Research in

ISSN : P-2409-0603, E-2409-9325

AGRICULTURE, LIVESTOCK and FISHERIES

An Open Access Peer-Reviewed International Journal

Article Code: 415/2023/RALF
Article Type: Research Article

Res. Agric. Livest. Fish.
Vol. 10, No. 2, August 2023: 207-218.

Acute Effects of Malathion 57 EC Pesticides on The Histology, and Blood Cell Structure of Silver Barb (*Barbonymus gonionotus*) in The South-West Region of Bangladesh

Rabeya Akter*, Redwan Amin and Fatema Jannat Raina

Department of Fishery Biology and Genetics, Fisheries and Ocean Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh.

***Corresponding author:** Rabeya Akter; E-mail: rabeya.kau@gmail.com

ARTICLE INFO

Received
05 August, 2023

Revised
29 August, 2023

Accepted
30 August, 2023

Online
September, 2023

Key words:
Pesticide
Organophosphate
Malathion 57 EC
B. gonionotus
Haematology

ABSTRACT

Pesticides used in agriculture that wash off have a detrimental effect on freshwater fish in Bangladesh. The purpose of this research is to assess the effects of the organophosphate insecticide Malathion 57 EC on the silver barb (*Barbonymus gonionotus*). The effects of Malathion 57 EC at dosages of 0.001 and 0.005 ppm, respectively, were assessed using a histological bio-assay of the gill, liver, kidney, and RBC count of *B. gonionotus*. For *B. gonionotus*, the LC_{50} value of Malathion 57 EC was estimated to be 0.08 ppm. The notable alterations included gill clubbing, missing gill lamellae, hyperplasia, nuclear hypertrophy, vacuolation, glomerular enlargement, increasing the width of renal tubules, bleeding, necrosis, and pyknosis were found in the histological analysis. Large lymphocytes, dead cells, tear-shaped cells, a fusion of cells, binucleated cells, ghost cells, senile cells, and irregular cell shapes were among the abnormalities detected in peripheral nuclear erythrocytes. In *B. gonionotus*, the RBC count was considerably lower (P<0.01) at lower pesticide doses compared to higher ones. The outcome of the study shows that the organophosphorus pesticide has a negative impact on the histology and haematology of various organs in *B. gonionotus*.

To cite this article: Akter R., R. Amin and F. J. Raina, 2023. Acute effects of malathion 57 EC pesticides on the histology, and blood cell structure of Silver barb (*Barbonymus gonionotus*) in the South-west region of Bangladesh. Res. Agric. Livest. Fish. 10(2): 207-218.

DOI: https://doi.org/10.3329/ralf.v10i2.68784

INTRODUCTION

The world's population is expanding significantly each day. Food is in greater demand right now than it has ever been. Because of this, the agriculture sector is making great efforts to boost productivity. Pesticides are utilized. Pest control is essential to increasing output since pests can endanger human health, harm crops and food production, parasitize livestock, and cause nuisance. Pesticides are an important component of plant protection for Bangladeshi farmers. Without the application of pesticides, it will be difficult to cultivate crops of good quality, and the production will decrease by 30 to 40 percent (Hasanuzzoha, 2004). Pesticides found in aquatic ecosystems are primarily derived from agriculture. These agricultural pesticides pollute both surface and groundwater (Ray et al., 2012). Numerous pathways exist for pesticides to enter the atmosphere, but the common ways are spray drift and volatilization from soil or water. Pesticides or toxicants reach the aquatic environment from point sources including discharges by manufacturing plants, hazardous waste disposal sites, municipal garbage, water treatment plants, etc. as well as non-point sources including agricultural runoff from land, polluted groundwater, and bottom sediments, urban runoff, dredging sediment disposal, and atmospheric fallout (Hasan et al., 2008). The long-term effect of pesticides is the residue concentration (Higher or lower) found in animals in enclosed ponds, free-flowing creeks, and larger water bodies like lakes and estuaries (Naqvi and Cruz, 1973). Such pesticides have been pointed out as the catalyst of the gradual deterioration of aquatic ecosystems (Konar, 1975). Pesticides cause toxicity in numerous aquatic organisms, and fish are the most common (Ullah et al., 2014). Pesticides enter mainly through the gills or epithelial tissues, hence into the blood, and subsequently to different organs or systems. If the toxin reaches the fish's body, it can damage the organs, causing physiological and pathological problems (Ogundiran et al., 2007). The kidney, liver, gills, stomach, brain, muscles, and reproductive organs of fish are often affected by pesticide residue (Islam et al., 2019). Pesticides at high concentrations affect fish survival, growth, and reproduction (McKim and Benoit, 1975). Sublethal concentrations of pesticides in aquatic environments cause structural and functional changes in aquatic organisms, which is more common than mortality (Sancho et al., 2003).

Currently, the three main types of pesticides used to control pests are carbamates, organophosphorus, and chlorinated hydrocarbons. Since the elimination of organochlorine pesticides owing to their persistently negative effects, organophosphate (OP) insecticides have been selected as the most common insecticide in today's world to eradicate the crop of pests for enhanced output. An organophosphate pesticide known as malathion (O, O-dimethyl dithiophosphate of diethyl mercapto succinate) is effective against sucking and chewing insects on fruits and vegetables. Additionally, it works well against head and body lice, flies, household insects, and animal parasites (ectoparasites). Malathion harms fish in a variety of ways, even at low concentrations. For example, it alters fish development parameters, haematological traits, and swimming ability, and depletes various biochemical parameters (including glycogen, cholesterol, and total protein) (Huculeci et al., 2009). Toxicity tests can be used to determine the adverse effects of environmental contamination on aquatic species. Fish is an important protein source for humans; it is essential to investigate the hazardous effects of pesticides on fish and must be monitored, though, as aquatic species are extremely hazardous to them. Toxins cause death when present in fatal quantities, but when present in sublethal concentrations, they make animals, particularly fish, and other heterotrophic cold-blooded organisms, incapable of maintaining their life. When respiration, excretion, or detoxification processes are hampered by environmental contaminants, histopathological indicators can be very useful in determining the structure of major organs such as kidneys, liver, and gills (Gernhofer et al., 2001). Due to the presence of even low concentrations changes and in aquatic toxicology, any variations in the blood serve as biomarkers (Maurya et al., 2019). The physiological status of fish and other vertebrates has also been evaluated using hematological parameters as health indicators (Chandra and Chandra, 2013). The hematologic index can be used effectively to monitor the response of fishes to various toxicants reflecting the ecological status of the habitat and is a common method to determine the sub-lethal effects of the pollutant (Rakhi et al., 2013). Hematologic parameters in fish are influenced by factors like sex, age, size, reproductive stage, health, and external factors like seasonal dynamics, water temperature, ecological sustainability, food, and strain. As a result, blood parameters are used as indexes for predicting organisms' health and toxicological signs, particularly fishes. As fish is so directly associated with the aquatic environment, hematological research is crucial for toxicological research, environmental monitoring of fish and their health complications during culture. The aim of this study is to understand the effects of commonly used OPs, Malathion 57 EC on selected carp fish species by assessing their morphological changes, histopathological abnormalities in different organs, and the changes occurring in blood cells. The study describes the effects of Malathion and observed for different exposure periods with the parameters of mortality, toxicity, and physical behaviors.

MATERIALS AND METHODS

Materials collection

Malathion 57 EC, an organophosphate pesticide, was selected because it is widely used in agricultural cropland in the Dumuria area, Khulna in Bangladesh. It was collected from an authorized dealer in Khulna, Bangladesh. Fries of *Barbonymus gonionotus* were collected from the selected coastal area and were acclimated in laboratory conditions prior to the experiment.

Determination of Water Quality Parameters

The water quality parameters of aquariums were measured daily. Temperature and pH were measured using a mercury centigrade thermometer and a pH meter (Model: pH ep Tester, Romania). Over the experimental time frame, dissolved oxygen (DO) in the aquarium was traced using a dissolved oxygen meter (Model: HI 9146- DO meter, Romania).

Experimental Design

The research was carried out in triplicates in glass aquariums located in the Wet Laboratory of the Department of Fisheries Biology and Genetics, Khulna Agricultural University, Khulna, Bangladesh. Prior to the experiment, healthy juveniles of *B. gonionotus* uniform size were collected from the selected coastal region in Khulna. Before the experiment, the fish were acclimated, and following thorough cleaning, 35 L of chlorine-free tap water was added to glass aquariums, and *B. gonionotus* was acclimated for two days. Fish were kept in pesticide-free water as the pesticide concentrations (0.001, 0.005, 0.03, 0.08, and 0.10 ppm) were changed. The acute toxicity experiments were used to determine the LC_{50} for the fish at 96 hours.

Determination of LC_{50} value

Over the duration of 96 hours, standard LC_{50} experiments were conducted. A single dosage of Malathion 57 EC or multiple doses delivered by daily water changes made up the five-day period of acute exposure, which was sampled on days 1, 3, and 5. Weekly sampling was done throughout the 4 weeks of sub-acute exposure. Assuming a model water depth of 20 cm in a rice field, the acceptable dose for agriculture for Malathion 57 EC was determined as 0.08 ppm. Fish that had not been exposed to pesticides were compared to a control. Fish were watched to see how their exterior behavior changed, and dead fish were counted and destroyed as soon as they ceased moving around on their own. Fish deaths were seen after 24, 48, 72, and 96 hours of exposure. For a 96-hour exposure period, the LC_{50} values for *B. gonionotus* were determined using a probit analysis in SPSS.

Organ specific histology of pesticide treated fish

B. gonionotus was exposed to Malathion 57 EC at levels below 0.001 to 0.005 ppm at the agricultural recommended dose in glass aquariums for a period of seven days in order to detect the histopathological effects. The appropriate dosage for agriculture was determined using a 20 cm water depth in a rice field. The gills, liver, and kidney were removed after exposure and kept in 10% neutral buffered formalin for additional investigation. Using a micro-tome machine (Leica JUNG RM 2035), the paraffin wax-embedded samples were divided into 5 m sections. As a result, the sections were stained with eosin and hematoxylin. A series of different chemicals with varying concentrations and timing are used to remove eosin stains. Once stained, for a permanent slide, the portions were mounted with Canada balsam and left overnight. Photomicrography using a photomicroscope to examine the stained samples (OLYMPUS CX41, Japan).

Alterations in the blood cell structure of Organophosphorus Pesticide treated fish

a) Morphological Alterations

To analyze the morphological changes of erythrocytes, blood smears from fresh blood were prepared on glass slides. The blood-smeared slides were air-dried, methanol-fixed, and stained with Wright's Giemsa. A computer-attached microscope with a 400×lens (OLYMPUS-CX41) was used to collect pictures while immersion oil microscopy was used for examining blood corpuscles.

b) Estimation of blood parameters

Fish were split into two groups; with five fish each, after acclimation. The control group was Group 1. Malathion 57 EC at sublethal levels was administered to fish in group 2 for a period of 7 days. Approximately 2mL of Blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5 mg ethylene diamine tetra acetic acid (EDTA) an anticoagulant; it was properly mixed and used for hematological analysis. Take it into the RBC pipette and fill it to a concentration of 0.5 ppm to count the red blood cells. Hayem's solution was then placed on the pipette, and the solution was drawn up to 101 markings. The '8' knot motion effectively blended the pipette's contents. At least two drops of the mixture were expended after mixing, and the third drop was added to the chamber's side and covered with a cover slip. The cells from the four chambers and the center square, each of which comprised 16 small squares, were counted after two minutes. A 40x high-power objective lens was used to count. The following formula was used to determine the number of RBC per cubic millimeter:

$$\text{Total RBC } (mm^3) = \frac{\text{No. of cells } \times \text{dilution factor} \times \text{depth factor}}{\text{Total No. of small squares}}$$

Statistical analysis

To determine the LC_{50} values, data from acute toxicity tests were analyzed statistically using the probit analysis method.

RESULTS

Physicochemical parameters

During the experimental period, temperature, DO, and pH levels were regularly taken. Average values for temperature, DO, and pH were 27.0±3.0ºC, 7.5±1.0 ppm and, 9.25±2.1, respectively. Water quality measurements were not affected by the dissolution of Malathion 57 EC.

LC_{50} value of Malathion 57 EC and Morphometric and Behaviour Changes of *B. gonionotus*

The LC_{50} value of Malathion 57 EC for *B. gonionotus* was determined to be 0.073 ppm (Table 1). After being exposed to *B. gonionotus* for 96 hours, several morphometric alterations were noticed. Their caudal section of the vertebral column was bent and displayed aberrant swimming. Throughout the testing period, the tested fish's actions were observed. Due to the impacts of pesticides, a number of anomalous behaviors were noticed, including anxiety, circle movements, loss of balance, excessive operculum activity, severe spasms, and paralysis. The fish was then finally placed at the aquarium's base. After settling, sluggish operculum movement was seen, followed by the gradual demise of fish.

Table 1. LC_{50} of *B. gonionotus*

Conc. (ppm)	No. of dead fish	Mortality (%)	LC_{50} ($P < 0.05$)	χ^2 value
0.01	0	0	0.073	16.02
0.001	2	8.33		
0.005	3	12.5		
0.03	7	29.17		
0.08	8	50.33		
0.10	4	16.67		

Histopathological Observation of Fish Exposed to Pesticides

Malathion 57 EC was administered to *B. gonionotus* at two different concentrations 0.001 ppm and 0.005 ppm. Gill, liver, and kidney structural abnormalities were noticed and compared to those of the control group. The control group's main and secondary gill lamellae and gill arch did not show any pathology, but at a dosage of 0.001 ppm, blood congestion, hyperplasia, secondary lamellae curling, bleeding, epithelial hyperplasia, clubbing, and necrosis were identified in the gill (Figure 1). At the dose of 0.005 ppm Malathion 57 EC, similar pathologies were also observed, like vacuolation, missing secondary gill lamellae, hyperplasia, and clubbing (Figure 2). At the dosage of 0.001 ppm Malathion 57 EC, mild modifications including nuclear hypertrophy, cytoplasmic vacuolation, vacuole, pyknotic region, hemorrhage, and necrotic area were discovered (Figure 3). In the control group, renal tubules and hematopoietic cells were arranged in their regular, systematic, and normal ways. However, at the same pesticide dosage, the more severe modification of the kidney histology was seen, including glomerular enlargement, an increase in the renal tubule's diameter, necrosis, pyknosis, vacuolation, and bleeding (Figure 5). However, compared with different doses, pathologies were found almost similar in the liver and kidneys in both doses (0.005 ppm) (Figure 4 and 6).

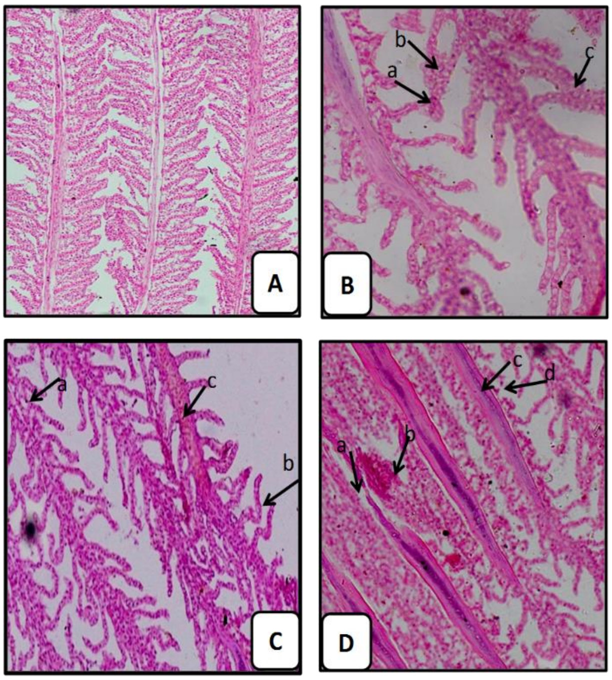

Figure 1. Photomicrographs of the gill of *B. gonionotus* after 7 days of exposure to 0.001 ppm Malathion 57 EC. (A)- control – normal epithelial cell and secondary lamellae were found; (B)- blood congestion (a), hyperplasia (b), curling of secondary lamellae (c); (C)- epithelial hyperplasia (a), necrosis (b) and clubbing (c); (D)- vacuolation (a), hemorrhage (b), pyknosis (c) were observed.

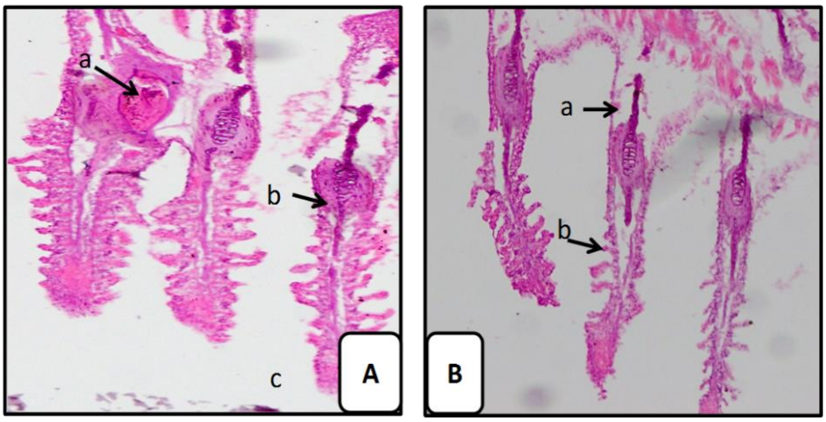

Figure 2. Photomicrographs of gills of *B. gonionotus* after 7-day exposure to 0.005 ppm Malathion 57 EC. (A)- vacuolation (a) and hyperplasia (b); (B)- missing of secondary gill lamellae (a), and clubbing (b) were observed

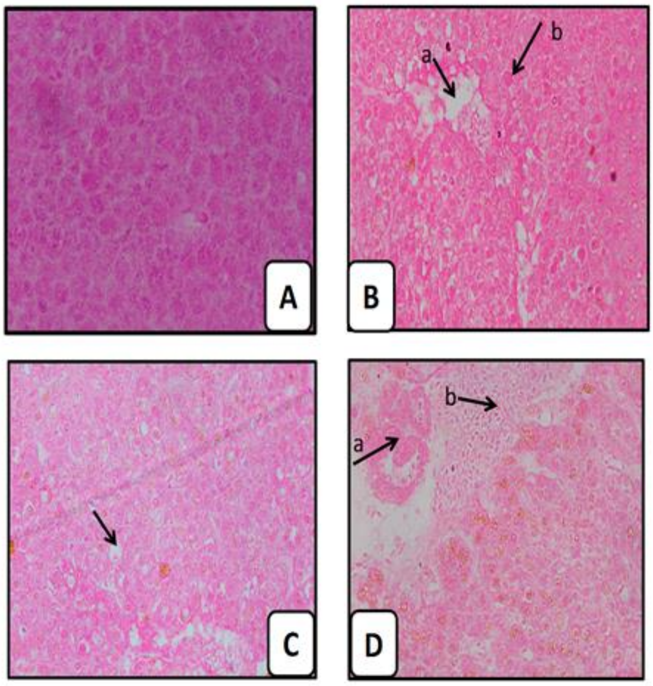

Figure 3. Photomicrographs of the liver of *B. gonionotus* after 7 days of exposure to 0.001 ppm Malathion 57 EC. (A)- control – normal regular and systematic arrangement of hepatocytes were found; (B)- cytoplasmic vacuolation (a) and nuclear hypertrophy (b); (C)- vacuole, pyknotic area; (D)- hemorrhage (a), necrotic area (b) were observed.

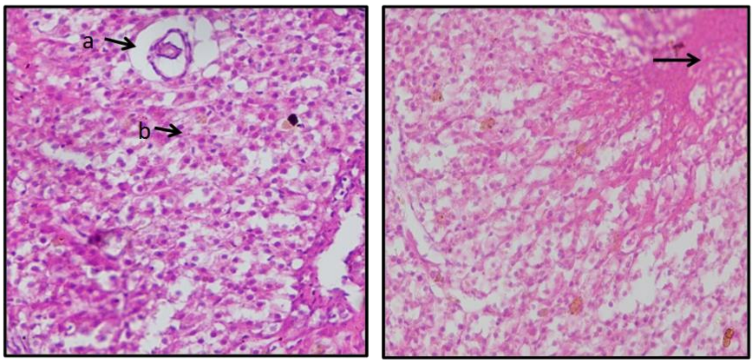

Figure 4. Photomicrographs of the liver of *B. gonionotus* after 7-day exposure to 0.005 ppm Malathion 57 EC. Severe (A) - nuclear hypertrophy and (B) - vacuolation (a) and cytoplasmic vacuolation (b) were observed.

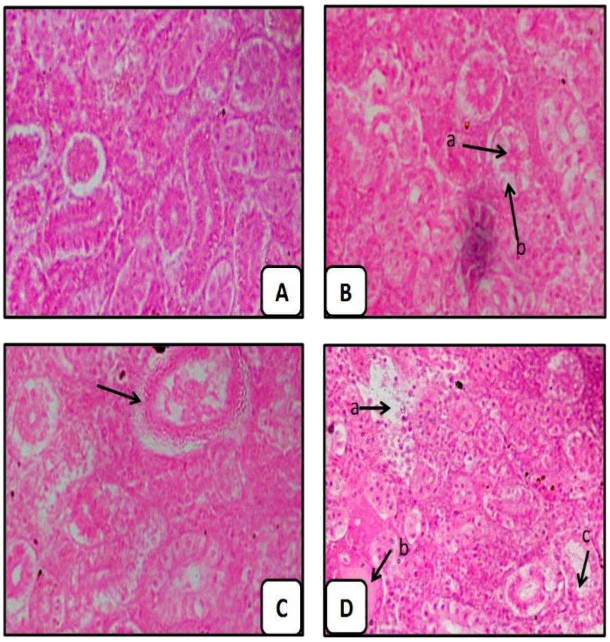

Figure 5. Photomicrographs of the kidney of *B. gonionotus* after 7 days of exposure to 0.001 ppm Malathion 57 EC. (A)-Control—normal regular and systematic arrangement of kidney tubules and hematopoietic cells were found; (B)- glomerular expansion (a) and increasing the diameter of the renal tubule (b); (C)-necrosis; (D)- vacuolation (a) and hemorrhage (b) pyknosis (c) were observed.

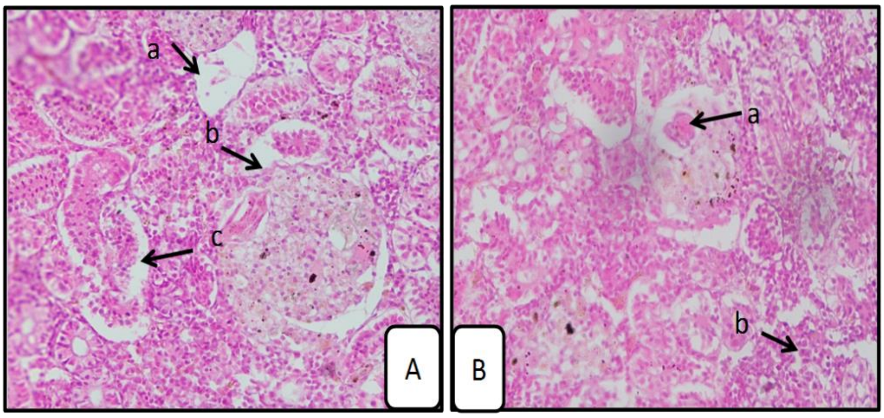

Figure 6. Photomicrographs of the kidney of *B. gonionotus* after 7-day exposure to 0.005 ppm Envoy 50 SC. Severe (A)- glomerular expansion (a) and cellular degeneration (b); (B)- increasing the diameter of the renal tubule (a) and vacuolation (b) were observed.

Morphological Alterations of Erythrocytes of Blood Smears Exposed to Malathion 57 EC

Each erythrocyte was identified by uniform blood smears from healthy, unpolluted samples of normal fish as being an oval-shaped cell with a concentric nucleus at the cell's outer border. At a dosage of 0.001 ppm Malathion 57 EC, binucleated cells, tear-shaped cells, senile cells, absence of a nucleus, irregular cell shape, ghost cells, and big lymphocytes were discovered (Figure 7).

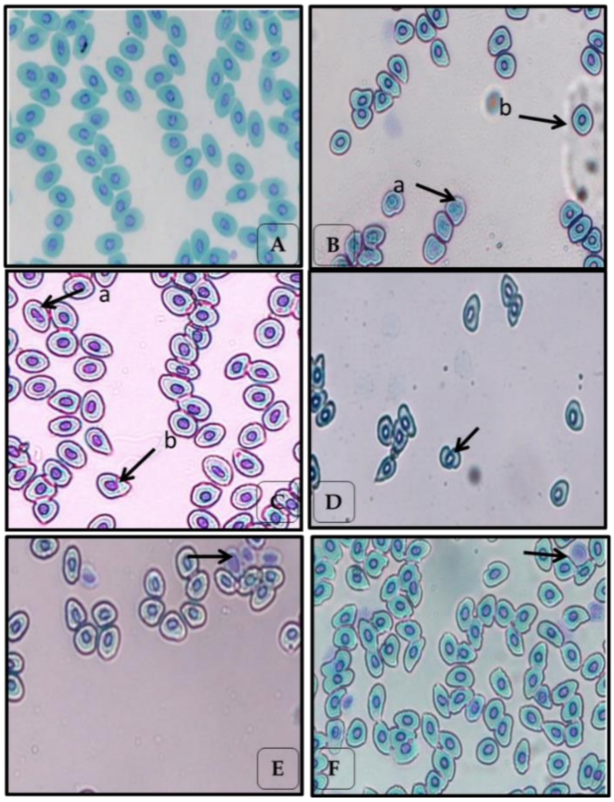

Figure 7. Photomicrograph of blood smears of *B. gonionotus* after 7 days of exposure to 0.001 ppm Malathion 57 EC. (A)- control – normal regular and systematic arrangement of the nucleus of erythrocytes were found; (B)- binucleoted cell, tear-shaped cell; (C)- senile cell; (D)- an-absence of the nucleus; (E)- abnormal shape of cell ; (F) ghost cell, large lymphocyte were observed.

Determination of Red Blood Cell Count of Fish Exposed to Malathion 57 EC

Red blood cells were measured for two sublethal doses of Malathion 57 EC after 10 days of exposure. When fish were treated with Malathion 57 EC, the mean red blood cell count was considerably ($P<0.01$) lower (Table 2).

Table 2. Mean RBC count of *B. gonionotus* at different concentrations of Malathion 57 EC

Treatment	Concentration (ppm)	Cell count (Million RBC)
Control	-	6.52 ± 0.36
Malathion 57 EC	0.001	5.10 ± 0.55**
	0.005	2.91 ± 0.87**

**P<0.01 vs control

DISCUSSION

LC_{50} Values of Malathion 57 EC for *B. gonionotus*

In these experiments, the concentration that kills 50% of the animals is called an LC_{50} rather than an LD_{50}. When an LC_{50} value is reported, it should also state the kind of test animal studied and the duration of the exposure. In the present study, the LC_{50} value of Malathion 57 EC was 0.073 ppm for *B. gonionotus*. In our previous studies, we found quite similar results: the calculated LC_{50} value of Kinalax 25 EC for *B. gonionotus* was 0.071 ppm (Akter et al., 2019). However it was lower than the LC_{50} value of Envoy 50 SC for the fish was found to be 0.151 (0.014-0.198) ppm (Akter et al., 2020). The LC_{50} value of Malathion was found to be 15.13 ppm for *B. gonionotus* (Mrong et al., 2021). Deka and Mahanta (2012) found that the LC_{50} value of Malathion was 0.98 ppm for *Heteropneustes fossilis* at 96 h. exposure. The LC_{50} value of malathion in the freshwater fish *Ophiocephalus punctatus* was found to be 16 ppm (Pugazhvendan et al., 2009). Hossain et al. (2000) estimated the LC_{50} value of Diazinon as 2.97 ppm for *L. rohita* at 96 h. exposure whereas Hossain et al. (2001) found that LC_{50} values were 0.3530 and 1.2809 ppm for Diazinon 60 EC and Dimecron 100 SCW at 48 h exposure on zooplankton, *Diaptomus*. Additionally, the LC_{50} values for *Anabas testudineus*, *Channa punctatus*, and *Barbodes gonionotus* on Dimecron 100SCW at 96 h were 6.75, 22.95, and 375.26 ppm, respectively (Hossain et al., 2002). The differences in the LC_{50} values are caused by its dependence on a number of parameters, including species, size, and susceptibility to the toxicants, concentration, and length of exposure.

Histopathological Observation in Pesticide Treated Fish

Histopathological alterations have been utilized as markers in evaluating the health of fish exposed to toxins in laboratory and field studies. These organ alterations can act as early indicators of an animal's disease because they are typically easier to spot than functional changes. It is an important biomarker to assess fish health in a stressed environment. The main and secondary gill lamellae, as well as the gill arch, in the control group in this investigation, did not exhibit any pathology, but at a high dosage of Malathion 50EC, blood congestion, hyperplasia, secondary lamellae curling, bleeding, epithelial hyperplasia, clubbing, and necrosis were found in the gill. The outcome is comparable to that reported by Akter et al. (2019) for Kinalax 25 EC for *B. gonionotus*. Marchand et al. (2009) described changes to the tissues of different fish organs in the polluted ecosystem. Gill changes in *B. gonionotus* that had been exposed to the pesticide Malathion 57 EC ranged from mild to severe. In this instance, the pathological symptoms were obvious at greater pesticide doses. The results are similar to those seen in a study conducted by Zodrow et al. (2004) on zebrafish gill hypertrophy and fusion, which were found to be dependent on the dosage. Additionally, Nile tilapia subjected to an organophosphate pesticide had telangiectasis at the tip of secondary gill lamellae, according to Çaglan et al. (2010). Reza et al. (2017) also discovered considerable gill clubbing, bleeding, and pyknosis in *Labeo rohita* at 0.058 ppm of an organophosphate pesticide, however, the gills were discovered in virtually normal conditions in *B. gonionotus* save for few secondary gill lamellae missing.

In the current investigation, the liver tissues showed ultrastructural damage in comparison to the control, including glomerular enlargement, dilation of Bowman's space, cellular degeneration, swelling of the renal tubule diameter, pyknotic region, and melanomacrophage for *B. gonionotus*. This result is similar to that reported by Akter et al. (2019) for Kinalax 25 EC for *B. gonionotus*. Similar results also were found by Hossain et al. (2002) and Rahman et al. (2002) from the liver of three fish species exposed to organophosphate pesticide. Zodrow et al. (2004) found lipidosis and hepatocyte hypertrophy. Oropesa et al. (2009) and Karaca et al. (2014) observed necrotic foci and lipid droplets in the liver of Cyprinus carpio and interactions between antioxidant enzyme activity and lipid peroxidation effect on pesticides. Reza et al. (2017) also discovered mild hemorrhage, fatty degeneration, and vacuole alterations in the liver tissues of *L. rohita* treated with 0.058 ppm Envoy 50 SC, whereas significant hemorrhage, fatty degeneration, and lipid droplets were discovered for the same fish species at 0.108 ppm.

The kidney tissues in the current investigation showed moderate to severe damage when compared to the control. The findings for *B. gonionotus* are remarkably comparable to those from Akter et al. (2019). Since Hossain et al. (2002) and Rahman et al. (2002) found substantially more diseases in *B. gonionotus*, the results are somewhat consistent with their findings. Reza et al. (2017) discovered minor vacuoles, degenerating kidney tubules, and bleeding in *Labeo rohita* at 0.058 ppm, but lower than that dose such as 0.01, the kidney tissues of *B. gonionotus* appeared normal but had some melanin pigment and vacuoles. In the kidney of fish treated with 0.108 ppm Envoy 50 SC, *Labeo rohita* was observed to have moderate bleeding, pyknosis, and hyaline, and B. gonionotus had moderate vacuoles, pyknosis, and necrosis. Zebrafish exposed to 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin did not exhibit any alterations in kidney tissue, however (Zodrow et al., 2004). The fact that *L. rohita* had more structural damage than *B. gonionotus* indicated that *L. rohita* was significantly more susceptible to pesticide exposure. Therefore, Sogut and Percin (2011) investigated the toxicant effects on the kidney, the target organ of bluefin tuna. At 10 gL of atrazine exposed to rare minnow *(Gobiocypris rarus*) for 28 days, Yang et al. (2010) identified lesions in kidney tissues including an extensive expansion in the lumen, degenerative and necrotic alterations of tubular epithelia, and shrinkage of the glomeruli.

Hematological Alteration of Pesticide Treated Fish

Hematological indicators are regarded as useful biomarkers of changes in metabolism or physiology since the blood participates directly or indirectly in many physiological processes of the body. Fish exposed to pesticides can be identified by blood parameters such as hematological and biochemical indices, which are significant markers of their structural and functional, state (Evans and Claiborne, 2005). Blood provides a valuable profile for the investigation of the toxicological effects on animal tissues. Depending on the level of stress and other environmental circumstances, blood values can frequently vary. According to much research, a drop or rise in specific blood parameters can be related to the toxicants and the species' toxicity. Fish anemia is the result of a large reduction in the hematocrit value, which is caused by the decrease in the hematological variables (PCV, Hb, and RBC) of the exposed fish. This reduction may be caused by hemolysis and RBC shrinkage by QP. The decrease in RBC count could alternatively be caused by an increase in RBC breakdown or a decrease in RBC synthesis (Mostakim et al., 2015).

In the present investigation, many modifications in peripheral erythrocytes i.e. binucleated cells, tear-shaped cells, senile cells, absence of nucleus, irregular shape of the cell, ghost cell, and big lymphocytes were discovered owing to sublethal exposure of Malathion 57 EC. Akter et al. (2019) also found the same alterations due to sublethal exposure to Kinalax 25 EC and also found that the RBC count was decreased in the fish treated with Kinalax 25EC which is partially similar to the sublethal exposure to Malathion 57 EC resulting in significantly lower RBC count in the higher dose than in the lower in this study. Saravanan et al. (2011) also investigated that the RBC count was decreased in the pesticide-treated fish.

CONCLUSION

This study unquestionably shows that the presence of regularly used organophosphate pesticides in freshwater reservoirs may have harmful effects on a very hardy and strong fish's early life stages, demonstrating the danger that pesticides may pose to other vulnerable wild species. Organic pesticides, especially organophosphates, have an effect on the ability of fish and aquatic invertebrates to ward off predators. The impact of Malathion on fish is dosage-dependent based on the data made in the aforementioned study. The number of behavioral changes grows along with concentration. The survival of the fish in their natural environment may be negatively impacted by the sublethal amounts of Malathion. Even at modest doses, Malathion 57 EC exposure changed some hematological characteristics and stressed the fish. Fish that have been exposed to pesticides for a long period provide ongoing health risks to the general public. The risk to the human population has increased due to the consumption of this hazardous seafood. To protect both people and wildlife, measures must be taken when applying pesticides.

ACKNOWLEDGEMENTS

We thank the Senior Upazila Fisheries Officer in Dumuria, Khulna for providing information and the local community for their cooperation during data collection and sampling.

CONFLICT OF INTEREST

The author states that they do not have any conflicts of interest regarding their research.

REFERENCES

1. Akter R, M A Pervin, H Jahan, S F Rakhi, A H M Reza and Z Hossain, 2020. Toxic effects of an organophosphate pesticide, envoy 50 SC on the histopathological, hematological, and brain acetylcholinesterase activities in stinging catfish (*Heteropneustes fossilis*). The Journal of Basic and Applied Zoology, 81(1): 1-14.
2. Akter R, A Parvin, H Jahan and Z Hossain, 2019. Impact of Kinalax 25 EC on vital organ histomorphology, blood cell structure and brain acetylcholinesterase activity in silver barb (*Barbonymus gonionotus*). Aquaculture Studies, 19(2): 103-111.
3. Çaglan A, K Benli and A Ozkul, 2010. Acute toxicity and histopathological effects of sublethal fenitrothion on Nile tilapia, *Oreochromis niloticus*. Pesticide Biochemistry and Physiology, 97: 32-35.

4. Chandra S and H Chandra, 2013. Role of haematological parameters as an indicator of acute malarial infection in Uttarakhand state of India. Mediterranean Journal of Hematology and Infectious Diseases, 5(1).
5. Deka S and R Mahanta, 2012. A study on the effect of organophosphorus pesticide malathion on hepato-renal and reproductive organs of *Heteropneustes fossilis* (Bloch). Science, 1(1): 1-13.
6. Evans D H and J B Claiborne, 2005. The Physiology of Fishes, Boca Raton, Fla, USA, CRC Press.
7. Gernhofer M, M Pawet, M Schramm, E Müller and R Triebskorn, 2001. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. Journal of Aquatic Ecosystem Stress and Recovery, 8(3-4): 241–260.
8. Hasan M M, M M Islam and M K Ahmed, 2008. Toxic effects of Malathion (57EC) on *Heteropneustes fossilis* (Bloch, 1822). Bangladesh Journal of Zoology, 35(1): 153-160.
9. Hasanuzzoha, 2004. Environment friendly use of pesticides in field crop protection in Bangladesh and pre/postsafety measures for farmers. Ph.D. Thesis. IPM Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh, 366.
10. Hossain Z, M Z Rahman and M F A Mollah, 2002. Effect of Dimecron 100 SCW on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus*. Indian Journal of Fisheries, 49(4): 405–417.
11. Hossain Z, M Z Rahman and I V F A Mollah, 2001. Effects of Two Organophosphorus Pesticides Diazinon-6O EC and Dimecron-1 OO SCW on a Zooplankton, Diaptomus. Pakistan Journal of Biological Sciences, 4(11): 1403-1405.
12. Hossain Z, G C Haldar and M F A Mollah, 2000. Acute toxicity of chlorpyrifos, cadusafos and diazinon to three Indian major carps (*Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*) fingerlings.
13. Huculeci R, D Dinu, A C Staicu, M C Munteanu, M Costache and A Dinischiotu, 2009. Malathion-induced alteration of the antioxidant defence system in kidney, gill, and intestine of *Carassius auratus gibelio*. Environmental Toxicology: An International Journal, 24(6): 523-530.
14. Islam M S, M M Haque, M N Uddin and M D Hasanuzzaman, 2019. Histopathology in the fish *Channa punctatus*, *Heteropneustes fossilis*, and *Anabas testudineus* exposed to diazinon. World Applied Sciences Journal, 37: 512-521.
15. Karaca M, L Varisli, K S Korkmaz, O Özaydin, F Perçin and H Orhan, 2014. Organochlorine pesticides and antioxidant enzymes are inversely correlated with liver enzyme gene expression in *Cyprinus carpio*. Toxicology Letters, 230: 198-207.
16. Konar S, 1975. Pesticides and aquatic ecosystems. Indian Journal of Fisheries, (22): 80-85.
17. Marchand M J, J C Van Dyk, G M Pieterse, I E J Barnhoorn and M S Bornman, 2009. Histopathological alterations in the liver of the sharptooth catfish *Clarias gariepinus* from polluted aquatic systems in South Africa. Environmental Toxicology: An International Journal, 24(2): 133-147.
18. Maurya P K, D S Malik, K K Yadav, N Gupta and S Kumar, 2019. Haematological and histological changes in fish *Heteropneustes fossilis* exposed to pesticides from industrial waste water. Human and Ecological Risk Assessment: An International Journal, 25: 1-28.
19. McKim J M and D A Benoit, 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Board of Canada, 28(5): 655-662.
20. Mostakim G M, M M Zahangir, M M Mishu, M K Rahman and M S Islam, 2015. Alteration of blood parameters and histoarchitecture of liver and kidney of silver barb after chronic exposure to quinalphos. Journal of toxicology, 2015.
21. Mrong C E, M R Islam, K Kole, N N Neepa, M J Alam, M R Haque, U O Rahman and G M Mostakim, 2021. Malathion-induced hematoxicity and its recovery pattern in *Barbonymus gonionotus*. Journal of Toxicology, 2021.
22. Naqvi S M and A A Cruz, 1973. Effects on Mirex insecticides on selected freshwater invertebrates. The Bulletin of the Association of Southeastern Biologists, (19): 8.
23. Oropesa A L, J P G Cambero, L Gómez, V Roncero and F Soler, 2009. Effect of long-term exposure to simazine on histopathology, haematological, and biochemical parameters in *Cyprinus carpio*. Environmental Toxicology, 24(2): 187–199.

24. Ogundiran M A, O O Fawole, S O Adewoye, 2007. Effects of soap and detergent effluents on the haematological profiles of *Clarias gariepinus*. BBC Science Focus, 12(1): 84–88.
25. Pugazhvendan S R, N J Narendiran, R G Kumaran, S Kumaran and K M Alagappan, 2009. Effect of malathion toxicity in the freshwater fish *Ophiocephalus punctatus*-A histological and histochemical study. World Journal of Fish and Marine Sciences, 1(3): 218-224.
26. Rahman M Z, Z Hossain, M F A Mollah and G U Ahmed, 2002. Effects of Diazinon 60 EC on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus*. Naga, 25(2): 8-12.
27. Rakhi S F, A Hakim, M S Hossen, M M Reza and Z Hossain, 2013. Alterations in histopathological features and brain acetylcholinesterase activity in stinging catfish *Heteropneustes fossilis* exposed to polluted river water. International Aquatic Research, 7.
28. Ray B P, M A Baten and M K Saha, 2012. Effects of some selected pesticides on the mortality of tilapia fish (*Oreochromis niloticus*). Journal of Biological Chemistry Research, 29: 189-205.
29. Reza A H M M, S F Rakhi, M S Hossen and Z Hossain, 2017. Organ specific histopathology and brain acetylcholinesterase inhibition in Rohu, *Labeo rohita* and Silver Barb, *Barbonymus gonionotus*: Effects of three widely used organophosphate pesticides. Turkish Journal of Fisheries and Aquatic Sciences, 17(4): 821-832.
30. Saravanan K, P Kumar and M Ramesh, 2011. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pesticide Biochemistry and Physiology, 100: 132-141.
31. Sancho E, C Fernandez-Vega, M D Ferrando and E Andreu-Moliner, 2003. Eel ATPase activity as biomarker of thiobencarb exposure. Ecotoxicology and environmental safety, 56(3): 434-441.
32. Sogut O and F Percin, 2011. Trace elements in the kidney tissue of Bluefin Tuna (*Thunnus thynnus* L. 1758) in Turkish seas. African Journal of Biotechnology, 10: 1252- 1259.
33. Ullah R, A Zuberi, S Ullah, I Ullah, D F Ullah, 2014. Cypermethrin induced behavioral and biochemical changes in mahseer, *Tor putitora*. Journal of Toxicological Sciences, 39(6): 829–836.
34. Yang L, J Zha, W Li, Z Li and Z Wang, 2010. Atrazine affects kidney and adrenal hormones (AHs) related genes expressions of rare minnow (*Gobiocypris rarus*). Aquatic Toxicology, 97: 204-211.
35. Zodrow J M, J J Stegeman and R L Tanguay, 2004. Histological analysis of acute toxicity of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) in zebrafish. Aquatic Toxicology, 66(1): 25-38.