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Haematological status of common carp, *Cyprinus carpio* L. exposed to sublethal dose of organophosphorus pesticide, monocrotophos

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Abstract
The introduction of organophosphorus insecticide into the aquatic ecosystem through agricultural runoffs, rainwater seepage adversely affects many non-target organisms including fishes. The chronic effect of organophosphorus pesticide, Monocrotophos on the haematological parameters of freshwater fish, *Cyprinus carpio* has been investigated in the present study. *Cyprinus carpio* were procured and their sublethal, median lethal and lethal concentrations for 120 hrs were assessed. Two sub-lethal concentrations (T2) and (T3) were selected to which the fishes were exposed for 30 days. Control set up without monocrotophos (T1) was run simultaneously. Blood from anaesthetised fishes were collected after 30 days of exposure period. Hemoglobin (Hb), Red Blood Cells (RBCs) and hematocrit (Hct) significantly decreased with the increasing concentration of monocrotophos during the experimental periods. But White Blood Cells (WBCs) significantly increased in the treated fishes when compared to the control depicting negative effects of monocrotophos on the hematological parameters of *Cyprinus carpio*.

Keywords: *Cyprinus carpio*, chronic effect, haematology, monocrotophos, sublethal concentration

1. Introduction

Applications of pesticides have been increasing ever since the onset of green revolution to feed the overgrowing population of the world. This phenomenon is evident over the last few decades especially in tropical countries like India, where majority of the population is reliant on agriculture for their livelihood. Now the use of pesticides has become inevitable in current agriculture practices to ensure increased productivity through the pest control. However, the indiscriminate use of pesticides ultimately leads to pollution of aquatic environment and becomes hazardous to the non - target aquatic life. Among the innumerable aquatic organisms, fishes occupy an important position in the field of aquatic toxicology (Giulio and Hinton, 2008) [7] not only because of their position at topmost trophic level of the aquatic food chain but also due to the high accumulation of pesticides in their body by bioaccumulation and biomagnification processes. Hence different concentrations of insecticides and pesticides present in water bodies found their way to fishes affecting these non-target species (Talebi, 1998; Uner *et al.*, 2006; Banaee *et al.*, 2008) [18, 20, 1].

Various stressors and pollutants generally cause rapid changes in biological characteristics of fish (Sahan *et al*., 2007) [15]. These changes can be measured and used as indicators or biomarkers for effects of such toxicants. These biomarkers enable the rapid assessment of the health of the organisms and warn about environmental risks associated with the toxicants. Among biological changes, haematological parameters are considered potential biomarkers of exposure to toxicants, since the latter can induce an increase or decrease in the various haematological parameters (Oost *et al*., 2003) [12]. Blood is the most important and abundant body fluid acting as a vehicle for quickly mobilizing defense against trauma and diseases. Its composition often reflects the total physiological condition of the body. The main route of entry for any pesticide is through the gills from which it gets transported to various parts of the body via the blood stream. Henceforth blood provides an ideal medium for toxicity studies and haematological variables of fish under stress are of great significance in assessing the impacts of pollutants in the biota of a particular ecosystem.

Among the various pesticides used in farming practices, the organophosphate pesticide, Monocrotophos often referred to as the "farmer's benevolent pesticide" is most preferable by farmers because of their low cumulative ability, high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment. Though much studies have been carried out on the acute toxicity of monocrotophos on different aspects of fishes, the research on its chronic effects are scanty. To ascertain the same, the current study has been undertaken wherein the commonly available freshwater fish, *Cyprinus carpio* were exposed to sublethal doses of monocrotophos for a period of 30 days to assess the haematological alterations induced by it. Such a study is vital as it not only assesses the health of fish subjected to changing environmental conditions but also to monitor the long term deleterious effects of the so called sublethal doses of various pesticides on aquatic life.

2. Materials and Methods

2.1 Selection of test animals

Live and healthy Common carp *(C. carpio*) fingerlings of length 10 ± 0.9 cm; weight 20 ± 0.8 g were collected from a commercial fish farm at Maruthur village in Nagapattinam District, Tamil Nadu and brought to the laboratory in plastic bags with sufficient air without causing any injury. The fishes were disinfected by treatment of 0.05% potassium permanganate, stocked and maintained in aquaria under a controlled natural photo-regimen (14/10 h, light/dark) and suitable temperature to acclimatize for a period of at least 20 days before starting the experiment. The fingerlings were fed with commercial feeds twice a day respectively.

2.2 Selection of toxicant

The commercial grade formulations of Organophosphorous pesticide, Monocrotophos (36% SL) which is commonly used in agricultural fields was used as a toxicant in the present study. This pesticide was collected from a retailer shop at Mayiladuthurai.

2.3 Experimental design

A stock solution of Monocrotophos (36% SL) was prepared by dissolving 1 ml of pesticide in 1000 ml acetone to maintain the standard concentration of 1 ppt in the container from which different concentrations of Monocrotophos ranging from 5 ppm to 100 ppm with an interval of 10 ppm were prepared and ten fishes were subjected to each concentration. A control group having ten fishes kept in water without toxicant was run concurrently. The mortality of fishes were recorded at five different exposure periods viz. 3, 6, 12, 24, 48, 72, 96 and 120 hrs. The median lethal concentration LC_{50} for 3. 6, 12, 24, 48, 72, 96 and 120 hrs were computed by the Probit method (Zubair Ahmad, 2011). Based on the acute toxicity tests, two sublethal concentrations viz. 1/5th and 1/10th of 120 hrs LC_{50} were selected for chronic toxicity. The experiment was conducted with a control (T1), and two treatments 1/5th of 120 hr LC_{50} (T2) and 1/10th of 120 hr LC_{50} concentration (T3) respectively. each with three replications. At the end of 30 days of exposure period, five fishes from each set were sacrificed for assessing hematological parameters.

2.4 Blood sampling and measurement of hematological parameters

The fish specimens were anaesthetized with clove oil and 1 ml of blood was obtained by caudal vein puncture into citrated tuberculin syringes and placed in glass tubes containing EDTA while the fishes were sedated. Blood samples were immediately analyzed for the estimation of hematological parameters. Hemoglobin (Hb; g/dL) of samples was estimated by acid hematic method (Hawk *et.al*. 1965). Hematocrit (Hct; %) values were determined by micro-hematocrit centrifuge using hematocrit tube. The Red Blood Cell (RBC; $x10^6/mm^3$) and White Blood Cell (WBC; $x10^3/mm^3$) count was made using Neubauer haemocytometer. The mean corpuscular volume (MCV; μm^3), the mean corpuscular hemoglobin (MCH; pg) and the mean corpuscular hemoglobin concentration (MCHC; %) were calculated using the following formulas:

MCV = (% of Hct/RBC in millions) x 10 μm^3
MCH = (Hb in g/RBC in millions) x 10 pg
MCHC = (Hb in g/ (% of Hct) x 100 g per 100 mL

2.5. Statistical analysis

The haematological parameters were represented as mean ± standard error of mean (SEM). The differences in the haematological parameters among the fish group exposed to different sublethal concentrations of monocrotophos were subjected to one-way ANOVA followed by Duncan's multiple range test to determine the significant difference at 5% probability level.

3. Results

3.1. Acute toxicity

The LC_{50} values of Monocrotophos on *Cyprinus carpio* for 3, 6, 12, 24, 48, 72, 96 and 120 hrs were calculated by Probit method (Finney, 1971). In the present study the sublethal, median lethal and lethal concentration of Monocrotophos for 120 hrs in *Cyprinus carpio* were assessed as 100 ppm, 155.3 ppm and 250 ppm respectively. For chronic exposure, two sublethal concentrations, 31.0 ppm ($1/5^{th}$ of 120 hrs LC_{50}) and 15.5 ppm ($1/10^{th}$ of 120 hrs LC_{50}) were used to which the fishes were exposed for 30 days to assess haematological changes.

3.2. Hematological parameters

In the present study, the alterations in the haematological parameters were investigated in *Cyprinus carpio* after exposure to sublethal concentrations of Monocrotophos. The organophosphorus pesticide had shown considerable impact on the blood parameters. The changes induced by the toxicant exposure in different blood parameters were given in Table - 1. In the present study, a significant decrease was observed in RBC count and platelet count at the end of exposure period while the WBC count showed a significant increase in the two treatments when compared to the control ($p<0.05$). Similarly while the Hb value showed a significant decrease, the other blood indices such as MCV, MCH, MCHC showed a significant increase when compared to the control (Fig. 1).

Table 1: Changes in the haematological parameters of Cyprinus carpio at the end of 30 days of exposure to Monocrotophos

Parameters	Control sample (T1)	Treated (T2)	% change over control	Treated (T3)	% change over control	*p* value
WBC count x 10^3	145.7 ± 1.76	178.3 ± 0.67	22.37	155.0 ± 1.22	6.38	0.000
RBC count x 10^6	1.36 ± 2.74	0.8 ± 1.67	-41.17	1.1 ± 0.22	-19.11	0.000
Lymphocytes x 10^3	9.6 ± 0.48	35.7 ± 0.80	71.82	30.1 ± 1.09	13.54	0.000
Monocytes x 10^3	41.7 ± 0.66	55.7 ± 0.95	33.57	49.3 ± 0.88	18.22	0.000
Granulocytes x 10^3	93.6 ± 0.95	106.6 ± 1.64	13.88	101.6 ± 1.26	8.54	0.000
Haemoglobin	11.3 ± 0.88	8.3 ± 0.06	-26.54	10.1 ± 0.25	-10.61	0.027
Haematocrit	25.6 ± 0.75	15.7 ± 0.93	-38.67	21.3 ± 1.17	-16.79	0.000
MCV	175.5 ± 0.77	223.5 ± 1.47	27.35	193.0 ± 3.46	9.97	0.000
MCH	64.6 ± 0.08	143.7 ± 0.68	12.44	129.3 ± 1.99	10.15	0.000
MCHC	35.9 ± 0.89	65.0 ± 0.92	81.05	47.3 ± 2.89	31.75	0.000
Platelet count x 10^3	120.4 ± 0.20	165.4 ± 2.02	37.37	145.1 ± 5.83	20.51	0.000
MPV	9.3 ± 0.18	9.4 ± 0.23	1.075	9.2 ± 0.17	-1.07	0.757

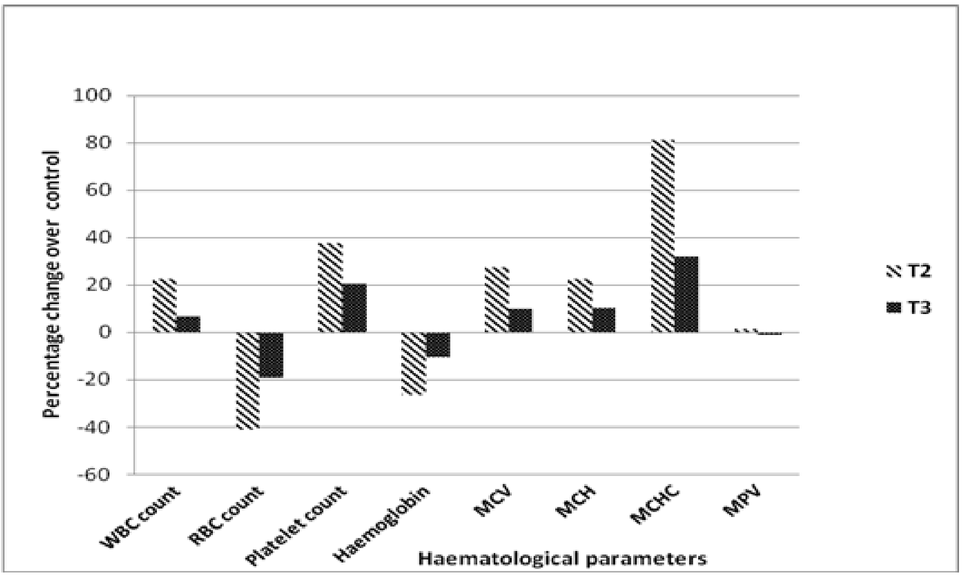

Fig 1: Haematological alterations in *Cyprinus carpio* exposed to sublethal concentrations of Monocrotophos

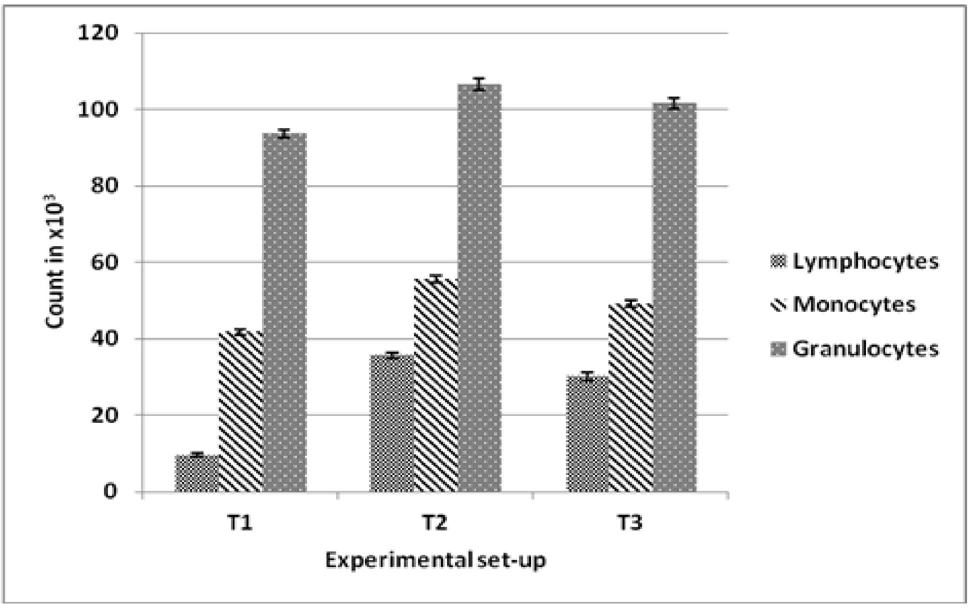

Fig 2: Changes in the differential count of WBC of *Cyprinus carpio* exposed to sublethal concentrations of Monocrotophos

The differential count of WBC revealed a highly significant increase in the lymphocytes, followed by monocytes and granulocytes in both the treatments T2 and T3 when compared to the control T1. All the parameters of WBC differential count showed a greater increase in the 1/5th concentration (T2) when compared to 1/10th concentration (T3) (Fig. 2).

4. Discussion

The use of haematological techniques in fish culture has growing importance for toxicological research, enironmental inspecting and fish health conditions. Several studies were carried out on the haematological changes in fish as a result of pesticides by Das and Mukherjee (2000), Kumar (2010) [11]. Ovie *et al*., (2012) [13] reported a significant variation in the blood parameters of diagnostic significance such as erythrocyte and leukocyte differential counts that readily responds to physical and environmental stress caused by the water quality. Thus any change in the haematologial parameters are good reflectors of physiological changes. The exposure of fish to chemical pollutants can either induce an increase or decrease in the haematological levels.

The results of the present study are in good agreement with earlier work that reported a decrease in RBC count, haemoglobin content and platelet count of freshwater fish exposed to toxicants (Blahova *et al.,* 2014) [3]. The RBC count, haemoglobin and PCV decreased in the Common carp fingerlings while other indices like MCV, MCH and MCHC values were increased in all exposures in this study. The decrease in RBC and Hb content indicates acute anemia in exposed fingerlings which may be either due to destruction of RBC or due to damage to the haemopoietic tissue. Decreased RBC count and Hb content in *Cyprinus carpio* after acute exposure to another organophosphorous pesticide, diazinon were also reported by Svoboda *et al.,* (2001) [17]. Other effective substances of organophosphorous pesticides also induce changes which give evidence for decreased hematopoiesis followed by anemia induction in fish. It justifies changes in erythrocyte profile induced by acute effect of dichlorvos in *Clarias batrachus* (Benarji & Rajendranath. 1990) [2], formothion in *Heteropneustes fossilis* (Shrivastava *et al.,* 1989) [16], malathion in *Cyprinion watsoni* (Khattak & Hafeez. 1996) [10], and trichlorphon in *Piaractus mesopotamicus* (Tavares *et al.,* 1999) [19]. The disruptive action of the pesticides on the erythropoietic tissue results in the loss of viability of the cells. A significant decrease in the haemoglobin and haematocrit values in the present study correlated with the results of Rahaman *et al*., 2002 in *C. punctatus* and *B. gonionotus*. It indicates that the fingerlings are under chemical stress which may lead to pathological conditions in the tissues.

Similarly white blood cells in fishes respond to various stressors including infections and chemical irritants. Thus increase in the number of white blood cells are a normal reaction to a toxicant, which demonstrates the protective responses of immune system under toxic conditions (Guedenon *et al.,* 2012) [8]. The increase in the WBC count can be correlated with an increase in antibody production, which helps in survival and recovery of the fish exposed to sublethal concentrations of pesticide (Joshi and Tsai, 2002) [9]. Another type of hematological response to the effect of organophosphorous compounds was a significant increment of MCV and MCH. The alterations in erythrocyte constants such as MCV, MCH and MCHC are may be due to defense against the toxic effect of toxicant. The increase in these parameters in the present study clearly indicates the reduction in cellular blood iron resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis. MCH is a good indicator of RBC swelling. The significant increase in the MCHC values in the present study is may be due to direct or feedback responses of structural damage to red blood cell membranes resulting in hemolysis and impairment in hemoglobin synthesis and stress related release of red blood cells from the spleen and hypoxia induced by exposure to a toxicant (Patel *et al.,* 2009) [14].

5. Conclusion

Blood parameters are often measured when clinical diagnosis of fish physiology is applied to determine the sub-chronic effects of pollutants. The use of haematological parameters (haemoglobin, haematocrit, blood cell counts, glycemia and ion concentrations), can indicate a physiological response to a contaminated environment (Dethloff *et al.,* 2001) [5]. In the present study the changes in the haematological parameters of monocrotophos exposed *Cyprinus carpio* fingerlings reveals a negative impact of the toxicant. Finally it can be concluded that the long term exposure of even sublethal concentration of this toxicant can cause countless abnormalities and reduce the life span of aquatic organisms.

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