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Effects of Cypermethrin and Sumithion Exposure on the Hematological Profiles of a Minor carp, *Labeo bata* (H)

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ABSTRACT

The study was conducted to investigate the effects of cypermethrin and sumithion exposure on the hematological profiles of *Labeo bata* in glass aquaria for 96 hrs through two experiments under three treatments (T1, T2 and T3). T1 was treated as control fish (for both experiment) and in the first experiment, fish were exposed to 0.001µg/L (T2) and 0.003 µg/L (T3) of cypermethrin whereas in the second experiment, fish were exposed to 0.75 mg/L (T2) and 1.50 mg/L (T3) of sumithion. During this study, behavior and hematological profiles (Total WBC and RBC, Hb, PCV, MCV, MCH and MCHC) of the fish were analyzed by standard methods. When the fish were exposed to the pesticides, primarily decreased their food intake, exhibited restlessness and irregular swimming behavior and then showed lethargic swimming. After 96 hr of cypermethrin exposure, WBC and MCV increased and RBC, Hb, PCV and MCHC decreased significantly whereas after sumithion exposure, WBC and RBC, Hb, PCV and MCV increased and MCHC decreased significantly. The study indicated that exposure of cypermethrin and sumithion produce an adverse effect in hematological profiles that might affect normal behavior, immunity and growth of *L. bata*.

Introduction

About 300 types of pesticides and insecticides are used for crop protection in agriculture of Bangladesh. Water bodies like ponds, beels and lakes are continuously getting polluted due to indiscriminate use of these pesticides. Contamination of water bodies by pesticides can lead to fish mortality, reduced productivity and elevated concentration of undesirable chemicals in edible fish tissue which can affect the human health. Fishes are very sensitive to pesticide contaminated water and impairment of certain physiological and biochemical processes may occur when pesticides enter into the different organs of fish (Tulasi et al. 1992).

Pyrethroid pesticides are widely used for controlling the insect pests all over the world. Cypermethrin is a pyrethroid pesticide that is highly toxic insecticide and used to control insect pest in agriculture (Jee et al. 2005). Cypermethrin has a high rate of absorption in the gill of fish at very low concentration in the water. Like all pyrethroids, cypermethrin is highly toxic to fish and aquatic insects. Organophosphate like sumithion is also

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most commonly used pesticide in crop pest control (Senanayake and Johnson, 1992) and also in aquaculture for eradication of insect earlier to release of fish larvae. However, the toxicity of pesticides in aquatic organisms can be studied by evaluating the changes in the hematological parameters (Saravan et al. 2011). Hematological studies are frequently and routinely applied in the diagnosis of diseases in aquaculture (Ranzani-Paiva et al. 2008). A number studies (Binukumari et al. 2013; Jasmin et al. 2017; Khatun et al. 2014) have shown that different pesticides in aquatic environment have adverse effects on hematological profile of fish.

Labeo bata (Hamilton) is one of the important minor carp species in polyculture and rich-fish culture system in Bangladesh due to its faster growth and deliciousness. Thus, it is necessary to study the deleterious effects of pesticide on the hematology to provide data on pollution and health condition of this species. Though, many researchers have been reported alteration in the blood parameters in different fish species due to pyrethroid and organophosphate pesticides exposure in Bangladesh (Jasmin et al. 2017; Khatun et al. 2014; Mostakim et al. 2015) but, the researches on the effects of pyrethroid and organophosphate pesticides on the hematology of minor carps are so scares. Therefore, the study was conducted to investigate the effects of two selected pesticides, cypermethrin and sumithion exposure on the hematological profiles of a minor carp species, *Labeo bata*.

Materials and Methods

Experimental unit and site

The study was conducted in glass aquaria in the laboratory of the Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. The aquaria were rectangular in size (90×40×45 cm) containing 80 liters of water and providing aeration facilities. Fine meshed nets were used to cover the aquarium to avoid jumping of fish.

Test chemicals

A synthetic pyrethroid insecticide, cypermethrin that contains 100g active ingredients per liter and an organophosphorous pesticide, sumithion that contains 150g active ingredients per liter were used in this study.

Experimental design

Two experiments were carried out for a period of 96 hours under three treatments *viz.* T1, T2 and T3 with three replicates in each treatment. T1 was treated as control fish (for both experiment) and in the first experiment, fish were exposed to cypermethrin at 0.001µg/L (T2) and 0.003 µg/L (T3) whereas in the second experiment, fish were exposed to sumithion at 0.75 mg/L (T2) and 1.50 mg/L (T3), respectively.

Collection and maintenance of test fish

The specimens of *L. bata* were collected from a local fish farmer at Banesshor, Rajshahi, Bangladesh. The mean weight of the fish was 70.93±11.43 g and the length was 16.52±1.92 cm. After collection, the fish were transferred to the laboratory and released in the rectangular glass aquaria for acclimatization (7 days). During the study period, 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 and was aerated continuously through aerator. The experimental water was renewed every 24 hours to remove fecal matters and to maintain the better environment for the experimental fish.

Study of water quality parameters

Water quality parameters such as temperature, dissolved oxygen (DO) and pH were monitored by Centigrade thermometer, HACH Kit and pH meter, respectively.

Study of fish behavior

Feeding behavior and swimming movement as well as body color of the fish were monitored regularly with eye observation and taking video recording.

Study of blood profiles

At the end of the experiments, fish were harvested and blood samples were collected in a hematocrit tube containing anticoagulant agent (EDTA). The blood profiles such as total count of white blood cell (WBC) and red blood cell (RBC), hemoglobin content (Hb), pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were analyzed. Total WBC and RBC were counted by an improved Neubauer hemocytometer (Shah and Altindag, 2005). Hb level and PCV were

determined by cyanmethemoglobin method (Lee et al. 1998) and microhematocrit method (Hesser, 1960), respectively. MCV was calculated from the hematocrit value and the erythrocyte count (Feldman et al. 2000). MCH was calculated from the hemoglobin content and the erythrocyte count (Stoskopf, 1993). MCHC was calculated by dividing the hemoglobin content by the PCV % of red blood cell (Stoskopf, 1993).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) to test for the level of significance through SPSS software. The significance difference among the mean values were determined by Duncan's multiple range test at level 5% ($P<0.05$).

Table 1. Water quality parameters during the cypermethrin exposure

Parameters	Treatment		
	T1	T_2	T_3
Temperature (^{0}C)	27.30±0.15^a	27.40±0.15^a	27.54±0.06^a
DO (mg/L)	7.00±0.10^a	7.10±0.25^a	7.10±0.11^a
pH	7.60±0.06^a	7.54±0.10^a	7.60±0.06^a

- T1: Control fish, T2: Fish group exposed to 0.001 µg/L of cypermethrin and T3: Fish group exposed to 0.003 µg/L of cypermethrin. Values are mean of triplicate determination. Values in the same row with same superscripts are not significantly different ($P<0.05$).

Table 2. Water quality parameters during the sumithion exposure

Parameters	Treatment		
	T1	T_2	T_3
Temperature (^{0}C)	27.42±0.23^a	27.55±0.21^a	27.40±0.21^a
DO (mg/L)	7.03±0.11^a	7.05±0.09^a	7.13±0.13^a
pH	7.65±0.15^a	7.70±0.10^a	7.50±0.10^a

- T1: Control fish, T_2: Fish group exposed to 0.75 mg/L of sumithion and T_3: Fish group exposed to 1.50 mg/L of sumithion. Values are mean of triplicate determination. Values in the same row with same superscripts are not significantly different ($P<0.05$).

Table 3. Changes in hematological profiles due to cypermethrin exposure

Hematological Profiles	Treatments		
	T1	T2	T3
Total WBC ($10^4\times$ mm^{-3})	5.07±1.07^c	6.70 ±0.61^b	7.60±0.51^a
RBCs ($10^6\times$mm^{-3})	3.41 ±0.16^a	2.98 ±0.25^b	2.82 ±0.13^b
Hb (g/dl)	11.21±0.47^a	10.05 ±0.65^b	9.28 ±0.62^c
PCV (%)	33.38±1.07^a	30.15 ±2.01^b	28.33 ±1.67^c
MCV (fl)	97.97±1.11^b	99.94 ±1.99^a	100.60 ±1.90^a
MCH (pg)	32.90±1.38^a	33.24±1.66^a	33.29 ±1.67^a
MCHC (g/dl)	33.57±1.28^a	31.14±1.11^b	30.70 ±1.23^b

- T1: Control fish, T2: Fish group exposed to 0.001 µg/L of cypermethrin and T3: Fish group exposed to 0.003 µg/L of cypermethrin. Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

Table 4. Changes in hematological profiles due to sumithion exposure

Hematological Profiles	Treatments		
	T1	T2	T3
Total WBC ($10^4\times$ mm^{-3})	5.30±0.43^c	7.40 ±0.88^b	8.80±0.37^a
RBCs ($10^6\times mm^{-3}$)	7.65±0.40^b	7.90±0.29^b	8.45±0.42^a
Hb (g/dl)	2.54±0.13^b	2.63 ±0.11^b	2.82 ±0.14^a
PCV (%)	22.66±1.23^b	24.54 ±1.12^a	25.38 ±1.55^a
MCV (fl)	89.21±2.01^b	92.01 ±2.34^a	93.25±2.53^a
MCH (pg)	30.08±1.04^a	30.52±1.25^a	30.63±1.11^a
MCHC (g/dl)	33.73 ±1.31^a	30.31 ±1.21^b	30.17±1.13^b

- T1: Control fish, T2: Fish group exposed to 0.75 mg/L of sumithion and T3: Fish group exposed to 1.50 mg/L of sumithion. Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different (P<0.05).

Result

Water quality parameters

The recorded mean values of water quality parameters (*viz.*, temperature, DO and pH) during the study period are given in the Table-1 and 2. The water quality parameters were more or less similar among the treatments in both experiments.

Changes in fish behavior

During the study period, no death occurred in the experimental fish when exposed to the tested doses of cypermethrin and sumithion. When the fish were exposed to cypermethrin, showed some abnormal behaviors, primarily decreased their food intake and the fish exhibited uneasiness, irregular swimming and then showed lethargic movement. Similarly, when the fish were exposed to sumithion, primarily their food intake decreased and the fish exhibited restlessness and asymmetrical swimming activities and loss of equilibrium, motionlessness then showed lethargic movement. All fish showed upward down movement and loss their appetite.

Changes in hematological profiles due to cypermethrin exposure

The changes in hematological profiles (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) of *L. bata* due to cypermethrin exposure are shown in Table-3. After 96 hr of cypermethrin exposure, it was observed that the total WBC count and MCV value of the fish increased significantly and MCH value showed slight increase whereas the total RBC count, Hb level, PCV and MCHC value decreased significantly when compared to the control fish.

Changes in hematological profiles due to sumithion exposure

The changes in hematological profiles (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) of *L. bata* due to sumithion exposure are shown in Table-4. From the data, it was observed that the total WBC and RBC count, Hb level, PCV and MCV value increased significantly and the MCH values showed slight increase whereas the MCHC value decreased significantly in the sumithion exposed fish when compared to the control fish.

Discussion

Water quality parameters

During the present study, the observed water quality parameters of test water did not show any significant variation among the treatments in both experiments. Range of the water parameters were: temperature 25.3 to 25.5^{0}C and 25.4 to 25.5^{o}C; dissolved oxygen 7.0 to 7.1 mg/L and 7.0 to 7.05 mg/L and pH 7.5 to 7.6 and 7.5 to 7.7 in cypermethrin and sumithion treated aquaria, respectively. The values of recorded water quality parameters were within the productive ranges for fish growth according to the findings of Jhingran (1991).

Changes in behavior of the fish due to pesticide exposure

Fish behavior is a suitable index in toxicological studies against the aquatic pollutants. Pesticides can affect the fish behavior and can be used as an index of stress (Dobsikova et al. 2006). In the present study, the fish showed some abnormal behaviors when they were exposed to cypermethrin and sumithion. The abnormal

behaviors were decrease in food intake, restlessness, loss of equilibrium, motionlessness, asymmetrical swimming activities and lethargic movement. Similar behavioral changes have been reported due to cypermethrin exposure in *Poecilia reticulate* (Li et al. 2005), in *Cyprinus carpio* (Svoboda et al. 2001). Similar behaviors have also been reported in *L. rohita* due to cypermethrin and diazinon exposure (Khatun et al. 2014) and due to difenoconazole and thiamethoxam exposure (Jasmin et al. 2017). Absorption of pesticides in gills with respect to the lipophilic nature of them can cause respiratory limitations (Masud and Singh, 2013) that consequently might be resulted in abnormal behavior of the fish (Singh et al. 2004).

Changes in hematological profiles due to cypermethrin exposure

In the present study, cypermethrin was found to be toxic to *L. bata* by reflecting hematological profiles. The white blood cells play different role in responding to the presence of foreign organisms/substances in the body. The increase in the total WBC counts in the present study due to cypermethrin exposure is supported by the reports of Maheswaran et al. (2008). Similar reports for total WBC count have also been reported by Hossain et al. (2014). In this study, a decrease in total RBC count was observed in the cypermethrin exposed fish. Decreased RBC counts have also been reported by Khatun et al. (2014). Similar findings have also been reported by Parma et al. (2007) due to exposure of pyrithiods pesticides. In the present study, the Hb level decreased significantly with increasing concentration of cypermethrin. Decreased Hb level in different fishes due to different pesticides exposures have been reported by Jasmin et al. (2017) and Khatun et al. (2014). A significant decrease in PCV value has been reported by Svoboda *et al.* (2001) in *Cyprinus carpio* due to difenoconazole exposure and Khatun et al. (2014) in *Labeo rohita* due to diazinon exposure. These reports are supportive to the present study.

The values of MCV, MCH and MCHC are fully dependent upon the total RBC count, Hb level and PCV value (Binukumari et al. 2013). As the total RBC count, Hb level and PCV values were changed due to cypermethrin exposure, the MCV, MCH and MCHC values were more or less fluctuated. In this study, MCV values increased with increasing concentration of cypermethrin which is similar with the report of Mostakim et al. (2015). In addition, MCH value was increased in the cypermethrin exposed fish which is supported by the finding of Khoshbavar-Rostami et al. (2005) who reported an increase value of MCH in diazinon exposed fish. Jayaprakash and Shettu (2013) reported a decrease in MCHC value due to deltamethrin exposure. Similarly, a decrease in MCHC value was found in the present study.

Changes in hematological profiles due to sumithion exposure

Sumithion has negative effects on the hematological profiles of fish (Hossain et al. 2014). The number of WBC in fish may increase or decrease considerably due to the pesticide exposure. An increase or decrease in lymphocyte number may be the completive response of lymphoid tissues (Shah and Altindag, 2005). In the present study, total WBC count increased rapidly due to sumithion exposure which is supported by the report of Hossain et al. (2014). Similar results have also been reported by Maheswaran et al. (2008). An increase in total RBC count, Hb level and PVC value was also observed due to sumithion exposure. Sasikala et al. (2011) reported significant increase in total RBC, Hb level and PVC in *Channa striata* due to metasystox exposure which is supportive to the present study. Similar findings have also been reported by Kathya and Cláudia (2010) who found an increase in RBC count, Hb level and PVC in *Prochilodus lineatus* due to Roundup Transorb (glyphosate-based herbicide) exposure. Contrary results have been reported by Jasmin et al. (2017) and Khatun et al. (2014) for organophosphate pesticides exposure which might be due to the differences in the active ingredient of the pesticides.

In the present study, MCV values increased in sumithion exposed fish which is similar to the report of Hossain et al. (2014) who found an increase value of MCV in *Cyprinus carpio* due to sumithion exposure. In addition, an increase in MCH value in the sumithion exposed fish was also observed which is accords with the report of Rostami et al. (2005)] who found an increase value of MCH due to diazinon exposure. The MCHC value was decreased significantly in the sumithion exposed fish which is similar to the report of Jayaprakash and Shettu (2013).

Conclusion

This study concluded that exposure of cypermethrin and sumithion even at low concentration resulted significant changes in hematological profiles of *L. bata*. These changes might be potentially disruptive to the normal behavior, immunity and growth of the fish. Long term studies are required to draw more accurate conclusion on the effects of pesticides exposure on the hematological profiles of fish.

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Authors' Contribution

Conceptualization, MMR and MMR; Methodology, MMR and MMR; Investigation, MMR, JKS and MMR; Write-up, MMR, JKS and MMR: Supervision, MMR and MMR. All authors have read and agreed to the published version of the manuscript.

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