

Sub-Lethal Effects of Monocrotophos on Some Haematological Indices of African Catfish *Clarias gariepinus* (Teugels)

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Abstract: The effects of sub-lethal concentrations, 2.51, 5.02 and 10.04 mg L⁻¹ of monocrotophos on some haematological indices of *Clarias gariepinus* were investigated in the laboratory. The packed cells volume and TEC were significantly reduced ($p > 0.05$) in fish exposed to the toxicant concentrations than in the control groups. However, the TLC, neutrophils and Lymphocytes were observed to be significantly higher ($p < 0.05$) in the exposed fish compared to the control groups. The study shows that sublethal concentration of monocrotophos is harmful to *Clarias gariepinus*, therefore, the usage close to water bodies for fish production is dangerous.

Key words: Monocrotophos, effects, haematological indices, *Clarias gariepinus*

INTRODUCTION

Aquatic pollution from pesticides results mainly from their widespread use in agriculture and in vector control programme. These may enter the aquatic environments directly by their introduction to attack a particular organism, such as in pest control programmes or indirectly, through atmospheric precipitation and surface runoff. Pesticides represent one group of agrochemicals that have a history of causing toxic effect in fish and other forms of aquatic life and represent one of the most popular used compounds that are xenobiotic in nature (Avoaja and Oti, 1997). Water pollution by pesticides is a serious problem in most aquatic fauna and flora and to a considerable extent man. The toxicities of insecticides vary depending on the type of insecticide and the animal species involved (Vasauthi *et al.*, 1989).

Monocrotophos (an organophosphate) insecticide is a widely use pesticide in Nigeria mainly applied against cotton, citrus, olives, rice, maize, sorghum sugarcane, vegetables, ornamentals and tobacco pests.

Scanty work has been done on the effect of pesticides on Nigerian fish species (Ita, 1993). Haematological parameters act as physiological indicators to changing external environment (Gill and Pant, 1981) as a result of their relationship with energetic (metabolic levels), respiration (haemoglobin) and defence mechanisms (leucocytes levels). These parameters provides an integrated measure of the health status of an organism which overtime manifest in changes in weight (growth). This study investigated the effect of monocrotophos on haematological indices of a common Nigerian fresh water fish *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental fish: Healthy juveniles of *Clarias gariepinus* (average weight 18.5 g and standard length 10.7 cm) of mixed sexes were obtained from river Galma in Zaria, Kaduna State, Nigeria. The fish were transported in cold plastic container to the laboratory in the Department of Biological Sciences, Ahmadu Bello University, Zaria. In the laboratory, the water from the river was gradually replaced with dechlorinated tap water in large baths of 160 L capacity at 24.5-27.5°C and acclimatized for two weeks. The natural day and night photoperiod were maintained, during this period, the fish were fed with pelleted feeds containing 35% crude protein twice per day at 5% body weight.

Sub-lethal bioassay: From the results of the 96h LC₅₀, sub-lethal concentrations of 2.51, 5.02 and 10.04 mg L⁻¹ of monocrotophos were obtained (Oladimeji and Ologunmenta, 1987; Mohammad, 1995). Ten *Clarias gariepinus* juveniles were randomly selected and exposed to each concentration per aquarium with dimensions 30.5×30.5 and 46.25cm for 8 weeks given a total of 120 specimen. The experiment was replicated three times with the control. Fresh solution was added at two days intervals to maintain the concentration level of the toxicant after the test water renewed and level of dissolved oxygen during the experiment (APHA, 1985).

Haematological studies: Two fish were sacrificed biweekly from each test group and blood collected by the severance of the caudal peduncle, 2 cm away from the caudal region (Blaxhall and Danself, 1973).

Microhaematocrit (packed cells volume): Blood from the severed caudal peduncle was drawn directly into the microhaematocrit tubes. The tubes were then centrifuged in a microhaematocrit centrifuge for 5 min. The readings were made with the aid of a Hawsley microhaematocrit reader and expressed as the volume of the erythrocytes per 1000 cm³.

Total Erythrocyte Counts (TEC): Blood from the caudal peduncle, was drawn into haemoglobin pipette just beyond 0.5 mark and wiped with cotton wool to adjust the volume to exactly 0.5 mark. The pipette was filled to 101 mark with Hendrick's solution and shaken for 30 min to ensure mixing. The diluted suspension of cells was drawn into Neubaur's chamber haemocytometer. The number of cells were counted and multiplied by $\times 10^6$, which gave the total number of cells per mm³.

Total Leucocyte Counts (TLC): Blood was drawn upto 0.5 mark on the stem of the white pipette. Shaw's solution 'A' was drawn to shaken the bulb of the pipette half way and then filled to 101 mark with solution 'B'. Few drops was dispensed into haemocytometer. The cells in the four large squares of the chamber were counted and multiplied by 500, which gave the total number of cells per mm³.

Leucocyte differential counts: One or two drops of blood from the severed caudal peduncle was placed on a slide and made into thin smear with another slide and left to dry. The smear was fixed with absolute methanol, stained with Giemsa's stain and buffered with distilled water. It was allowed to stand for about 20-30 min and washed again with buffered distilled water, then allowed to air dried. The slide was examined under the oil immersion objective of the microscope and the cell platellets counted with the aid of leucocytes counting machine.

Statistical analysis: Analysis of Variance (ANOVA) and Duncan multiple range test were used to test for test for differences between different levels of treatment and to separate means respectively (Duncan, 1955). Test of significance were at 95% probability.

RESULTS

It was observed that the means values of haematocrit (packed cell volume) and Total Erythrocyte Counts (TEC) were significantly higher in the control fish compared to the exposed groups (Table 1). The haematocrit and TEC were dose-dependent. The highest values were observed in the lowest concentration (2.51 mg L⁻¹) and lowest values in the highest concentration (10.04 mg L⁻¹). However, the total leucocyte counts was observed to be significantly lower

Table 1: The effects of sub-lethal concentrations of monocrotophos on haematological parameters of *Clarias gariepinus*

Parameters			
Conc. (mg e ⁻¹)	Haematocrit (%)	TEC ($\times 10^6$ mm ³)	TLC ($\times 500$ mm ³)
0.00	33.67 ^a	190.00 ^a	16900.00 ^d
2.51	33.50 ^a	168.33 ^b	22527.75 ^c
5.02	29.25 ^b	151.25 ^c	24832.00 ^b
10.04	21.75 ^c	126.25 ^d	32256.00 ^a

Means with the same superscript in the same column are not significantly different (p>0.05)

Table 2: The leucocyte differential counts of *Clarias gariepinus* exposed to sub-lethal concentrations of monocrotophos

Conc. (mg e ⁻¹)	Neutrophils (%)	Lymphocytes (%)
0.00	5.00 ^c	82.8 ^c
2.51	15.6 ^b	83.5 ^{bc}
5.02	16.40 ^{ab}	83.8 ^b
10.04	16.80 ^a	94.1 ^a

Means with the same superscript in the same column are not significantly different (p>0.05)

(p>0.05) in the control group than fish exposed to the toxicant. The lowest TLC value was recorded in fish exposed to 2.51 mg L⁻¹ and highest in fish exposed to 10.04 mg L⁻¹ (Table 1).

Table 2 shows the leucocytes differential counts of *Clarias gariepinus* exposed to the toxicant concentrations. Lower values of neutrophils and lymphocytes were obtained in the control fish compared to the exposed groups of the toxicant. Neutrophils and lymphocytes values were dose-dependent. The lowest values were obtained in fish exposed to 2.5 mg L⁻¹ and the highest in 10.04 mg L⁻¹ of the toxicant.

DISCUSSION

This study revealed that *Clarias gariepinus* exposed to sub-lethal concentrations of monocrotophos clearly recorded a dose-dependent reduction in packed cells volume and total erythrocyte counts as concentration of the toxicant increased, in which all were significantly lower (p>0.05) than the control groups. This is an indication of severe anaemia. The anaemic effect could be due an inhibition in erythrocyte production and haemodilution. Annes (1978) reported haematological abnormalities in *Channa puntatus* exposed to sub-lethal and chronic levels of three organophosphorus insecticides that the blood was affected prior to any other visible changes in fish. Anaemia associated with erythropenia was reported by Srivastava and Mishra (1979) in *Colisa fasciatus* after acute exposure to lead. Similar findings were reported on several fresh water fishes (Khalaf, 1999; Balathakur, and Bais, 2000; Rehulka, 2000; Gbem *et al.*, 2003). Seth and Saxena (2003), reported a reduction in RBC and HB in *Channa pantatus* exposed to sub-lethal concentrations of fenvalerate. However, the Total Leucocyte Counts (TLC) was lower in

the control fish compared to those exposed to the toxicant. The increased in TLC in the exposed fish was dose-dependent. The changes in leucocyte counts could be due to immunological reactions to produce antibodies to cope up with stress induced by monocrotophos. The increased in white blood cells count can be correlated with an increase in antibody production which helps in survival and recovery of fish exposed to sub-lethal concentrations of pesticides (Joshi *et al.*, 2003). Seth and Saxena (2003) reported an increased in TLC in *Channa punctatus* exposed fenvalerate intoxication.

Present investigation revealed lower values of neutrophils and lymphocytes in the control fish than the exposed groups, in which all are significantly lower ($p > 0.05$) than the exposed fish (Table 2). 2.51 mg L⁻¹ recorded lowest values of neutrophils and lymphocytes and highest values were obtained in 10.04 mg L⁻¹ of the toxicant. This could be probably related to increased immunity in the exposed groups. The changes in leucocytes differential counts gave an evidence for decreased level of non-specific immunity in fish after exposure to toxic substance (Svoboda *et al.*, 2003). It was also observed that lymphocytes were more numerous compared to neutrophils. Babatunde (1997) reported that lymphocytes constitute the majority of white blood cells present in the peripheral blood of *Oreochromis niloticus*.

This investigation revealed that, exposure of *Clarias gariepinus* to low concentration of monocrotophos affects the physiology of this fish, such as reduction in volume of red blood cells. Therefore, it is dangerous to use this pesticide close to water bodies used for fish production.

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