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Haematological effects of sublethal concentrations of tobacco leaf dust on the African catfish: *Clarias gariepinus* (Burchell 1822)

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ABSTRACT

The effect of sublethal concentrations of tobacco leaf dust on the haematological parameters of the African Catfish, Clarias gariepinus, mean weight 108.69± 4.02g and mean length 23.00±0.86cm was investigated using static renewal bioassay system during a 7days exposure period. The result obtained revealed significant difference (P<0.01) in the haematological parameters examined (haematocrit, haemoglobin, total erythrocytes count and mean erythrocyte haemoglobin concentration) depicting a proportional decrease with an increase in the toxicant concentration during the exposure period. The haematological parameters (mean erythrocyte volume, mean erythrocyte haemoglobin) showed insignificant differences in the exposed fish compared to the control. Haematological examination showed that there was destruction of the erythrocytes production and the concentration of haemoglobin in the RBC was much lower in the exposed fish compared to the control depicting an anaemic condition.

Keywords: *Nicotiana tobaccum*, tobacco leaf dust, haematocrit, haemoglobin, total erythrocytes count, haematological indice, *Clarias gariepinus*, Nigeria.

INTRODUCTION

Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters [1]. The use of haematological technique in fish culture has made it possible for researchers to use it in environmental monitoring and fish health conditions [2]. Blood analysis is crucial in many fields of ichthyological research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation [3]. Haematological indices are very indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases [4 – 5]. Sampath *et al* [6] noted that studies in fish blood lies in the possibility that

the blood will reveal conditions within the body of the fish long before there is any outward manifestation of disease.

The attraction of tobacco leaf dust as a biocide according to Aleem [7] is because of its inexpensiveness, local availability and easy degradability. Control of mollusk in fish pond can be accomplished by using tobacco waste [8]. In Taiwan, tobacco waste dust is applied at 1 ton/acre as a pesticides and fertilizer in fish ponds [9]. Tobacco dust, a by-product of the cigarette industry, is locally available, inexpensive, easy degraded and serves as an organic fertilizer. The active ingredient of tobacco is nicotine which is lipophytic in nature with high solubility in membrane lipid and fast influx into cells. It is easily absorbed in body and can penetrate the epithelial and blood cells [10].

The wide ranges of toxicological effects of tobacco leaf dust on various fish species and certain mammals have been studied. Tobacco leaf dust is a dry green leaf prepared which is widely used in aquaculture as pesticide and in food, health as medicine for the treatment of earache, toothache, as a poultice and locally, it could be used to scare snakes from the environment, textile and other industries for other purposes. It controls fungal attacks, protozoan infections and some other diseases caused by helminthes on a wide variety of fish and other aquatic organisms.

The use of *Nicotiana tobaccum* leaf dust to clear fish ponds of predators and fish weeds before stocking has been documented in Nigeria[11 – 13]. Tobor [14] reported that these predators are large enough to take significant toll on the stocked fish if not eradicated before the ponds are stocked thus the need to study the sublethal effects of the pesticide / piscicides on the biochemical parameters of the non-target organism.

In the study conducted by Omoniyi and his co-workers [12] reported 48 hours LC_{50} of tobacco leaf dust on the African catfish – *Clarias gariepinus* to be 626.0mg/L, sublethal leaf exposure showed progressive decrease in fish weight while haematological indices indicated that the fish became anaemic. Similarly, the LC_{50} of tobacco (*Nicotiana tobaccum*) leaf dust (2.00, 1.00, 0.50 and 0.25mg/L) on the Nile tilapia - *Oreochromis niloticus* was found to be 109.6mg/L [13].

The African catfish C*larias gariepinus* is a common freshwater mud fish that are frequently and widely cultured in ponds as they also occur freely in Nigerian natural freshwater bodies. Perhaps the most exciting feature of the catfish in terms of aquaculture is its potential for highly intensive culture without prerequisite pond aeration or high water exchange rates facilitated by its air-breathing ability and tolerance of poor water quality. *Clarias gariepinus* is very hardly, posses' accessory air breathing organs, which enable them to tolerate adverse aquatic condition which other cultivates fish species, cannot survive [15].

The aim of this study was to ascertain the haematological changes in the African catfish *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) for a period of 7days.

MATERIALS AND METHODS

Healthy live specimens of juveniles of the African catfish C*larias gariepinus* of the same broodstock; mean weight 108.68±4.02g and length 23.00±0.86cm, were purchased from Igbide fish farm, Igbide; Isoko South LGA, Delta State Nigeria. The fish were held in Animal and Environmental Biology laboratory in a large plastic aquarium of 60L capacity

with well-aerated bore-hole water. The fish were acclimatized for 14days and during which they were fed with commercial pellet fish feed (Coppens 2.0m). They were then transferred to the experimental plastic aquaria twelve (12) fish per 40L aquarium.

Tobacco leaf dust was obtained from a local store in Uzere in Isoko South LGA, Delta State as a dry leaf and was grinded into powder form. A stock solution of the toxicant (tobacco leaf dust) was prepared by diluting 1g of the toxicant into 100ml of distilled water. The concentrations used were arrived at after several preliminary tests to be 7.512, 3.756, 1.875 and 0.00g/l concentration which were introduced into the four (4) aquaria with replicates.

Forty (40) liters aquaria were maintained throughout the exposure period. Twelve (12) juvenile each were placed in the 40L plastic aquarium. Well-aerated borehole water was used during acclimatization and exposure period. Feeding regime was the same in acclimatization as in the experimental set up. In order to monitor the toxicant level of dissolved concentrations during experimental and reduce stress; 50% of toxicant was added after removing the exact volume of toxicant to the exposure set-up daily. The water quality parameters of the experimental set up bioassay, with tobacco leaf dust toxicant and control, were conducted at every sampling time according to the APHA [16] procedures. The water quality parameters measured included pH 6.58 $\pm$ 0.34, temperature 28.4 $\pm$ 1.2^{o}C, dissolved oxygen 7.89 $\pm$ 1.16mgL^{-1}, free carbon dioxide 4.95 $\pm$ 0.08 mgL^{-1} and total alkalinity 38.6 $\pm$ 1.34 mgL^{-1}.

At the end of the exposure period of seven (7) days the fish were taken from the control and test tanks, sacrificed and subjected to the analysis described below.

Five fish were caught individually in a small hand net from each container (test and control). After the preliminary investigation of the length and weight, the fish were then placed belly upwards and blood samples obtained from the caudal circulation with the aid of a heparinised 2cm^3 disposable plastic syringe and a 21 gauge disposable hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time [17]. The site chosen for puncture (about 3-4cm from the genital opening) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish and gently aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel. Blood was taken under gentle aspiration until about 1cm^3 has been obtained; then the needle was withdrawn and the blood gently transferred into heparinized plastic containers. The samples were then mixed gently but thoroughly. Some blood samples were used for the measurement of haematocrit, haemoglobin concentration and red blood cell count.

The haemoglobin concentration of the blood samples was determined in duplicate by the cyanmethaemoglobin method [18]. The haematocrit was determined by the microhaematocrit method of Snieszko [19]. The red blood cells were enumerated in an improved Neubaeur haemocytometer, using Hendricks diluting fluid. The total white blood cell counts were similarly enumerated in an improved Neubaeur haemocytometer using Shaw's diluting fluid. The haematological indices: Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the equations given by Anderson and Klontz [20].

$$\text{MCHC}(\%) = \frac{\text{Haemoglobin (g\%) x 100}}{\text{Haematocrit (\%)}}$$

$$\text{MCH } (\rho g) = \frac{\text{Haemoglobin (g\%) x 10}}{\text{Erythrocyte count (per/L)}}$$

$$\text{MCV } (\mu^3) = \frac{\text{Haematocrit (\%) x 10}}{\text{Erythrocyte count (per/L)}}$$

The results obtained were subjected to analysis for mean and standard error. The mean values of treatment were subjected to statistical analysis using one-way analysis of variance (ANOVA) to test for the level of significance between the various sublethal concentration of tobacco (*Nicotiana tobaccum*) leaf dust. Comparison of the means were analyzed the Bonferroni posttest. All statistical analyses were performed using the software programme (GraphPad Prism® Software version 5.0, San Diego, CA). Results were considered significant at the 0.05, 0.01 and 0.001 levels respectively.

RESULTS

The mean values of haemoglobin concentration of the fish exposed to sublethal concentrations of tobacco leaf dust after the seven (7) days exposure period are as presented in Fig. 1. The value was observed to decrease as the concentration as the concentration of tobacco leaf dust increased.

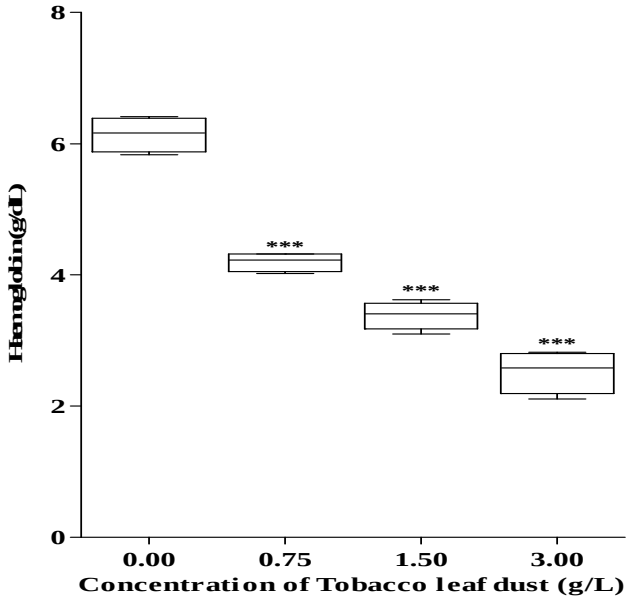

Fig 1: Mean values of haemoglobin of test fish *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) after 7 days exposure period. One, two and three asterisk(s) represents significant difference at 0.05, 0.01 and 0.001 levels respectively.

The mean total value of haemoglobin concentration of the exposed African catfish *Clarias gariepinus* after the 7days exposure period were valued at 6.1 (0.13), 4.2 (0.072), 3.4 (0.11), and 2.5 (0.16) g/dl for 0.00, 1.875, 3.756 and 7.512g/L concentration of tobacco leaf dust respectively. There was significance decrease ($P<0.001$) in the level of the examined haemoglobin concentration; showing significance decrease in the level of haemoglobin concentration in the exposed fish as the exposure period and the concentrations of tobacco leaf dust increased. Multiple comparison test Bonferroni showed that 7.512g/L of tobacco leaf dust was significantly different at 0.01% probability level compared to the control.

The mean values of packed cell volume of the fish exposed to the various sublethal concentrations of tobacco leaf dust after the seven (7) days exposure period is as presented in Fig. 2.

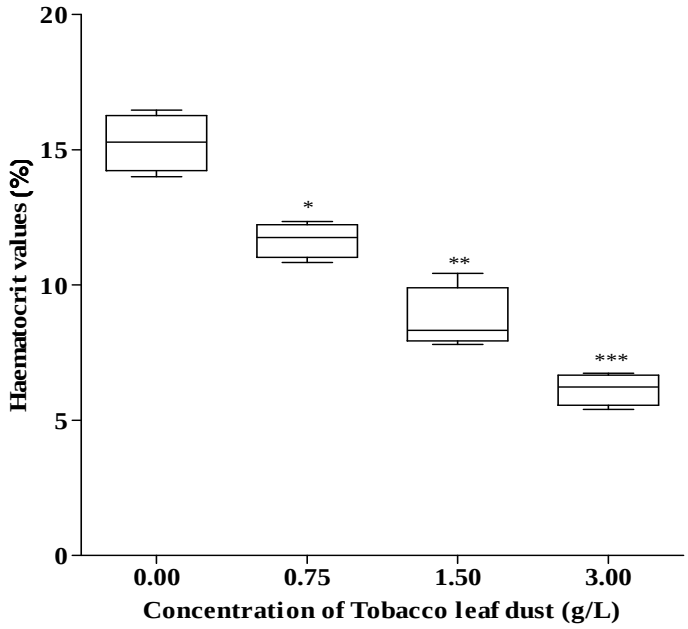

Fig. 2: Mean value and standard error of packed cell volume of test fish *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) after 7 days exposure period. Symbols as in Fig. 1.

The mean packed cell volume of the exposed Africa catfish C*larias gariepinus* after the seventh 7days exposure period were 15 (0.53), 12 (0.32), 8.7 (0.58) and 6.1 (0.58) for 0.00, 1.875, 3.756, and 7.512g/L respectively. There was significance decrease ($P<0.001$) in the level of the sublethal concentrations as tobacco leaf dust concentration increased. Multiple comparison test (Bonferroni) showed that 1.875, 3.756 and 7.512g/L of tobacco leaf dust were significantly different at 0.050 and 0.01, 0.001% probability level compared to the control respectively .

The mean values of the total erythrocytes count (TEC) of the fish exposed to sublethal concentration of tobacco leaf dust after the seven (7) exposure period is as presented in Fig. 3. The value of TEC was observed to decrease as the concentration of tobacco leaf dust increased. The mean total value of total erythrocytes count of the exposed fish after the 7days exposure period were 2.4 (0.084), 1.6 (0.10), 1.0 (0.029) and 0.79 (0.02).

There was significance difference ($P<0.001$) in the level of the examined total erythrocytes count; showing significance decrease in the level of total erythrocytes count in the exposed fish as the exposure period and the concentrations of tobacco leaf dust increased. Multiple comparison test (Bonferroni) showed that the 1.875 and 7.512mg/L of tobacco leaf dust were significantly different at 0.01% probability level compared to the control.

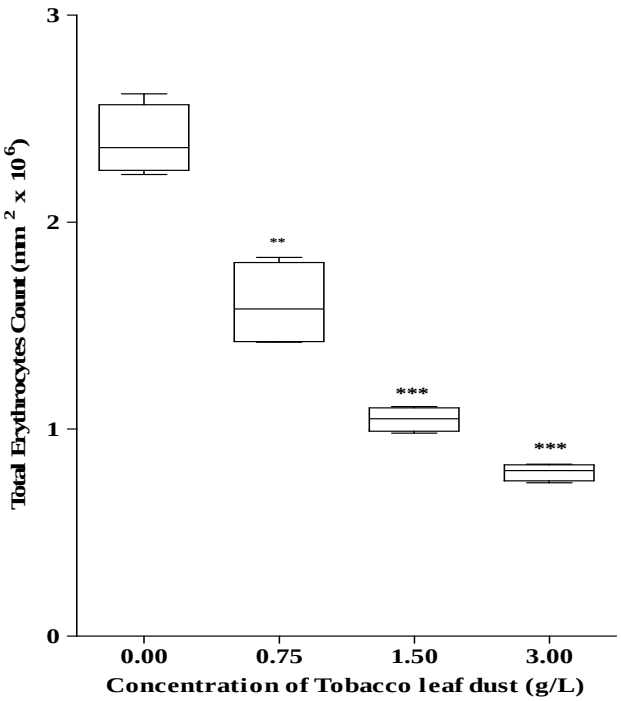

Fig 3: Mean values of total erythrocytes count of test fish *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) after 7 days exposure period. Symbols as in Fig. 1.

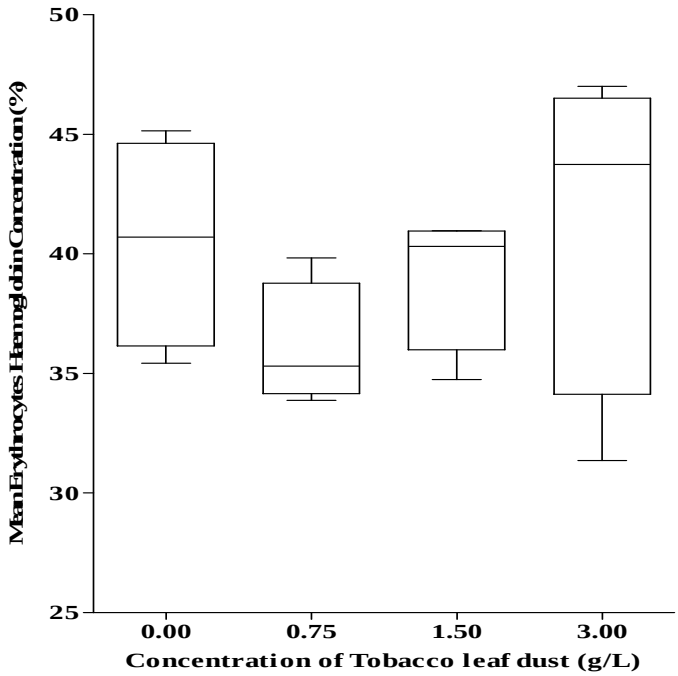

Fig. 4: Mean values of mean erythrocytes haemoglobin concentration of *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) after 7 days exposure period. Symbols as in Fig.1

The calculated level of mean erythrocyte haemoglobin concentration (MEHC) of the fish exposed to sublethal concentrations of Tobacco leaf dust after the seven (7)days exposure period is as presented in Fig. 4. The mean total value of mean erythrocyte haemoglobin

concentration of exposed fish *Clarias gariepinus* after the 7days exposure period were 40(2.2), 36(1.3), 39(1.5) and 41(3.5) μm^3 for 0.00, 1.875, 3.756 and 7.512/l of tobacco leaf dust concentration respectively. There was significant change (P<0.001) in the level of the calculated mean erythrocytes haemoglobin concentration. Multiple comparison tests showed that 7.512mg/l of Tobacco leaf dust was significantly different at 0.01% probability level compared to control.

The calculated level of mean erythrocyte haemoglobin (MEH) of the fish exposed to sub-lethal concentrations of tobacco leaf dust after the seven (7) days exposure period is as presented in Fig 5. The value of MEH of the exposed fish C*larias gariepinus* after the 7days exposure period revealed 26(1.1), 27(1.8), 32(0.69), 32(2.8) for 0.00, 1.875, 3.756 and 7.512g/l. there was significance difference (P<0.001) in the level of the calculated mean erythrocytes haemoglobin; multiple comparison tests showed that 3.756mg/l of Tobacco leaf dust concentration was significantly different at 0.01% probability level compared to the control.

The calculated level of mean erythrocytes volume (MEV) of the fish exposed to sublethal concentration of tobacco leaf dust after the seven (7) days exposure period is as presented in Fig. 6. The value of MEHC was observed to decrease as the concentration of the tobacco leaf dust increased. The mean total value of mean erythrocytes haemoglobin concentration of the exposed fish *Clarias gariepinus* after the 7days exposure period revealed 64(2.5), 74(6.2), and 78(4.0) for 0.00, 1.875, 3.756 and 7.512g/l concentration of Tobacco leaf dust respectively. There was insignificant increase (P<0.001) in the level of the calculate MEHC as the concentration of tobacco leaf dust increased as shown in the Fig 6.

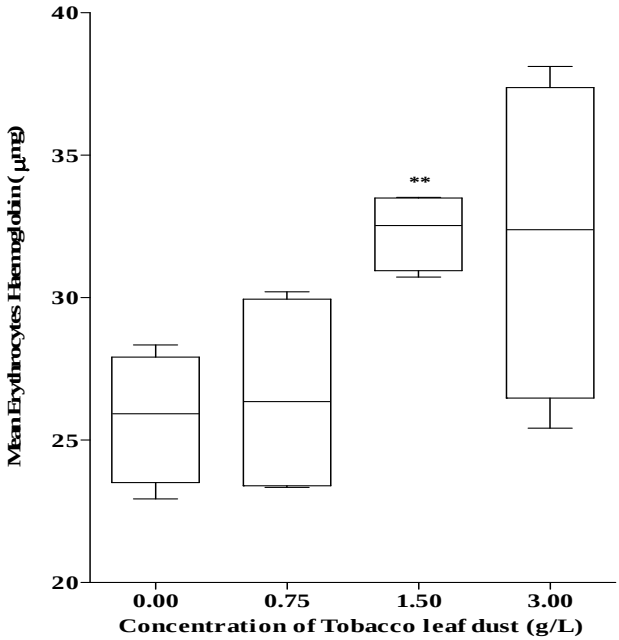

Fig 5: Mean values of mean erythrocytes haemoglobin of test fish *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) after 7 days exposure period. Symbols as in Fig. 1.

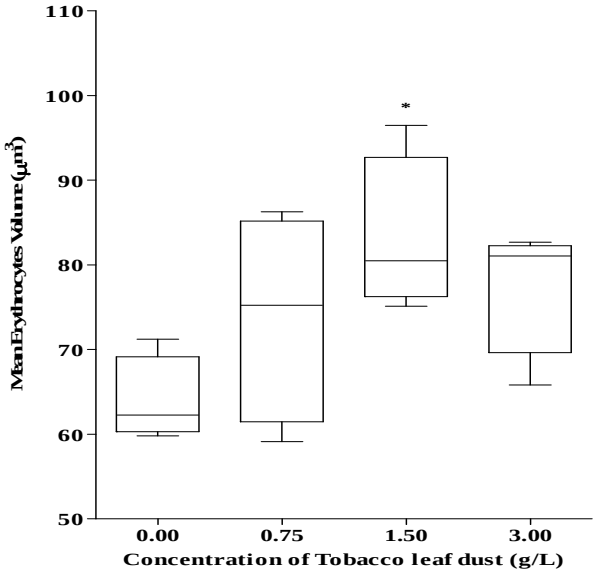

Fig. 6: Mean value and standard error of mean erythrocytes volume of test fish *Clarias gariepinus* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) after 7 days exposure period. Symbols as in Fig. 1.

DISCUSSION

The results above revealed an interesting pattern of response on the haematological variables in tobacco leaf dust-dosed fish, C*larias gariepinus*, in addition, the duration of exposure (7 days) to sublethal concentrations of tobacco leaf dust has resulted in an anaemic condition in C*larias gariepinus.* Studies have shown that when the water quality is affected by toxicants many physiological changes will be reflected in the values of one or more of the haemotological parameters [1]. Thus, water quality is one of the major factors responsible for individual variations in fish haematology since they live in close association with their environment and are sensitive to sight fluctuation that may occur within their internal milieu [21].

In the light of the present study, it is obvious that exposure of C*larias gariepinus* to Tobacco leaf dust caused a significant decrease in erythrocyte values which could be attributed to the destruction of the erythrocytes, thereby limiting their synthesis, similar trends in total erythrocytes count in fishes exposed to various toxicants have been observed by other workers, [22 – 23].

Consequently, haematocrit (Hct) and haemoglobin (Hb) decreased with increasing concentration of tobacco leaf dust. Decrease in haematocrit, an anaemic response has been reported in C*larias gariepinus* exposed to Malachite green [24]. Effect of sublethal concentration of formothion has been reported by Singh and Srivastava [25] with significant decrease in haematocrit and haemoglobin concentration in *Heteropneustes fossil* and decrease in haemoglobin has been reported by Santhakumar *et al.* [26] in *Anabas testudineus*. The significant reduction in the values of the haematocrit and haemoglobin as the concentration of the toxicant, tobacco leaf dust increases is an indication of severe anaemia in C*larias gariepinus*. The anaemic response could be as a result if destruction or inhibition of erythrocyte production [13].

The mean erythrocyte haemoglobin concentration (MEHC) is a good indicator of red blood cell swelling [27]. The MEHC, which is the ratio of blood haemoglobin concentration as opposed to the haematocrit is not influenced by the blood but can be interpreted incorrectly only when new cells with different haemoglobin concentration are released into blood circulation [28].

The significant decrease in MEHC after the 7days exposure period is probably an indication of red blood cells swelling and or to a decrease of red blood cells swelling and or to a decrease in haemoglobin synthesis. Bhagwant and Bhikajee [23] reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fish exposed to toxicant such as tobacco leaf dust.

Also, the fluctuation in the mean erythrocyte haemoglobin (MEH) and mean erythrocyte volume (MEV) in the study clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish that in the control thereby depicting an anaemic condition.

In conclusion, this research highlights the fact that sublethal concentration of tobacco leaf dust have deleterious effects on the haematological parameters of C*larias gariepinus*. These effects are directly proportional to the tobacco leaf dust concentrations. The use of this toxicant in fish ponds needs proper control to avoid reduction in fish production and aquatic fauna.

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