides doses was done every 24 hours during the whole experimental time period of 96 hours by completely changing the already existing water in each aquarium. The whole acute toxicity trials were carried out at consistent 25°C water temperature, 7.5 pH, and hardness (230 mg/L). Careful examination of test aquaria was done at equal intervals of 24, 48, 72 and 96 hours and if no opercular breathing is observed in any fish it was considered dead and immediately removed from the aquaria. The number of dead fishes during 96 hour experiment duration was counted that would be the end point of these acute toxicity tests. The 96 hr LC_{50} and LC_{100} of fenitrothion for *Oreochromis niloticus* and *Ctenopharyngodon idella* were calculated by analyzing the fish mortality data (Reichelt-Brushett and Michalek-Wagner 2005).

HISTOPATHOLOGICAL AND BEHAVIORAL EXAMINATION

The dead fishes collected after exposure to fenitrothion were dissected to remove liver and gills for histological examinations. These tissues were fixed with 10% formalin, dehydrated by passing through various alcoholic grades, washed with clearing solution and embedded in paraffin wax (molten). Fine sectioning of embedded tissue was performed by using a microtome, hemotoxylin and eosin staining. Under a high resolution microscope fitted with a digital camera, stained slides were observed and photography of fenitrothion exposed tissues with photomicroscope were carried out. The histopathological observations were classified into 5 categories ranging from no alteraions (-), mild alterations (+, < 10%), moderate alterations (++, 10-50%) and severe alterations (+++, > 50%). During the whole experiment various behavioural changes following exposure to fenitrothion were observed that was include erratic swimming, mucus discharge and swollen abdomen (Hinto *et al*., 1992).

RESULTS

Current study was design to evaluated the effect of insecticide,fenitrothion on liver and gills of *Oreochromis niloticus* and *Ctenopharyngodon idella.* A total number of 40 fingerlings of *Oreochromis niloticus*, were purchased from Manga Mandi

Fish hatchery. Four groups of fish were formed, group I was kept as a control group while group II, III and IV were treated with different concentration of fenitrothion 3mg/L, 4mg/L and 5mg/L respectively. Histopathological changes in liver and gills was observed after exposure with fenitrothion at 24, 48, 72 and 96 hours. Control mortality was zero results are shown.

OREOCHROMIS NILOTICUS

CONTROL GROUP GILLS

Histological analysis of control group gills have normal filaments. There was no malignancy, granuloma or any inflammation found.

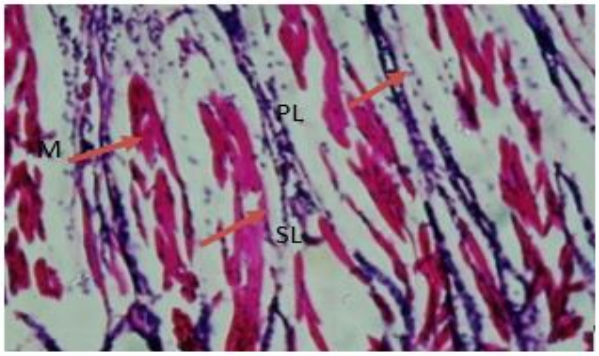

Fig:1 Histology of gills of control group of *Oreochromis niloticus,* M: Mucous cell, PL: Primary lamella, SL: Secondary lamella, P: pillar cell, E: epithelial cell, VS: sinus vein.

GILLS TREATED GROUP Histological appearance of the gills tissue after treatment of 5mg/L of fenitrothion

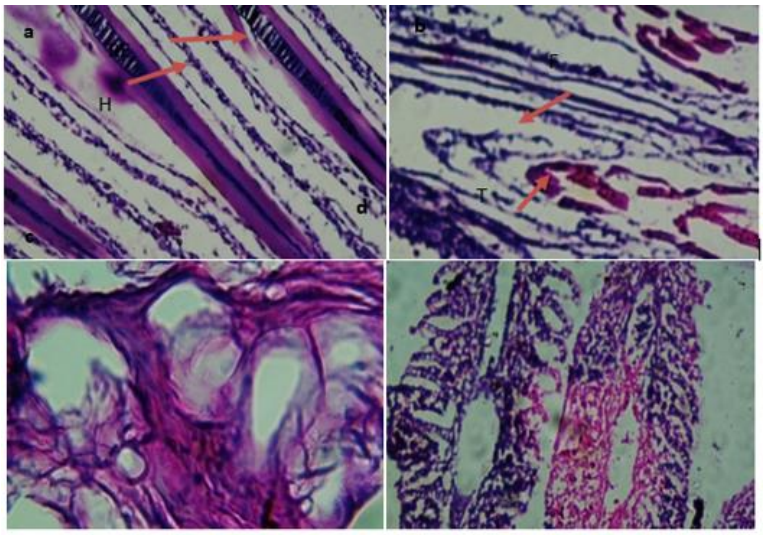

Fig2: Histology of gills of *Oreochromis niloticus* treated groups with fenitrothion at 5mg /L of dose (H and E stain, 10x). (a) after 24 h exposure respiratory epithelium damage and dearrange (b) after 48 h exposure telangiectasis (c) after 72 h exposure was occured hyperplasia (d) after 96 h exposure severe fusion. RE: respiratory epithelium, T: telangectiasis, H: hyperplasia, F: fusion.

LIVER CONTROL GROUP Histological analysis of control group liver has normal hapatic structure which show normal sinusoids, hepatocytes, and central vein. No inflammation diseased malignancy were observed.

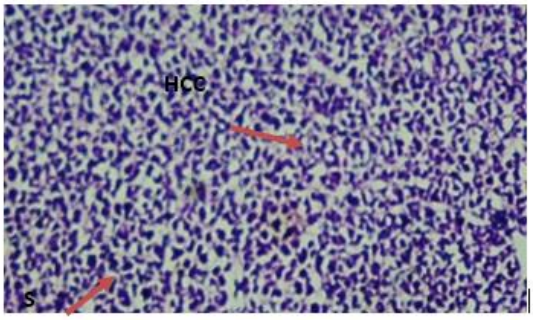

Fig 3: Histology of control group liver of *Oreochromis niloticus* (H and Estain, 10x).HC: hepatocytes, S: sinusoids, HN: hepatic necrosis, CV: central vein.

LIVER TREATED GROUP

Histological analysis of liver after treatment of 5mg/L fenitrothion showed central vein raptured, necrotic hepatocytes , inflammation in hepatocytes, dilution of sinusoids, vacuolar degeneration appear.

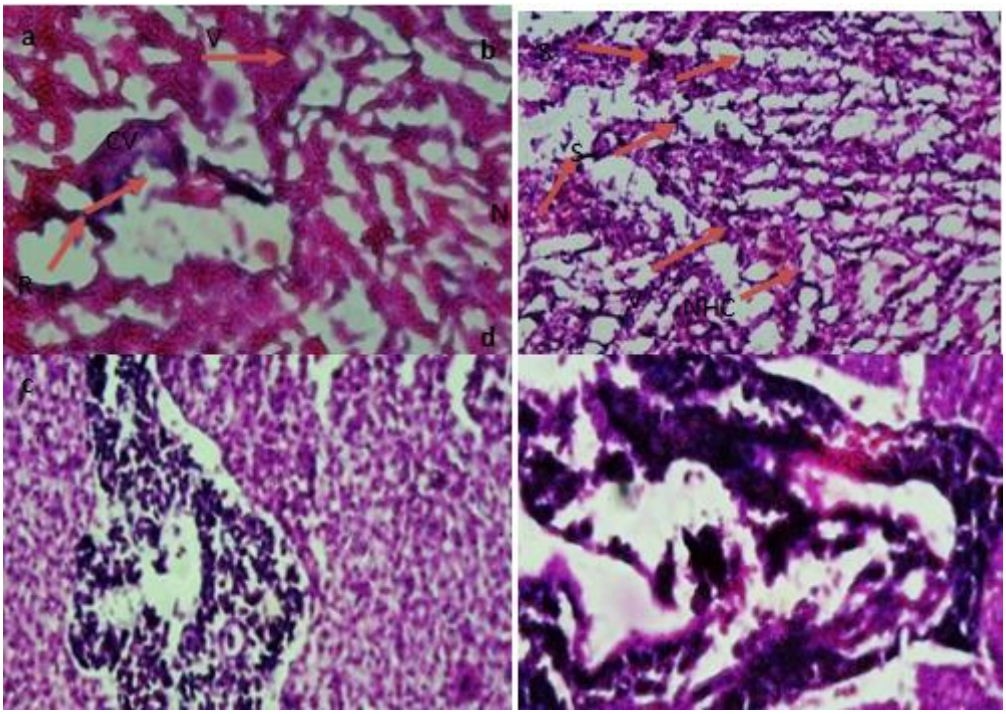

Fig4: Histology of liver treated group of *Oreochromis niloticus*(H and E stain, 10x).CV: central vein, N: necrosis, S: sinusoid, NH: necrotic hepatocytes, V: vacuolization, R: raptured central vein, n: nucleus. (a) after 24 h exposure central vein raptured (b) after 48 h exposure necrotic hepatocytes occured necrosis and damage sinusoid (c) after 72 h exposure severe alterations and raptured central veins (d) after 96 h exposure sinusoidal alterations severe in vacuolization alterations occurred.

Ctenopharyngodon Idella

Gills Control Group

Histological examination of gills reveals normal looking gills filaments. No evidence of any inflammation disease, granuloma or malignancy seen in gills of control group of fish.

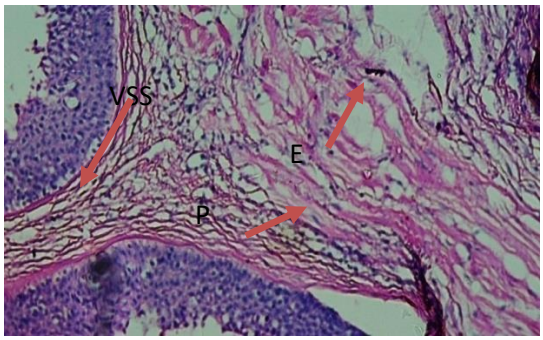

Fig5: Control group gills of *Ctenopharyngodon idella* (H and E stain, 10x). M: Mucous cells, PL: Primary lamellae, SL: Secondary lamellae, P: pillar cells, E: epithelial cell, VS : sinus vein.

Gills Treated Group

Histological appearance of the gills tissue after treatment of 5mg/L of fenitrothion.

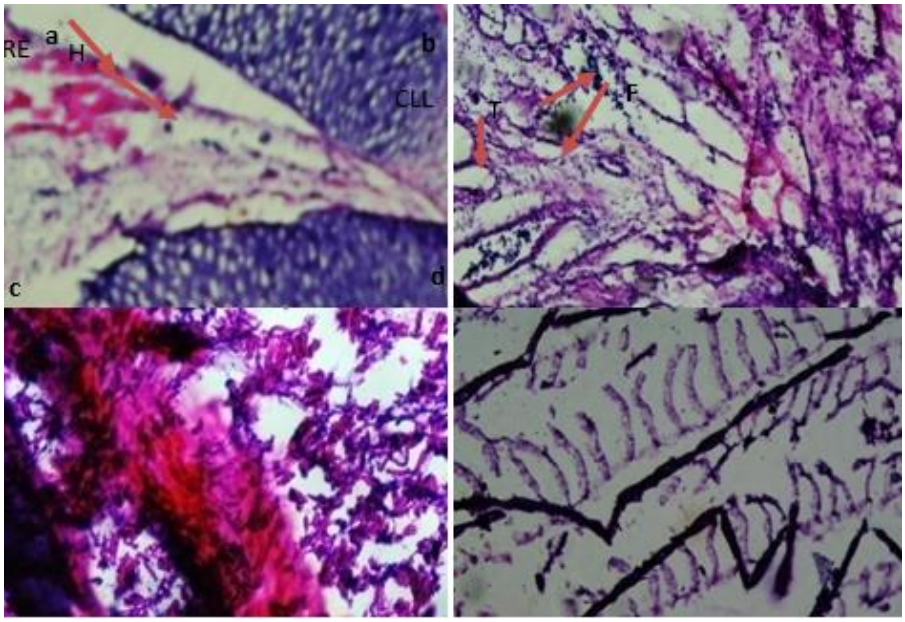

Fig 6: Histology of gills treated group *Ctenopharyngodon idella* (H and E stain, 10x). RE: respiratory epithelium, T: telangectiasis, H: hyperplasia, F: fusion, CL: central line.(a) after 24 h exposure damage respiratory epitheium (b) after 48 h exposure severe telangiectasis (c) after 72 h exposure hyperplasia occured (d) after 96 h exposure severe fusion occured and central line damage

LIVER CONTROL GROUP

Histological examination of liver tissue reveals normal hepatic architecture, normal size sinusoids and normal morphology of hepatocytes and hydrolysis. No evidence of any inflammation disease, granuloma or malignancy seen in liver of control group of fish.

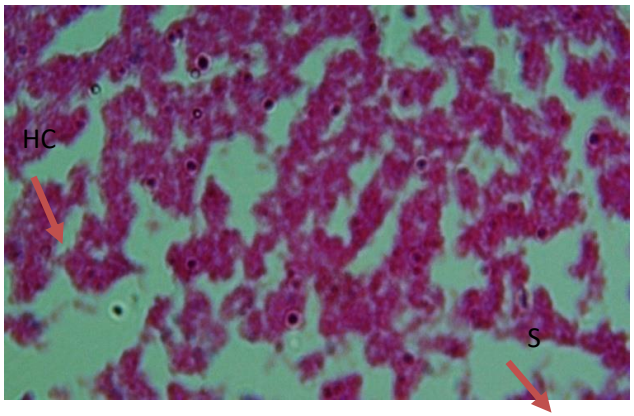

Fig 7: Histology of liver Control group of *Ctenopharyngodon idella* (H and E stain, 10x). HC: hepatocytes, S: sinusoids, HN: hepatic necrosis, CV: central vein.

LIVER TREATED GROUP

Histological appearance of the gills tissue after dose 5mg/L of fenitrothion. Histological analysis of liver showed central vein raptured, necrotic hepatocytes dissociation of hepatocytes, congestion of liver, inflammation in hepatocytes, dilution of sinusoids, vacuolar degeneration and giant cell formation.

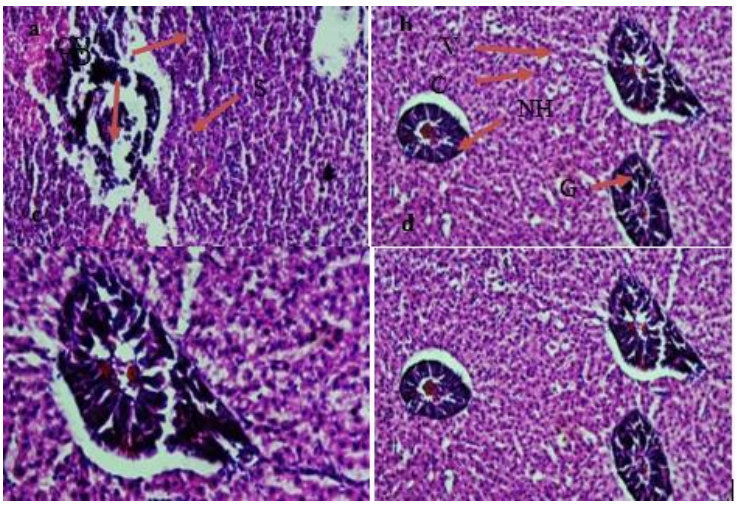

Fig8: Histology of liver treated group of *Ctenopharyngodon idella* (H and E stain, 10x).CV: central vein, NH: necrotic hepatocytes, C: congestion of liver, VD: vacuolar degeneration, S: sinusoid, V: vacuolation, G: giant cell. (a) after 24 h exposure central vein raptured (b) after 48 h exposure damage necrotic hepatocytes (c) after 72 h exposure vacuolar degeneration (d) after 96 h exposure vacuolation and giant cell formation.

DISCUSSION

The current study was planned to investigated the effect of fenitrothion pesticide in different organs of *Oreochromis niloticus* and *Ctenopharyngodon idella*. Fishes were separated in to four groups, each group contain 10 fishes. The group I was kept as control group and be placed in normal tap water without given any treatment. The group II, III and IV were exposed to three concentrations of fenitrothion (3mg/L, 4mg/L and 5mg/L) in 96 hours for LC_{50} of Nile tilapia was 3.764 mg/L, and Grass carp was 3.821 mg/L. Mortality in each group was observed throughout the experiment. After 96 hours, histopathological alterations were examined in gills and liver of Nile tilapia and Grass carp caused by fenitrothion.

In the present study results regarding histopathology of gills indicated that, during the trial period in control group no pathological changes were observed in gills of Nile tilapia. The gills showed normal morphology of mucous cells, pillar cells, epithelial cells, primary and secondary lamellae. No evidence of degeneration of lamellar epithelium, hyperplasia, necrotic changes, vacuolated lamellae, fusion of lamellae and hypertrophy of secondary lamellae were observed. The histological examination of fenitrothion treated Nile tilapia groups were showed that at low dose (3mg/L) gills tissue show mild alterations in uplifting of epithelium, clubbing of secondary lamellae and epithelial cells of gills tissue as compared to control group, and at dose (4mg/L) show moderate alterations in central line, pillar cells and primary lamellae at high dose (5mg/L) severe toxicity in gills tissue was observed in respiratory epithelium, telangiectasis, fusion and hyperplasia.

Our results are consistent with the hispathological work done by Marigoudar et al. (2018) on two fishes that showed highest dose of pesticide elicited changes in gill architecture of fish which included the hyperplasia, lifting and fusion of epithelium in secondary lamellae, degeneration cells in primary lamellae and necrosis. Similarly, at higher pesticide concentration, degeneration of cells and necrosis in primary lamellae, as well as lifting and fusion of epithelium in secondary lamellae were observed in *C. chanos* fingerlings. Furthermore, the gills showed varrying degrees of epithelial hypertrophy, telangiectasis with epithelial separation from basement mmbranes, general necrosis, and epithelial desquamation (Ly *et* al. 2012).

In the present study the liver tissue no changes in control group hepatocytes observed. Whereas, in group II exposed to 3mg/L dose of fenitrothion showed vacuolar degeneration. The histological examination of fenitrothion treated groups were showed that at low dose (3mg/L) liver tissue show mild alteration in sinusoids and hepatic necrosis as compared to control group, and second dose (4mg/L) show moderate alterations in hepatocytes, sinusoids, vacuolizations and necrosis at high dose (5mg/L) severe toxicity in liver tissue was observed as necrotic hepatocytes and raptured central vein of liver.

In the present study liver showed hydropic degenerations and pyknosis, liver being the main organ of several metabolic pathways, toxic effects of chemical usually appear in primarily in the liver. This, in turn provides important data on the chemical's toxicity and mode of action. Many organic compound induce toxicopathic lesions in the liver of fish species (Hinto et al. 1992). Similarly, our findings showed that acute pathological lesions in the liver such as hydropic degeneration, vacuolization, pyknotic nuclei and fatty infiltration of Nille tilapia.

The results indicated that toxicity increase in gills and liver of Nile tilapia tissues as dose increase. Similar findings were observed in deltamethrin exposed *Aphanius dispar* freshwater fish that show vacuolization, elevation of the lamellar

epithelium and secondary lamellae fusion in gills (Al-Ghanbousi et al. 2012). Histopathological abnormalties in fish gills were frequently associated with toxic components cause regressive changes (Vydrov and Van 2009).

Zeid and Khalil, (2014) stated that the mortality rate of Nile tilapia exposed to acute exposure of fenitrothion, was the toxic effect of FNT in short exposure (3/10, 5/10, 9/10 for 96 hrs LC_{50}). Similarly evaluation the toxicity of organophosphate insecticides, fenitrothion (FNT), on *Oreochromis niloticus*fingerlings by determining its 96 h median lethal concentration (LC) showed damaging the effect in gills and liver tissues. Acute low and high sublethal fenitrothion exposure, showed histological changes occurred in the gills and liver. Mortality was studied in fenitrothion exposed fish after 96 hours at doses ranging from 0 to 7mg/L. The 96-hour LC was calculated to be 4.7 mg/L. Depending on the dosage level of fenitrothion, histological changes in gills and liver were seen

In the present study results of histopathology of gills tissue of *Ctenopharyngodon idella* indicated that, during the trial period in control group no pathological alterations were observed in gills of Grass carp. Gills showed normal morphology of mucous cells, pillar cells, epithelial cells, and sinus vein of Grass carp. No evidence of degeneration of gills tissue was observed. The histological examination of fenitrothion treated Grass carp groups showed mild alterations in gills filaments, vacuolization and epithelial cells of gills at low dose (3mg/L) as compared to control group, and second dose (4mg/L) show moderate alterations in central line, pillar cells and primary lamellae, at high dose (5mg/L) severe toxicity in gills tissue was observed in respiratory epithelium, telangiectasis, fusion and hyperplasia.

According to Strehl et al. (2014), the histological alterations identified in the fish gills structure was an adaptive response to the pesticide impact. The histological alterations identified were due to exposure of pesticide that cause notably proliferative changes due to toxicant penetration. As a result, the organ's whole physiological condition would be effected by exposure to various doses of pesticide (Georgieva et al. 2014).

In the present study, The liver tissue of Grass carp show no chnages in control group hepatic necrosis and sinusoid are normal in their morphology. The histological examination of fenitrothion treated groups were showed exposure to low dose (3mg/L), liver tissue show mild alteration in dissociation hepatocytes and sinusoids as compared to control group, and the dose (4mg/L) show moderate alterations in periportal vacuolation, necrotic hepatocytes and sinusoid of liver tissue at high dose (5mg/L) severe toxicity in liver tissue was observed in congestion of liver, vacuolar degenration, rcentral vein and vacuolation of liver tissue of Grass carp. The presence of histopathological abnormalties in the liver is frequent and the structural changes might impede the circulatory and digestive systems FNT is classified as toxic material, which means it mostly effects the gills, liver and kidney (Boran et al. 2012).

CONCLUSION

After exposure to fenitrothion both fishes show prominent variation in treated groups as compare to control group. Histological study of organs. i.e gills and liver of *Oreochromis niloticus* and *Ctenopharyngodon idella* showed prominent variations in these organs and showed toxic effect. It is further recommended to ban the use of fenitrothion in Agriculture practices and find alternative novel biological insecticides.

LITERATURE CITED

Abdul kareem, S. I., Ajetunmobi, M. O., and Yekini, Y. O. (2020). Histopathological Alterations in some Tissues of *Heteroclarias* Juveniles Exposed to Lethal and Sub-lethal Concentrations of Chlorpyrifos and Ameliorative Potentials of Vitamin E to the Toxicity of the Pesticide. Tanz. J Agri Sci, 19(2), 67-77.

Al-Ghanbousi, R., Ba-Omar, T., and Victor, R. (2012). Effect of deltamethrin on the gills of *Aphaniusdispar*: a microscopic study. *Tissue and Cell*, 44(1), 7-14.

Arcury, T. A., Talton, J. W., Summers, P., Chen, H., Laurienti, P. J., and Quandt, S. A. (2016). Alcohol consumption and risk for dependence among male Latino migrant farmworkers compared to Latino nonfarmworkers in North Carolina. *Alcoholism:* Clin Exp Res. 40(2), 377-384.

Awual, M. R., Hasan, M. M., Islam, A., Rahman, M. M., Asiri, A. M., Khaleque, M. A., and Sheikh, M. C. (2019). Offering an innovative composited material for effective lead (II) monitoring and removal from polluted water. J Clean Prod. 231, 214-223.

Boran, A., Shawaheen, M., Khader, Y., Amarin, Z., and Hill Rice, V. (2012). Work-related stress among health professionals in northern Jordan. Occup Med. 62(2), 145-147.

Boran, A., Shawaheen, M., Khader, Y., Amarin, Z., and Hill Rice, V. (2012). Work-related stress among health professionals in northern Jordan. *Occupational Medicine*, 62(2), 145-147.

Coward, S., Clement, F., Benchimol, E. I., Bernstein, C. N., Avina-Zubieta, J. A., Bitton, A., and Kaplan, G. G. (2019). Past and future burden of inflammatory bowel diseases based on modeling of population-based data. *Gastroenterology*, 156(5), 1345-1353.

.Ehsan, S., Ali, S., Noureen, S., Mahmood, K., Farid, M., Ishaque, W., and Rizwan, M. (2014). Citric acid assisted phytoremediation of cadmium by *Brassica napus*. Ecotoxicol. Environ. Saf. 106, 164-172.

Franzellitti, S., Buratti, S., Capolupo, M., Du, B., Haddad, S. P., Chambliss, C. K., and Fabbri, E. (2014). An exploratory investigation of various modes of action and potential adverse outcomes of fluoxetine in marine mussels. Aquat Toxicol, 151(2), 14-26.

Goessens, T., De Baere, S., Deknock, A., De Troyer, N., Van Leeuwenberg, R., Martel, A., and Croubels, S. (2022). Agricultural contaminants in amphibian breeding ponds: Occurrence, risk and correlation with agricultural land use. Sci. Total Environ, 806, 150661.

Guedes, T. D. A., Moreira-de-Sousa, C., Lima, H. M. S., Grella, T. C., Socolowski, P. C., and Fontanetti, C. S. (2020). Cytoprotective and anti-apoptotic action of HSP70 stress protein in *Oreochromisniloticus* exposed to residual dilutions of insecticides with fipronil and ethiprole. J Environ Sci Health, 55(8), 687-693.

Hinto, J., Kangawa, K., Kozawa, H., Minamino, N., and Matsuo, H. (1992). Isolation of four novel tachykinins from frog (*Ranacatesbeiana*) brain and intestine. *Regulatory Peptides*, 19(2): 67-7

Itoh, H., Tago, K., Hayatsu, M., and Kikuchi, Y. (2018). Detoxifying symbiosis: microbe-mediated detoxification of phytotoxins and pesticides in insects. Nat. Prod. Rep, 35(5), 434-454.

Jaramillo, M. C., and Zhang, D. D. (2013). The emerging role of the Nrf2–Keap1 signaling pathway in cancer. Genes Dev, 27(20), 2179-2191.

Kamal, M. S., Razzak, S. A., and Hossain, M. M. (2016). Catalytic oxidation of volatile organic compounds (VOCs)–Areview. Atmos Environ, 140, 117-134.

Lakshmaiah, G. (2017). Brain histopathology of the fish *Cyprinuscarpio* exposed to lethal concentrations of an organophosphate insecticide phorate. Brain, 2(5), 668-672.

Ly, K. N., Xing, J., Klevens, R. M., Jiles, R. B., Ward, J. W., and Holmberg, S. D. (2012). The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. Ann. Intern. Med. 156(4), 271-278.

Marigoudar, S. R., Nagarjuna, A., Karthikeyan, P., Mohan, D., and Sharma, K. V. (2018). Comparative toxicity of chlorpyrifos: Sublethal effects on enzyme activities and histopathology of *Mugilcephalus* and *Chanoschanos*. Chemosphere, 211, 89-101.

Marinowic, D., Viola, F. S., Majolo, F., Zanirati, G. G., Azevedo, P. N., Zanatta, A., and Da Costa, J. C. (2021). Influence of Zika Virus in mechanisms related of cytotoxicity, cell adhesion, apoptosis and inflammatory markers on glioblastoma cells. Oncotarget, 9(13): 10995-11008.

Ozkara, B. Y., and Bagozzi, R. (2021). The use of event related potentials brain methods in the study of conscious and unconscious consumer decision making processes. J. Retail, 58, 102202.

Pucher, J., Steinbronn, S., Mayrhofer, R., Schad, I., El-Matbouli, M., and Focken, U. (2013). Improved sustainable aquaculture systems for small-scale farmers in northern Vietnam. In *Sustainable land use and rural development in southeast Asia:* Innovations and policies for mountainous areas. Springer, Berlin, Heidelberg.6, 281-317.

Reichelt-Brushett, A. J., and Michalek-Wagner, K. (2005). Effects of copper on the fertilization success of the soft coral, *Lobophytumcompactum*. Aquat. Toxicol, 74(3): 280-284.

Reitsma, M. B., Fullman, N., Ng, M., Salama, J. S., Abajobir, A., Abate, K. H., and Patton, G. C. (2017). Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease Study 2015. Lancet, 389(10082), 1885-1906.

Reza, A. M., Rakhi, S. F., Zhu, X., Tang, Y., and Qin, J. (2022). Visualising the Emerging Platform of Using Microalgae as a Sustainable Bio-Factory for Healthy Lipid Production through Biocompatible AIE Probes. *Biosensors*, 12(4), 208.

Sabra, F. S., and Mehana, E. S. E. D. (2015). Pesticides toxicity in fish with particular reference to insecticides. Asian J Agric Food Sci, 3(1), 2321-1571.

Shahjahan, M., Islam, S. M., Bablee, A. L., Siddik, M. A., and Fotedar, R. (2021). Sumithion usage in aquaculture: benefit or forfeit?. Rev Aquac, 13(4), 2092-2111.

Strehl, C., Fangradt, M., Fearon, U., Gaber, T., Buttgereit, F., and Veale, D. J. (2014). Hypoxia: how does the monocyte-macrophage system respond to changes in oxygen availability?. J.Leukoc Bio. 95(2), 233-241

Strzyzewska, E., Szarek, J., and Babinska, I. (2016). Morphologic evaluation of the gills as a tool in the diagnostics of pathological conditions in fish and pollution in the aquatic environment: a review. Vet Med, 61(3), 255-256.

Sumon, K. A., Ritika, A. K., Peeters, E. T., Rashid, H., Bosma, R. H., Rahman, M. S., and Van den Brink, P. J. (2018). Effects of imidacloprid on the ecology of sub-tropical freshwater microcosms. Environ Pollut, 236, 432-441.

Topal, A., Atamanalp, M., Oruc, E., Beydemir, S., Isık, A., and Demir, Y. (2014). In vivo changes in carbonic anhydrase activity and histopathology of gill and liver tissues after acute exposure to chlorpyrifos in rainbow trout. Arh Hig Rada Toksikol, 65(4), 377-385.

Zahran, E., Risha, E., Awadin, W., and Palic, D. (2018). Acute exposure to chlorpyrifos induces reversible changes in health parameters of Nile tilapia (*Oreochromisniloticus*). Aquat. Toxicol, 197(3), 47-59.

Zeid, E. H. A., and Khalil, A. S. A. (2014). Effects of acute fenitrothion insecticide exposure on DNA damage and oxidative stress biomarkers and health of Nile tilapia fingerlings, *Oreochromisniloticus*. World, 6(4), 361-370.