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Haematological Changes in *Labeo rohita* (H.) due to Exposure of Pesticides, Difenoconazole and Thiamethoxam

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Abstract:

As pesticide's pollution is a very common phenomenon in aquatic environment of Bangladesh, an attempt has been made to investigate the changes in haematological parameters of *Labeo rohita* (H.) due to exposure of two pesticides, difenoconazole and thiamethoxam for a period of 96 hrs (from 12th to 15th August, 2016) in the Laboratory of Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. Two experiments were conducted in eighteen glass aquaria under 3 treatments (T1, T2 and T3) in each experiment. In both experiments, T1 was assigned to the fish treated as control; in experiment-1, T2 and T3 were assigned to the fish treated with difenoconazole at 0.002 and 0.0.004 ml/L, respectively and in experiment-2, T2 and T3 were assigned to the fish treated with thiamethoxam at 0.05 and 0.10 mg/L, respectively. In this study, physico-chemical parameters (temperature, dissolved oxygen and pH) of test water, behaviour and haematological parameters (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) of the fish were analyzed with standard methods. During the study period, there was no significant difference in the physico-chemical parameters of test water among the treatments and were within the suitable ranges. When the fish exposed to the pesticides, their food intake decreased primarily, exhibited restlessness and sluggish swimming behavior. When the fish exposed to Difenoconazole, the total count of WBC and RBC, and the values of Hb, PCV and MCV decreased whereas the MCH and MCHC values increased in the fish of T2 and T3 compared to the control fish. On the other hand, when the fish exposed to Thiamethoxam, the total count of WBC and RBC decreased in the fish of T2 and T3 compared to the control fish. The Hb level in the fish of T2 showed minute decrease but in the fish of T3 significantly increased. The values of PCV, MCH and MCHC in the fish of T2 and T3 were significantly increased but MCV values showed decrease in the fish of T2 and T3. Therefore, the present study concluded that the exposure of difenoconazole and thiamethoxam pesticides produce an adverse effect in haematological parameters of the fish.

Key words: *Haematology, Change, Exposure, Pesticides, Difenoconazole, Thiamethoxam*

Introduction:

Due to injudicious and indiscriminate use of pesticides in crops field, water bodies are continuously getting polluted. Pollution due to pesticides, especially in aquatic ecosystems has become a severe problem. Direct or indirect contamination of water by pesticides can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of human [1]. Fishes are very sensitive to water contamination by

pesticides and may damage certain physiological and biochemical processes when pesticides enter the different organs of fish [2].

Among different pesticides, the synthetic pyrethroids are widely used in crop protection in the world. These pesticides are extremely toxic to fish [3]. Difenoconazole is a synthetic pyrethroid which is most extensively used against pests and this chemical is potentially more toxic to fish and other aquatic organisms [4]. Another pesticide, thiamethoxam is a systemic pesticide in the class of neonicotinoids that cause deleterious effect in fish [5]. Since, water contamination by pesticides imbalance the aquatic system and affect the fish health, hence, it is important to examine the toxic effects of pesticides on fish. Toxic effects of pesticides on aquatic organisms can be investigated by testing the changes in haematological parameters [6]. Studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the haematological parameters [7]. Blood is a pathophysiological reflector of the whole body and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants [8].

Labeo rohita is an Indian major carp which spawns in the marginal areas of flooded rivers in Bangladesh. During spawning season, they are particularly susceptible for pesticides pollution. Higher consumer preference and market demand have led to increase the culture practices of this fish species in Bangladesh. Thus, it is necessary to study the deleterious effects of pesticide on the haematology of this species. A number of studies have been reported on the effects of pesticides on haematology of various fish species in different parts of the world [9, 10, 11, 12, 13]. But, the researches on the effects of pesticides on the haematology of Indian major carp, *L. rohita* in Bangladesh are so scares.

Therefore, this study was conducted to investigate the changes in haematology of *L. rohita* due to exposure of two selected pesticides, difenoconazole and thiamethoxam.

Materials and Methods:

Experimental site and design:

The study was conducted in eighteen glass aquaria in the laboratory of the Department of Fisheries, University of Rajshahi, Bangladesh. Two experiments were conducted for a period of 96 hours (from 25th to 28th July 2016) under three treatments viz. T1, T2 and T3 with three replicates in each treatment. In both experiments, T1 was assigned to the fish treated as control (without pesticides). In experiment-1, T2 and T3 were assigned to the fish treated with 0.002 ml/L and 0.004 ml/L Difenoconazole and in experiment-2, T2 and T3 were assigned to the fish treated with 0.05 mg/L and 0.10 mg/L Thiamethoxam, respectively.

Maintenance of experimental fish:

The juvenile of *L. rohita* (body weight 111.25±12.13 g and length 17.31±2.20 cm) were collected and acclimatized in glass aquaria (90×40×45 cm) containing 90L of water for seven days. During the study period, the fish were fed with artificial feed twice daily. The water in the aquaria was aerated continuously with aerators. Constant amount of the test chemical and test water were renewed every 24 hours. Waste matters were removed regularly to maintain the better environment of the experimental units.

Study of physico-chemical parameters of water:

Physico-chemical parameters of water such as water temperature, dissolved oxygen (DO) and pH were monitored by Centigrade thermometer, HACH Kit and pH meter, respectively.

Study of fish behavior:

Behaviour (feeding, movement and swimming) as well as body colour of the treated fish was monitored regularly with eye observation.

Study of blood parameters:

Blood samples were collected in a haeamatocrit tube containing anticoagulant agent (EDTA). The blood parameters, total count of white blood cell (WBC) and red blood cell (RBC), haemoglobin level (Hb), pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed. Total WBC and RBC were counted by an improved Neubaeur haemocytometer. Hb level and PCV were determined by cyanmethemoglobin method [14] and microhaematocrit method [15], respectively. MCV and, MCH and MCHC values were calculated according to Feldman *et al.* [16] and Stoskopf [17], respectively.

Statistical analysis:

The mean values 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 (DMRT) at level 5% ($P<0.05$).

Results:

Physico-chemical parameters:

The physico-chemical parameters of test water including temperature, DO and pH were more or less similar among the treatments in both experiments. The recorded mean values of these parameters during the experimental period are given in the Table-1.

Table-1: Physico-chemical parameters of test water in Experiment-1 and Expepriment-2

Parameters	Experiment-1			Experiment-2		
	T1	T2	T3	T1	T2	T3
Temperature (^{O}C)	28.7 ±0.12	28.8 ±0.10	28.8 ±0.14	28.7 ±0.10	28.8 ±0.09	28.7 ±0.15
DO (mg/L)	6.6 ±0.10	6.5 ±0.15	6.4 ±0.22	6.6 ±0.10	6.6 ±0.14	6.4 ±0.11
pH	7.8 ±0.19	7.6 ±0.17	7.5 ±0.21	7.8 ±0.13	7.6 ±0.18	7.6 ±0.16

Changes in behavior of fish:

During the study period, no death occurred in the experimental fish when exposed to tested doses of Difenoconazole and Thiamethoxam. When the fish were exposed to Difenoconazole, primarily their food intake decreased and the fish exhibited uneasiness and irregular swimming and then showed sluggish movement. When the fish were released to higher doses (0.006-0.009 ml/L) rather than the tested doses (0.002 ml/L and 0.004 ml/L), all the fish were died within a short time. Similarly, when the fish were exposed to Thiamethoxam, primarily their food intake decreased and the fish exhibited restlessness and asymmetrical swimming activities and then showed lethargic movement. Moreover, severe paleness was also observed over the body surface when fish treated with 0.10 mg/L of Thiamethoxam. In addition, when the fish were released to higher doses (0.15-0.20 mg/L) of Thiamethoxam rather than the tested doses (0.05 and 0.10 mg/L), all fish showed upward down movement and loss their appetite but no fish were died.

Changes in haematological parameters due to difenoconazole exposure:

The changes in the haematological parameters (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) of the fish due to Difenoconazole exposure are shown in the Table-2. From the data, it was observed that the total count of WBC and RBC, and the value of Hb, PCV and MCV decreased significantly in the fish of T2 and T3 compared to the control fish though the decreased rate was higher in the fish of T2 than the T3, whereas the MCH and MCHC values showed an increase in the fish of T2 and T3 compared to the control fish though the increase rate was higher in the fish of T2 than the fish of T3.

Table-2: Haematological changes due to difenoconazole exposure (Experiment-1)

Parameters	Treatments		
	T1	T2	T3
WBC ($10^4\times$ mm^{-3})	6.64 ± 1.12^{a}	2.82 ± 0.12^{c}	4.42 ± 0.01^{b}
RBCs ($10^6\times$mm^{-3})	1.01 ± 0.10^{a}	0.81 ± 0.06^{c}	0.92 ± 0.08^{b}
Hb (g/dl)	2.66 ± 0.21^{a}	2.20 ± 0.20^{b}	2.30 ± 0.12^{b}
PCV (%)	14.0 ± 1.15^{a}	9.80 ± 0.40^{c}	11.30 ± 0.32^{b}
MCV (fl)	137.2 ± 8.65^{a}	86.10 ± 4.56^{c}	121.0 ± 5.37^{b}
MCH (pg)	26.3 ± 4.21^{b}	28.4 ± 0.80^{a}	26.5 ± 0.86^{b}
MCHC (g/dl)	18.9 ± 1.11^{c}	28.1 ± 2.10^{a}	22.1 ± 1.12^{b}

* T1 assigned the fish treated as control; T2 and T3 assigned to the fish treated with 0.002 and 0.004 ml/L of Difenoconazole, respectively. Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

Changes in haematological parameters due to Thiamethoxam exposure:

The changes in the haematological parameters (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) of the fish due to thiamethoxam exposure are shown in the Table-3. From the data, it was observed that the total count of WBC and RBC decreased significantly in the fish of T2, but it decreased slightly in the fish of T3 compared to the control fish. The Hb level in the fish of T2 showed minute decrease but in the fish of T3 significantly increased compared to the fish of T1 and T2. The values of PCV, MCH and MCHC in the fish of T2 and T3 significant increased whereas the MCV values showed significant decrease in the fish of T2 and T3 compared to the control fish.

Table-3: Haematological changes due to Thiamethoxam exposure (Experiment-2)

Parameters	Treatments		
	T1	T2	T3
Total WBC (10^4× mm^{-3})	6.64±1.12[a]	2.32±1.15[b]	5.57±1.08[a]
RBC (10^6×mm^{-3})	1.01±0.10[a]	0.73±0.10[b]	0.97±0.13[a]
Hb (g/dl)	2.66±0.21[b]	2.64±0.24[b]	3.50±0.42[a]
PCV (%)	14.0±1.15[c]	19.5±1.35[a]	16.1±1.41[b]
MCV (fl)	137.2±8.65[a]	124.1±3.32[b]	123.8±3.50[b]
MCH (pg)	26.3±1.21[b]	28.7±1.40[a]	26.9±1.36[b]
MCHC (g/dl)	18.9±1.11[c]	23.1±1.45[a]	21.7±1.50[b]

* T1 assigned the fish treated as control; T2 and T3 assigned the fish treated with 0.05 mg/L and 0.10 mg/L of Thiamethoxam, respectively. Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different (P<0.05).

Discussion:

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 [7]. Thus, water quality is one of the major factors, responsible for individual variations in fish haematology since they are sensitive to slight fluctuation that may occur within their internal environment [18]. To ignore the effect of physico-chemical parameters of water on fish haematology, all the parameters must be maintained within suitable ranges. In the present study, the values of temperature, DO and pH among the treatment did not show any significant variation in both experiments (Table-1). The values of temperature, DO and pH in water of both experiments were within in suitable range According to Jhingran [19], Bhuiyan [20] and Swingle [21], respectively.

Changes in behaviour of fish due to pesticide exposure:

Pesticides in aquatic environment can affect the behaviour of fish and can be used as an index of stress [22]. By changing behaviour, fish try to reduce the effects of pesticides enter in to the body from the medium or to minimize the damage of their body tissues. During this study, Difenoconazole and Thiamethoxam treated fish exhibited uneasiness, decrease food intake and sluggish swimming which is accordance with the report of Bradbury and Coats [23]. Moreover, severe paleness was also observed over the body surface of treated fish during the study period. The observed changes in behaviour of *L. rohita* might be due to the effect of pesticides on the central nervous system or the disturbances in physiological mechanism [24]. Similar behavioural changes have also been reported in *Cyprinus carpio* for Diazinon [25] and in *L. rohita* for cypermethrin and diazinon exposure [13].

Haematological changes due to Difenoconazole exposure:

Difenoconazole is proved to be toxic for fish due to poor metabolize ability [12]. In our study, Difenoconazole was found to be toxic to *L. rohita* by changing haematological parameters. The number of WBC in fish may increase or decrease considerably due to pesticide exposure [26]. In this study, significant decrease in total WBC count was observed during exposure to Difenoconazole. Decrease in total WBC count reflects a state of stress in fish and points to the role of Difenoconazole as a potential environmental stressor. Stress-induced lowering of total WBC count has been reported in *Cyprinus carpio* due to cypermethrin exposure [27]. The decrease in total WBC count in the present study is supported by the findings of Khoshbavar-Rostami *et al.* [28] and Pourgholam *et al.* [29]. Like WBC count, a decrease in total RBC count was also observed in the difenoconazole treated fish. Due to the pesticide exposure, similar results for total RBC count have been reported by Santhakkumar *et al*. [30] and Das and Mukherjee [31]. Adhikari *et al*. [8] and Parma *et al*. [32] also reported significant decrease in total RBC in freshwater fish due to cypermethrin exposure, which is supportive to the present study.

In our study, the Hb level showed a decrease in the difenoconazole treated fish. According to Kocabatmaz and Ekingen [33], pesticide had a negative effect on Hb level which is identical to the present study. Similar results have also been reported by Dobsicova *et al*. [22] and Deka and Dutta [34]. The observed decrease in Hb level in the present study might be due to either an increase in the rate at which haemoglobin is destroyed or a decrease in the rate of Hb synthesis [35]. In case of PCV value, a significant decrease was observed in the difenoconazole treated fish which is supported by Svoboda *et al.* [25] and Pourgholam *et al.* [29]. The decreased value of PCV might be due to the fish loses its appetite or it is poisoned by pesticide [36].

The MCV, MCH and MCHC values are completely dependent upon the RBC count, Hb, PCV and also

due to stress condition [37]. In the present study, RBC count, Hb level and PCV values were changed in the difenoconazole treated fish. So, the values of MCV, MCH and MCHC were affected indirectly. The MCV values decreased significantly in the difenoconazole treated fish, which is supported by Atamanalp and Yanik [38]. Moreover, an increase in MCH values was observed in the difenoconazole treated fish, which is similar to the findings of Parma *et al*. [32]. Francesco *et al*. [39] described the increase of MCHC values for Grey Mullet when treated with pesticide which is supportive to the present study. Significant decrease in MCHC values due to pesticides exposure was also observed by Parma *et al*. [32]. The changes in MCHC values might be due to the stress condition created by the pesticide [40].

Haematological changes due to Thiamethoxam exposure:

The pesticide, thiamethoxam was found to be toxic to fish and other aquatic life causing notable changes in haematological parameters [41]. In our study, total count of WBC decreased rapidly when fish treated with thiamethoxam at 0.05 mg/L, but it slightly decreased when treated at 0.10 mg/L. An increase or decrease in lymphocyte number may be the compensatory response of lymphoid tissues [42]. Such lymphocyte response might be due to the presence of toxic substances or pollutants [43]. The exposure of thiamethoxam caused decrease in RBC count in the treated fish. Decreased RBC counts have also been reported in *Cyprinion watsoni* [9] and in *Piaractus mesopotamicus* [44] due to use of various pesticides.

During this study, Hb level in the treated fish decreased at 0.05 mg/L but increased at 0.10 mg/L of thiamethoxam. The increase or decrease in haemoglobin concentration might be due to either an increase or decrease in the rate of haemoglobin synthesis [35]. Similar findings have also been reported by Shamoushake *et al.* [45] and Far *et al.* [46] using various pesticides. The exposure of thiamethoxam showed an increase in PCV values which are similar with the findings of Aziz *et al.* [47] who found an increase of PCV in *Tilapia mossambica* exposed to mercuric chloride.

In addition, MCV values decreased in thiamethoxam treated fish which is similar with the findings of Atamanalp and Yanik [38]. Soltani and Khoshbavar-Rostami [48] investigated the effects of diazinon on *Acipenser gueldenstaedtii* and found lower MCV value in the experimental fish which is supportive to the present findings. Furthermore, an increase in MCH value in the treated fish was found which is supportive by the findings of Khoshbavar-Rostami *et al*. [28] who reported an increase value of MCH in diazinon treated fish. The MCHC value was also increased in the thiamethoxam treated fish. Similar result was reported by Chandrasekar and Jayabalan [49]. The changes in MCHC values might be due to the decrease in Ht level in treated fish caused by haemolysis or stress condition [40].

Conclusions:

Exposure of difenoconazole and thiamethoxam produces an adverse effect in haematological parameters which affect the normal behaviour of the fish. These changes might be potentially disruptive to the survivability of the fish in their natural environment. The measuring of haematological parameters in the present study provides valuable information which might be helpful for fishery managers and biologists in the assessment of fish health.

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