



*Research Paper*

**CHANGES IN HAEMATOLOGICAL PARAMETERS OF SILVER BARB  
(*Barbonymus gonionotus*) DUE TO EXPOSURE OF PESTICIDES, LAMDA-  
CYHALOTHRIN AND DIMETHOATE**

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**Abstract**

The study was carried out to investigate the changes in haematological parameters of silver barb (*Barbonymus gonionotus*) due to exposure of pesticides, lamda-cyhalothrin and dimethoate in glass aquaria. In this study, three batches of fish were kept in clean water treated as control fish (T1); six batches of fish were exposed to two concentrations of lamda-cyhalothrin as 0.001 ml/L (T2) and 0.003 ml/L (T3); and six batches of fish were exposed to two concentrations of dimethoate as 0.001 ml/L (T4) and 0.003 ml/L (T5) for 96 hours. During this study, physico-chemical parameters (temperature, oxygen and pH) of test water, behaviour and haematological parameters (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) of the fish were analyzed. Physico-chemical parameters of test water were within the productive ranges. When the fish exposed to pesticides, they showed less activity, loss of equilibrium, motionlessness, erratic swimming and loss of appetite. WBC, RBC, Hb, PVC and MCV were found significantly decreased and MCHC insignificantly increased. MCH was found decreased insignificantly in lamda-cyhalothrin exposed fish but significantly in dimethoate exposed fish. Therefore, the study stated that lamda-cyhalothrin and dimethoate pesticides produce an adverse effect in haematological parameters which might affect normal physiology and immunity of fish.

*Key words: Changes, haematological, parameters, exposure, lamda-cyhalothrin, dimethoate.*

**INTRODUCTION**

A variety of agricultural chemicals such as pesticides, insecticides, fungicides, herbicides and other biocides are used to control crop pests in agricultural production [2]. In Bangladesh, more than 300 types of pesticides and insecticides are used for agricultural crop protection. Due to injudicious and indiscriminate use of these pesticides, water

bodies like ponds, lakes, river and low lying water areas are continuously getting polluted through surface run off, sediment transportation from treated soil and direct application as spray for controlling pests [33]. Pollution of surface water caused by pesticides used in agriculture and their effects on non-target organisms are problems of global importance [25]. Pesticides may be directly toxic, deteriorate the water quality and cause ecological imbalance leading to health hazards to aquatic flora and fauna [23]. In acute cases, there are records of catastrophic mortality of the entire aquatic biota [33].

Pyrethroid pesticides are widely used for controlling the insect pest all over the world. Pyrethroid pesticides are extremely toxic to fish [21] and other aquatic animals due to impaired metabolism. Lambda-cyhalothrin is a synthetic pyrethroid used for the eradication of insects in agricultural fields [22]. Like all pyrethroids, lambda-cyhalothrin is a neurotoxin [5] insoluble in water and very toxic to fish [39]. It reduces packed cell volume, red blood cell count and haemoglobin concentration in fish [56]. Organophosphorus pesticides are extensively being used in agriculture and account for approximately 50% of the global pesticides use [29]. Occurrence of organophosphates into water bodies causing acute and chronic toxicity to fish fauna [1 and 42]. Dimethoate is an organophosphorus pesticide possessing contact and systemic properties [26] that is used in worldwide agriculture. The haematological effects of dimethoate reported are reduction of erythrocyte counts and haemoglobin concentration [3].

There are many reports available related to the toxicity of pesticides on different fish species [18]. Toxicity of pesticides in aquatic organisms can be studied by evaluating the changes in the haematological parameters [49]. Haematological studies are procedures frequently and routinely applied in the diagnosis of diseases in aquaculture [45]. However, a numbers of studies have been conducted on the effect of pesticides on the haematological parameters throughout the world [7, 11, 14, 17, 48, 55 and 57] but in Bangladesh, such studies are much limited in number [4, 27, 31 and 40]. However, the knowledge on the pesticide's pollution and health condition of fish still needs to be expanded to provide data for different fish species that will be helpful for fish health management in promising aquaculture practices in Bangladesh. As the information on the health conditions of silver barb related to lamda-cyhalothrin and dimethoate pesticides

pollution are not available in Bangladesh, therefore, the study was conducted to find out the effects of lamda-cyhalothrin and dimethoate pesticides on haematological parameters of silver barb as a model test organism and a candidate species for rice-fish culture in Bangladesh.

## **2. MATERIALS AND METHODS**

### **2.1. Experimental unit and site**

The study was conducted in glass aquaria in the wet laboratory of the Department of Fisheries, University of Rajshahi, Bangladesh. The aquaria were rectangular in size (90×40×45 cm) containing 90 liters of water and providing aeration facilities. Fine meshed nets were used to cover of aquarium to avoid jumping of fish.

### **2.2. Test pesticides**

Two pesticides, Fighter 2.5 EC, a commercial pyrethroid pesticide contains lamda-cyhalothrin and Tafor 40 EC, a commercial organophosphorus pesticide contains dimethoate were used.

### **2.3. Experimental design**

The study was carried out for a period of 96 hours. In this study, three batches of fish (12 fish in each batch) were kept in clean water (without pesticides) treated as control fish (T1); six batches of fish were exposed to two concentrations of lamda-cyhalothrin as 0.001 ml/L (T2) and 0.003 ml/L (T3); and at the same time, six batches of fish were exposed to two concentrations of dimethoate as 0.001 ml/L (T4) and 0.003 ml/L (T5).

### **2.4. Collection and maintenance of test fish**

The test fish, *B. gonionotus* (Body weight 80.93±11.43 g) were collected from local fish farm and were transferred in the aquaria in the laboratory for acclimatization. During the period of acclimatization and experiment, the fish were fed with a commercial pellet feed twice daily to satiety for avoiding the effects of starvation. Tap water was used throughout the course of the experimental period. The aquarium water was aerated continuously. Constant amount of the test chemical and test water were renewed every

24 hours to remove fecal matter and waste metabolites of fish and also for maintaining constant concentration of test pesticides.

### **2.5. Physico-chemical parameters**

The physico-chemical parameters, viz., water temperature, dissolved oxygen and pH were monitored regularly with a Centigrade thermometer, HACH Kit and pH meter, respectively.

### **2.6. Behaviour of fish**

Behaviour of the test fish was monitored by eye observation and taking video.

### **2.7. Blood samples collection and analysis**

At the end of the experiment, blood samples from the fish were collected in a haematocrit tube containing anticoagulant agent (EDTA). During the study, total count of White Blood Cells (WBC) and Red Blood Cells (RBC), Haemoglobin (Hb), Pack Cell Volume (PCV) or Hematocrit (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were analyzed. Total WBC and RBC were counted by using an improved Neubaur Haemocytometer [51]. Hb concentration was determined by the cyanmethemoglobin method [35]. PVC (%) was determined by the microhematocrit method [24]. The value of MCV was calculated from the haematocrit value and the erythrocyte count [19]. MCH value was calculated from the haemoglobin value and the erythrocyte count [54]. MCHC value was calculated by dividing the haemoglobin content in g/dl by the PCV % of red blood cell [54].

### **2.8. Statistical analysis**

The data were analyzed using one-way ANOVA test. The mean values were compared to see the significant difference from the Duncan's Multiples Range Test (DMRT) set as  $P \leq 0.05$ .

## **3. RESULTS AND DISCUSSION**

### **3.1. Physico-chemical parameters of water**

When physico-chemical parameters of water are affected by toxicants then any physiological changes may be reflected in the values of one or more of haematological parameters [58]. During this study, the observed physico-chemical parameters of test

water did not showed any significant variation among the treatments and were recorded as water temperature 22.7 to 22.8°C and 22.2 to 22.3°C; dissolved oxygen 6.3 to 6.8 mg/L and 6.4 to 6.8 mg/L and pH 7.5 to 7.8 and 7.6 to 7.9 in lamda-cyhalothrin and dimethoate treated aquaria, respectively. The values of observed parameters were within the productive ranges for fish growth according to the different research reports [6, 10 and 28].

### 3.2. Changes in behaviour of test fish

Behaviour is an appropriate index in toxicological studies, because behavioural changes in fish are good index of organism response toward the aquatic pollutants [60]. Presence of pesticides in water can affect the behaviour of fish and can be used as an index of stress [16]. In the present study, the fish showed some abnormal behaviours when they exposed to lamda-cyhalothrin and dimethoate pesticides. The abnormal behaviours were decrease in food intake primarily, restlessness, asymmetrical swimming activities, loss of equilibrium, motionlessness and lethargic movement. Similar behavioural changes have been reported due to cypermethrin exposure in *Poecilia reticulata* [36], in *Cyprinus carpio* [55]. Identical behaviours have also been reported in *L. rohita* due to cypermethrin and diazinon exposure [31] and due to difenoconazole and thiamethoxam exposure [27]. Absorption of pesticides in gills with respect to the lipophilic nature of them can cause respiratory limitations [39] that consequently might be resulted in abnormal behaviour of the fish [53].

### 3.3. Changes in haematological parameters due to lamda-cyhalothrin exposure

The changes in hematological parameters of silver barb due to lamda-cyhalothrin exposure are shown in Table 1. Compared to the control fish, the hematological response of silver barb exposed to lamda-cyhalothrin was a significant decrease in WBC, RBC, Hb, PVC, MCV and an insignificant decrease in MCH and insignificant increase in MCHC. These results are more or less consistent with the findings of other studies investigating hematological response of different fish species exposed to other pyrethroid pesticides.

Decrease in total WBC count in the present study might be due to the role of lamda-cyhalothrin as a potential environmental stressor. Decrease in WBC counts have been reported by Masud and Singh [37] in *Cyprinus carpio* and by Khatun *et al.* [31] in *Labeo*

*rohita* using cypermethrin pesticide which are consistent to the present study. The total count of RBC in present study decreased with the increase in concentration of lamda-cyhalothrin (Table 1). Significant decreases in total RBC counts have also been reported in *Channa punctatus* by Saxena and Seth [50] and in *Labeo rohita* by Khatun *et al.* [31] when treated with cypermethrin. These reports are supportive to the results of the present study. Lowering of RBC count might be due to the disruptive action of pesticides on the erythropoietic tissue [34]. According to Kocabatmaz and Ekingen [32], use of pesticides had a negative effect on haemoglobin concentration which is supportive to the present study. Decreased concentration of Hb in different fishes due to pyrethroids pesticides exposures have also been reported by Deka and Dutta [15], Khatun *et al.* [31] and Jasmin *et al.* [27]. The decreased concentration of Hb in the present study might be due to either increase in haemoglobin destroyed or decrease in haemoglobin synthesis [38].

Blood cell indices like MCV, MCH and MCHC seem to cause changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with RBC count, hemoglobin concentration and PCV [9]. A similar response was noted in the present study, when the fish exposed to lamda-cyhalothrin pesticide. A significant decrease in PCV value has been reported in the difenoconazole exposed fish [27, 43 and 55] and in the cypermethrin exposed fish [31]. These reports are consistent to the present study. MCV values decreased significantly whereas MCH decreased insignificantly in lamda-cyhalothrin exposed fish. These findings are more or less supported by the report of Khatun *et al.* [31] and Chandrasekar and Jayabalan [12]. Moreover, the increased value of MCHC in the lamda-cyhalothrin exposed fish is supported by the report of Jasmin *et al.* [27] who described the increase in MCHC value for *Labeo rohita* when exposed to difenoconazole. Increase in MCHC value due to pesticide exposure is also supported by the report of Fransesco *et al.* [20]. The changes in MCHC value in the fish might be due to the stress condition created by the pesticide [13].

**Table 1: Changes in haematological parameters due to lamda-cyhalothrin exposure**

Parameters	Treatments		
	T1	T2	T3
<b>Total WBC (<math>10^4 \times \text{mm}^{-3}</math>)</b>	5.78±0.21 <sup>a</sup>	5.39±0.14 <sup>b</sup>	4.63±0.18 <sup>c</sup>
<b>RBCs (<math>10^6 \times \text{mm}^{-3}</math>)</b>	1.53±0.08 <sup>a</sup>	1.33±0.11 <sup>b</sup>	1.27±0.09 <sup>b</sup>
<b>Hb (g/dl)</b>	3.20±0.19 <sup>a</sup>	2.75±0.23 <sup>b</sup>	2.58±0.19 <sup>b</sup>
<b>PCV (%)</b>	8.67±0.27 <sup>a</sup>	7.29±0.18 <sup>b</sup>	6.71±0.23 <sup>c</sup>
<b>MCV (fl)</b>	56.67±1.10 <sup>a</sup>	54.88±1.03 <sup>b</sup>	52.83±1.05 <sup>c</sup>
<b>MCH (pg)</b>	20.91±0.38 <sup>a</sup>	20.67±0.43 <sup>a</sup>	20.31±0.57 <sup>a</sup>
<b>MCHC (g/dl)</b>	36.90±1.85 <sup>a</sup>	37.72±1.64 <sup>a</sup>	38.45±1.67 <sup>a</sup>

\* T1 assigned the fish didn't expose to lamda-cyhalothrin (treated as control). T2 assigned the fish exposed to 0.001 ml/L of lamda-cyhalothrin; and T3 assigned the fish exposed to 0.003 ml/L of lamda-cyhalothrin. Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ( $P \leq 0.05$ ).

### 3.4. Changes in haematological parameters due to dimethoate exposure

The changes in haematological parameters of silver barb due to dimethoate exposure are shown in Table 2. Compared to the controlled fish, the hematological response of silver barb exposed to dimethoate was a significant decreases in WBC, RBC, Hb, PVC, MCV and MCH whereas insignificant increased in MCHC. These results are consistent with the findings of other studies investigating hematological response of different fish species exposed to other organophosphorus pesticides.

The decrease in the leukocyte counts in the present study due to dimethoate pesticide exposure is supported by the reports of Rostami *et al.* [46], Pourgholam *et al.* [44], Far *et al.* [17], Khatun *et al.* [31] and Jasmin *et al.* [27] using different organophosphorus pesticides. Decreased RBC count have also been reported in *Heteropneustes fossilis* by Singh and Srivastava [52], in *Cyprinion watsoni* by Khattak and Hafeez [30], in *Piaractus mesopotamicus* by Tavares *et al.* [57] and in *Labeo rohita* by Khatun *et al.* [31] due to

exposure of various organophosphorus pesticides. The decrease found in Hb concentration might be due to either an increase in the rate of Hb destroying or a decrease in the rate of Hb synthesis. This assumption is supported by Matkovies and Witas [38]. Decreased Hb concentration in *L. rohita* has also been reported by Jasmin *et al.* [27] due to exposure of an organophosphorus pesticide, thiamethoxam.

Significant decreased PCV value has been reported by Svoboda *et al.* [55] in *Cyprinus carpio* and Khatun *et al.* [31] in *Labeo rohita* due to diazinon exposure which is supportive to the present study. The reduction of PVC might be due to the fish suffers from haemodilution [59]. Significantly decreased values of MCV and MCH in dimethoate exposed fish in the present study are supported by Far *et al.* [17] and Khatun *et al.* [31]. More or less similar results have also been reported by Binukumari *et al.* [8] Mostakim *et al.* [40] and Muralidharan [41]. Moreover, the increased value of MCHC in the present study is consistent with the finding of Jasmin *et al.* [27] who recorded increased MCHC value in *L. rohita* due to thiamethoxam exposure. Increased values of MCHC have also been reported by Khatun *et al.* [31] in *L. rohita* due to diazinon exposure and Chandrasekar and Jayabalan [12] in *C. carpio* due to endosulfan exposure. The changes in MCHC value might be due to the decrease in Ht % caused by haemolysis or stress condition [47].

**Table 2: Changes in haematological parameters due to dimethoate exposure**

Parameters	Treatments		
	T1	T4	T5
Total WBC ( $10^4 \times \text{mm}^{-3}$ )	5.78±0.21 <sup>a</sup>	3.41±0.46 <sup>b</sup>	2.48±0.21 <sup>c</sup>
RBCs ( $10^6 \times \text{mm}^{-3}$ )	1.53±0.08 <sup>a</sup>	1.17±0.17 <sup>b</sup>	1.06±0.13 <sup>b</sup>
Hb (g/dl)	3.20±0.19 <sup>a</sup>	2.25±0.21 <sup>b</sup>	2.05±0.35 <sup>b</sup>
PCV (%)	8.67±0.27 <sup>a</sup>	6.02±0.46 <sup>b</sup>	5.29±0.34 <sup>c</sup>
MCV (fl)	56.67±1.10 <sup>a</sup>	51.45±1.78 <sup>b</sup>	49.91±1.52 <sup>b</sup>
MCH (pg)	20.91±0.38 <sup>a</sup>	19.23±0.39 <sup>b</sup>	19.33±0.26 <sup>b</sup>
MCHC (g/dl)	36.90±1.85 <sup>a</sup>	37.37±1.43 <sup>a</sup>	38.75±1.87 <sup>a</sup>

\* T1 assigned the fish didn't expose to dimethoate (treated as control). T4 assigned the fish exposed to 0.001 ml/L of dimethoate; and T5 assigned the fish exposed to 0.003

ml/L of dimethoate. Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ( $P \leq 0.05$ ).

From the results of this study, it can be said that both pesticides, lambda-cyhalothrin and dimethoate are hazardous to silver barb and resulted significant changes in haematological parameters. More significant changes were observed in dimethoate exposure than lambda-cyhalothrin exposure. These changes might be potentially disruptive to the health of silver barb in their natural environment.

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