

## HAEMATOLOGICAL INDICES OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) JUVENILE EXPOSED TO DDVP (DICHLORVOS) INSECTICIDE

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### ABSTRACT

This study investigated the impact of DDVP (Dichlorvos) insecticide on the haematological indices of juvenile *Clarias gariepinus*. The research focused on evaluating the mortality rates and haematological parameters of fish exposed to various concentrations of DDVP. Juvenile African catfish were procured and acclimated for two weeks in dechlorinated water under natural conditions. A total of 150 fish were randomly allocated into five treatment groups (T1-T5) in triplicate, each containing 10 fishes, and exposed to various concentrations of DDVP based on a 96-hour LC50 of 0.00056 ml/L. The experimental groups included T1 (control, 0.00000 ml/L), T2 (0.000112 ml/L), T3 (0.000224 ml/L), T4 (0.000336 ml/L), and T5 (0.000448 ml/L). Temperature and pH of experimental water were monitored daily. Fish mortality was assessed at multiple time points throughout the experiment, with dead fish removed promptly to prevent oxygen depletion. Haematological analysis was performed by collecting blood samples through caudal ablation, with parameters including red and white blood cell counts, packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV) evaluated. Quality assurance was ensured through standard operating procedures and use of accredited laboratories. Results showed that mortality rates were significantly higher in the highest concentrations (T4: 85.00 ± 7.07% and T5: 100.00 ± 0.00%) compared to lower concentrations (T1: 0.00 ± 0.00%, T2: 15.00 ± 7.07%, T3: 20.00 ± 14.14%) ( $p < 0.05$ ). T5 exhibited the highest mortality rate among all treatments. Haematological analysis revealed significant alterations in the blood parameters of *C. gariepinus* exposed to DDVP. The packed cell volume (PCV) was notably reduced in the T3 group (20.33%) compared to other groups, suggesting adverse effects on erythropoiesis. The red blood cell (RBC) count was lowest in T2 ( $2.00 \pm 0.00 \times 10^6/L$ ), correlating with decreased PCV, indicating potential anaemia or reduced red blood cell production. Elevated mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in T2 pointed to macrocytic anemia, while mean corpuscular haemoglobin concentration (MCHC) remained relatively stable. Haemoglobin levels were significantly lower in T3 ( $7.44 \pm 0.69$  g/dl), supporting the reduced PCV and RBC counts. The white blood cell (WBC) count was markedly increased in T2 ( $4.03 \pm 0.06 \times 10^9/L$ ), suggesting an inflammatory or stress response. Granulocyte levels were significantly higher in T2 ( $32.77 \pm 0.83\%$ ), reflecting a shift toward a more inflammatory response, while lymphocytes decreased in T3. This study highlights the detrimental effects of DDVP on the health and blood parameters of *Clarias gariepinus*, with higher concentrations leading to increased mortality and significant haematological disturbances.

**Keywords:** DDVP (Dichlorvos) insecticide, Haematological indices, African catfish, Mortality

### INTRODUCTION

*Clarias gariepinus*, commonly known as the African catfish, is notable for its exceptional tolerance to environmental pollutants. This species can thrive in a variety of polluted aquatic environments due to several key adaptations. It possesses a high degree of metabolic and physiological resilience, allowing it to cope with contaminants such as heavy metals and pesticides (Amaeze *et al.*, 2020). The ability of catfish to perform aerial respiration, alongside its gill-based breathing, enables it to survive in oxygen-depleted waters often associated with pollution (Mishra and Gupta, 2014). Additionally, *Clarias gariepinus* is frequently used as a bio-indicator in environmental monitoring due to its ability to indicate the presence and impact of pollutants on aquatic ecosystems (García and Martínez, 2017).

Pesticides are synthetic organic and inorganic compounds used to kill or contain the activities of pests. According to Doherty *et al.* (2016), pesticides affect all members of an ecosystem from the smallest vertebrates to birds and humans, and their toxicities to both urban and agricultural settings are responsible for the death of many fishes, birds and smaller aquatic animals that fish depend on for food. In their separate studies, Chakraborty *et al.* (2017) and Amaeze *et al.* (2020) showed that aquatic contamination by pesticides causes acute and chronic poisoning of fish as well as severe damage to vital organs. It has also been shown that humans are at risk of

exposure to pesticides either through skin, oral consumption or respiration (Jayaraj, 2016). There is evidence of bio-accumulation of pesticides at higher trophic levels with possible deleterious effects. The major insecticides that are usually used are organophosphate, organochlorine, carbamates, pyrethroids and neocotenoïdes (Agbohesi, 2015).

The effects of insecticides and pesticides on the haematological indices of catfish can be quite significant, reflecting broader concerns about their impact on aquatic ecosystems. Exposure to pesticides often leads to a reduction in the RBC count in catfish. Chemicals such as organophosphates and carbamates can cause hematopoietic disorders, leading to anemia or decreased red blood cell production. This reduction impacts the fish's ability to transport oxygen efficiently, which can affect overall growth and survival. Haemoglobin levels are another critical parameter affected by pesticide exposure. Pesticides can interfere with hemoglobin synthesis or stability, leading to decreased oxygen-carrying capacity of the blood. This condition may result in hypoxia, adversely affecting the fish's metabolic processes and overall health (Singh and Sharma, 2018). Hematocrit values, which measure the proportion of blood volume occupied by red blood cells, can be significantly altered by pesticide exposure. Pesticides may cause either an increase or decrease in hematocrit levels, reflecting stress or pathological changes within the fish (Zhao and Zhou, 2015).

DDVP (Dichlorvos) insecticide is one of the most currently and widely used insecticides in this part of the world and several studies have detected its presence in water bodies at levels above the limits determined by local and international authorities (for example the Maximum Contaminant Level of 3µg/L by United State Environmental Protection Agency, 2019). Knowledge on the nature of these alterations would be pivotal to accurate diagnosis of pesticide toxicity. The nature of the haematological, biochemical, morphological and histopathological alterations associated with DDVP (Dichlorvos) insecticide poisoning remains to be clearly defined in aquatic animals, hence the need for this study. The findings from this study would provide information that are of relevance in setting out environmental limits as regards use of DDVP (Dichlorvos) insecticide.

## MATERIALS AND METHODS

### Experimental Design

A 12-week completely randomized design (CRD) feeding trial was conducted at the site of Fisheries Technology Department, Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria. Juveniles of African catfish (*C. gariepinus*), were procured from the Teaching and Research farm of the Federal Polytechnic Ado Ekiti, Ekiti State Nigeria. Juvenile fish were used in the experiment because they are more susceptible to environmental changes than older/more mature fishes. 10 healthy juveniles of *Clarias gariepinus*, regardless of sex were randomly selected, weighed, distributed and replicated thrice into five (5) plastic aquarium tanks (42.5 × 30.5 × 22.5cm) contained 50 liters of dechlorinated water (one hundred and fifty (150) fish). For the range finding test, four healthy fish were selected into an aquarium tank containing 15L of water after which DDVP (Dichlorvos) insecticide were added to determine the LC<sub>50</sub>. The experiment was designed into five treatments with concentration of DDVP (Dichlorvos) insecticide below the determined LC<sub>50</sub> (0.00056ml/L) as T1 (0.00000; control), T2 (0.000112ml/L), T3 (0.000224ml/L), T4 (0.000336ml/L) and T5 (0.000448ml/L) as presented in **Table 1**.

**Table 1: Concentration of DDVP in each Treatment**

| T1 (control) | T2           | T3           | T4           | T5           |
|--------------|--------------|--------------|--------------|--------------|
| 0.0000ml/L   | 0.000112ml/L | 0.000224ml/L | 0.000336ml/L | 0.000448ml/L |

### Experimental Procedure

The fish were acclimatized for two weeks in two plastic tanks of 50L capacity each and fed daily with commercial feeds containing 42-45% crude protein. In order to sustain a good hygienic condition and to avoid pollution, fecal wastes and other devastating materials were siphoned off every day. Deceased fishes were removed with the help of plastic forceps to prevent further or possible deterioration of the water quality and eminence. For the duration of acclimatization, water in the tanks were renewed every day with well aerated tap water. The feeding was discontinued 24 hours before the experimental run to prevent interference of feces. For the present study, DDVP (Dichlorvos) insecticide was obtained as a commercially available insecticide from an agrochemical outlet in Ado Ekiti

### Water Quality Monitor

Temperature and pH of the test solution and the control were monitored every 24 hours using mercury in glass thermometer and pH meter (American Public Health Association, 2005).

### Sub-lethal toxicity bioassay

Based on the results of the range finding test, the 96-hour LC<sub>50</sub> were calculated as 0.00056ml/L and used to determine the concentrations of DDVP (Dichlorvos) insecticide used during the sub-lethal toxicity bioassay (Ali *et al.*, 2018; Amaeze *et al.*, 2020). Fresh specimens were exposed to concentrations below the LC<sub>50</sub> (0.00056ml/L) in triplicates, at a stocking density of 10 fish per aquarium tank. A total of one hundred and fifty fishes were used i.e. five treatments (T1 (0.00000; control), T2 (0.000112ml/L), T3 (0.000224ml/L), T4 (0.000336ml/L) and T5 (0.000448ml/L) in triplicates (Table 1). 15 plastic aquaria were used for the sub-lethal experiment. Fish were fed with pelleted commercial feed at 5% body weight daily which were split into 2 rations.

### Mortality

Examination of the experimental set-up for fish mortality were made at the end of 1, 2, 4, 6 and 12 hours post exposure and then twice daily before termination of the experiment (Olusegun, 2011). Juveniles of *Clarias gariepinus* were certified dead when there is cessation in opercular movement and a gentle prodding elicited no response. The number of dead fish were recorded against the time of death in a tabular form in each treatment (Oluah *et al.*, 2020). Dead fish were immediately removed to avoid dissolved oxygen depletion (Kumari, 2020).

### Haematological Analysis

#### Data Collection

Blood samples were collected by the caudal ablation method from both the control and exposed fishes at the end of the experiment. The blood samples were dispensed into tubes containing ethylenediaminetetraacetate (EDTA) anticoagulant and transported in ice-packed bags to the Microbiological Laboratory unit of Ekiti State University Teaching Hospital, Ado Ekiti for haematological analysis. Red blood cells and white blood cells were counted by Neubauers improved haemocytometer using Hayems and Turks solution as diluting fluids, respectively. Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration 30 (MCHC), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated respectively using standard formula described by Hussain *et al.* (2019).

#### Statistical analysis

The statistical analyses were carried out using Statistical package for social sciences version 20 (SPSS, 2014). The data were expressed as mean  $\pm$  standard deviation and multivariate analysis of variance were used to show significant variations at  $p < 0.05$ .

## RESULTS

### Mortality rates of fish exposed to DDVP insecticide

The mortality rates of fish exposed to different concentrations of DDVP insecticide are presented in Table 2. The table revealed that the mortality rates in T4 (85.00  $\pm$  7.07%) and T5 (100.00  $\pm$  0.00%) are significantly high compared to T1 (0.00  $\pm$  0.00%), T2 (15.00  $\pm$  7.07%), and T3 (20.00  $\pm$  14.14%). Specifically, T4 and T5 showed significantly elevated mortality rates ( $p < 0.05$ ) when compared to the other treatment groups, with T5 exhibiting the highest mortality rate.

**Table 2: Mortality rates of *Clarias gariepinus* exposed to DDVP insecticide**

|            | T1                           | T2                            | T3                             | T4                            | T5                             |
|------------|------------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| Mortality  | 0.00 $\pm$ 0.00 <sup>a</sup> | 1.50 $\pm$ 0.71 <sup>a</sup>  | 2.00 $\pm$ 1.41 <sup>a</sup>   | 8.50 $\pm$ 0.71 <sup>b</sup>  | 10.00 $\pm$ 0.00 <sup>b</sup>  |
| %Mortality | 0.00 $\pm$ 0.00 <sup>a</sup> | 15.00 $\pm$ 7.07 <sup>a</sup> | 20.00 $\pm$ 14.14 <sup>a</sup> | 85.00 $\pm$ 7.07 <sup>b</sup> | 100.00 $\pm$ 0.00 <sup>b</sup> |

Means $\pm$ SD on the same row with homogenous superscripts indicate no significant difference ( $p > 0.05$ ).

### Haematology of *Clarias gariepinus* exposed to DDVP insecticide

The haematological parameters of *Clarias gariepinus* juveniles exposed to different concentrations of DDVP (Dichlorvos) insecticide are presented in Table 3. The table reveals significant variations in the haematological parameters of *Clarias gariepinus* juveniles exposed to different concentrations of DDVP. The PCV was significantly reduced in T3 (20.33%) compared to other groups, suggesting a potential adverse effect of higher DDVP concentrations on erythropoiesis. The RBC count in T2 (2.00  $\pm$  0.00  $\times 10^6$ /L) was the lowest, correlating with the observed decrease in PCV, which indicates possible anemia or reduced red blood cell production in response to the pesticide.

The MCV and MCH were elevated in T2, reflecting possible macrocytic anemia, while the MCHC remained relatively stable across groups, indicating that the overall concentration of hemoglobin in the red cells was not significantly affected. The hemoglobin level was significantly lower in T3 (7.44  $\pm$  0.69 g/dl), aligning with the

observed decrease in PCV and RBC counts. This suggests that exposure to higher DDVP concentrations may impair hemoglobin synthesis or red cell turnover. There was a marked increase in WBC count in T2 ( $4.03 \pm 0.06 \times 10^9/L$ ), which could be indicative of an inflammatory or stress response due to DDVP exposure. Granulocytes were significantly higher in T2 ( $32.77 \pm 0.83\%$ ), suggesting a shift towards a more inflammatory response, while lymphocytes and monocytes varied, with lymphocytes decreasing in T3 (Table 3).

**Table 3: Haematological parameters of *Clarias gariepinus* exposed to DDVP insecticide**

| Parameter                        | T1                      | T2                       | T3                      | T4                       |
|----------------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
| PCV %                            | 29.00±1.00 <sup>b</sup> | 28.00±1.00 <sup>b</sup>  | 20.33±0.58 <sup>a</sup> | 31.00±1.00 <sup>c</sup>  |
| RBC ( $\times 10^6/L$ )          | 4.00±0.00 <sup>b</sup>  | 2.00±0.00 <sup>a</sup>   | 3.67±1.15 <sup>b</sup>  | 3.00±0.00 <sup>ab</sup>  |
| MCHC (g/dl)                      | 33.27±0.38 <sup>a</sup> | 33.40±0.53 <sup>a</sup>  | 34.33±1.53 <sup>a</sup> | 32.97±0.88 <sup>a</sup>  |
| MCV (fL)                         | 80.82±0.24 <sup>b</sup> | 117.19±0.64 <sup>d</sup> | 69.56±2.69 <sup>a</sup> | 110.57±1.50 <sup>c</sup> |
| MCH (pg/cell)                    | 27.21±0.50 <sup>b</sup> | 39.13±0.33 <sup>d</sup>  | 24.12±1.80 <sup>a</sup> | 36.23±1.06 <sup>c</sup>  |
| Haemoglobin (Hbg/dl)             | 10.19±0.50 <sup>b</sup> | 10.48±1.07 <sup>b</sup>  | 7.44±0.69 <sup>a</sup>  | 10.42±1.07 <sup>b</sup>  |
| WBC ( $\times 10^9/L$ )          | 1.10±0.10 <sup>a</sup>  | 4.03±0.06 <sup>d</sup>   | 2.20±0.20 <sup>c</sup>  | 1.63±0.32 <sup>b</sup>   |
| Granulocytes ( $\times 10^9/L$ ) | 0.30±0.01 <sup>a</sup>  | 1.25±0.14 <sup>d</sup>   | 0.72±0.05 <sup>c</sup>  | 0.51±0.01 <sup>b</sup>   |
| GRA %                            | 24.30±0.52 <sup>a</sup> | 32.77±0.83 <sup>b</sup>  | 32.95±0.99 <sup>b</sup> | 32.43±1.12 <sup>b</sup>  |
| Lymphocytes ( $\times 10^9/L$ )  | 0.86±0.04 <sup>a</sup>  | 2.58±0.02 <sup>d</sup>   | 1.41±0.01 <sup>c</sup>  | 1.06±0.01 <sup>b</sup>   |
| LYMPH %                          | 73.33±1.53 <sup>c</sup> | 69.00±1.00 <sup>b</sup>  | 64.67±0.58 <sup>a</sup> | 66.00±1.00 <sup>a</sup>  |
| Monocytes ( $\times 10^9/L$ )    | 0.06±0.01 <sup>c</sup>  | 0.00±0.01 <sup>a</sup>   | 0.04±0.00 <sup>b</sup>  | 0.04±0.01 <sup>b</sup>   |
| MON %                            | 4.00±0.00 <sup>c</sup>  | 0.00±0.00 <sup>a</sup>   | 2.00±0.00 <sup>b</sup>  | 2.33±0.58 <sup>b</sup>   |

Means±SD on the same row with homogenous superscripts indicate no significant difference ( $p>0.05$ ).

## DISCUSSION

### Mortality rates of fish exposed to DDVP insecticide

The results demonstrated a clear dose-response relationship between DDVP exposure and fish mortality. At lower concentrations (T1, T2, and T3), the mortality rates remained relatively low, indicating a lower acute toxicity of DDVP at these levels. In contrast, higher concentrations (T4 and T5) resulted in substantially higher mortality rates, reflecting increased toxicity. The significantly higher mortality rates observed in T4 and T5 suggest that the concentration of DDVP at these levels is lethal to a large proportion of the fish population. This is consistent with the known effects of organophosphate insecticides, which are known to disrupt acetylcholine esterase activity, leading to neurotoxic effects and potentially high mortality rates in aquatic organisms.

The lower mortality rates in the control (T1), T2, and T3 may be attributed to the sub-lethal concentrations of DDVP, where the fish are exposed to levels that are not immediately fatal but could still induce stress or sublethal effects. This finding which showed a significant increase in fish mortality with higher concentrations of DDVP insecticide, align with the research of Khan *et al.* (2010), which demonstrated that exposure to various pesticides, including organophosphates, results in higher mortality rates among fish at elevated concentrations and that even sub-lethal doses can impact fish health and behavior.

The high mortality rates (85% and 100% in T4 and T5, respectively) recorded in this study, correspond with the findings of Singh and Sharma (2018) who reported that mortality rates in fish exposed to high doses of organophosphates often exceeded 70%, which is consistent with the mortality rates observed in our T4 and T5 treatments. The observation of lower mortality rates in T1, T2, and T3 at lower concentrations aligns with Mishra and Gupta (2014), who found that lower concentrations of pesticides could result in sub-lethal effects, such as stress and behavioral changes, without immediate high mortality. Mishra and Gupta (2014) emphasized that even sub-lethal doses could impact fish health, affecting growth and reproduction, which underscores the importance of not only considering lethal effects but also sub-lethal impacts in environmental assessments. The results suggest that high concentrations of DDVP pose a severe risk to fish populations, which aligns with García and Martínez (2017) who reported that pesticide exposure can lead to substantial mortality and disrupt aquatic ecosystems.

### Haematology of *Clarias gariepinus* exposed to DDVP insecticide

The haematological effects of DDVP (dichlorvos) on *Clarias gariepinus* juveniles were evident in the significant alterations observed in various blood parameters. These findings are consistent with and expand upon previous

research on the impact of organophosphates and other pesticides on aquatic organisms. The observed reduction in PCV and RBC counts in *Clarias gariepinus* juveniles, particularly in T3, aligns with findings from earlier studies on organophosphates. For instance, research by Nwani *et al.* (2012) demonstrated that exposure to organophosphates led to significant decreases in PCV and RBC counts in freshwater fish, suggesting that insecticides adversely affect erythropoiesis and can induce anemia. Similarly, the findings of Omwoyo *et al.* (2021) noted that high concentrations of organophosphates such as DDVP can disrupt normal blood cell production, causing anemia-like symptoms in fish.

The increase in MCV and MCH in T2, and the relatively stable MCHC, suggested that higher pesticide concentrations might lead to macrocytic anemia. This is consistent with the study by Shabana *et al.* (2018), which observed macrocytic changes in fish exposed to high levels of chlorpyrifos, another organophosphate. Shabana *et al.* (2018) found similar alterations in MCV and MCH, indicating that pesticides can impact the size and hemoglobin content of red blood cells. The significant decrease in hemoglobin levels in T3 supports the findings of similar studies where decreased hemoglobin was associated with pesticide exposure. Ikenweuwe *et al.* (2019) reported decreased hemoglobin levels in fish exposed to organophosphates, which they attributed to the toxic effects of these chemicals on erythropoiesis and hemoglobin synthesis. The reduction in hemoglobin in T3 suggests that high doses of DDVP significantly impair the fish's ability to maintain adequate oxygen-carrying capacity.

## CONCLUSION

This study investigated the impact of DDVP (dichlorvos) insecticide on the mortality rates and hematological properties of *Clarias gariepinus* juveniles. The study established that exposure of *Clarias gariepinus* to DDVP insecticide shows a clear dose-dependent increase in mortality rates. Increased pesticide concentrations were associated with significant reductions in PCV, RBC count, and hemoglobin levels, indicative of anemia. Additionally, alterations in white blood cell counts and differential leukocyte counts suggest an inflammatory or stress response. The findings revealed significant alterations in various blood parameters, highlighting the detrimental effects of pesticide exposure. This study recommends that it is crucial to monitor and regulate the use of DDVP in aquatic environments to prevent harmful effects on fish populations. Also, further studies should be conducted to elucidate the mechanisms underlying the hematological changes observed and to assess the long-term impacts of DDVP exposure.

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