
**“DICHLOROVOS - INDUCED NEUROENDOCRINE DISRUPTION
AND RECOVERY IN JUVENILE AFRICA CATFISH, *CLARIAS
GARIEPINUS*”**

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ABSTRACT

Due to anthropogenic activities, aquatic environments are increasingly contaminated with pesticides, often without adequate consideration of their ecological and toxicological impacts on aquatic organisms and, indirectly, human health even at low concentrations. This study investigated the effects of sublethal concentrations of Dichlorvos, a widely used organophosphate pesticide, on biochemical stress responses and short-term recovery in *Clarias gariepinus*. A total of 100 juveniles of *C. gariepinus* were acclimated for 14 days and subsequently subjected to a range-finding test followed by a 96-hour acute toxicity assay to determine LC_{50} using Probit analysis. Thereafter, fish were exposed to sublethal concentrations (0.10, 0.20, and 0.40 mg/L) of Dichlorvos for 28 days. Neurotoxicity was assessed using acetylcholinesterase (AChE) activity, while endocrine stress response was evaluated through plasma cortisol levels. Biomarkers were measured using standard spectrophotometric and ELISA techniques. Physicochemical parameters of the test media remained within WHO/FAO acceptable limits for freshwater fish survival (temperature 26.2–26.5°C, pH 7.1–7.3, dissolved oxygen 6.4–6.7 mg/L, ammonia 0.02–0.04 mg/L), indicating that observed effects were not influenced by water quality stressors. Results showed significant ($p < 0.05$) time- and concentration-dependent alterations in stress biomarkers. AChE activity decreased markedly, with the highest inhibition observed at 0.40 mg/L, while plasma cortisol levels increased progressively with exposure duration and concentration, consistent with WHO-recognized patterns of organophosphate neurotoxicity and physiological stress. Following a 72-hour depuration period, partial recovery of biomarkers was observed at lower concentrations (0.10 mg/L), while persistent impairment remained at

higher exposure levels, particularly in AChE activity. This indicates that full physiological recovery extends beyond 72 hours under higher pesticide loads. The findings demonstrate that Dichlorvos induces significant neurotoxic and endocrine disruption in *C. gariepinus*, with recovery potential strongly dependent on exposure concentration. The study highlights ecological risks associated with organophosphate contamination in aquatic systems and supports the need for stricter regulatory control and sustainable pesticide management in accordance with international environmental safety standards.

KEYWORDS: Dichlorvos, Oxidative stress, *Claria gariepinus*, Biomarkers, Depuration.

1.0 INTRODUCTION

Pesticides are important chemicals used in agriculture and public health management. They are widely applied to control pests and improve crop production. However, their extensive use poses serious environmental and health risks (Ghayyur et al., 2021). These chemicals often enter aquatic environments through runoff and leaching, thereby affecting aquatic organisms such as fish and invertebrates (Bojarski and Witeska, 2020). Pesticides can cause both direct toxicity and sublethal effects, including behavioural and reproductive alterations (Clements et al., 2019). The severity of these effects depends on the characteristics of the pesticide, the duration and concentration of exposure, and the sensitivity of the exposed organisms (Zhang et al., 2018). Therefore, sustainable agricultural practices and stronger regulations are necessary to protect ecosystems from pesticide pollution (Ghayyur et al., 2021).

Dichlorvos, a widely used organophosphate insecticide, is commonly applied in agriculture, public health, and domestic environments (WHO, 1989). Its physicochemical properties, such as high water solubility and volatility, enhance its effectiveness as an insecticide but also increase the risk of environmental contamination (Howard, 1991). Dichlorvos functions by inhibiting acetylcholinesterase, resulting in neurotoxic effects in pests and posing risks to non-target organisms, including fish (Jokanović, 2018). It is toxic to a broad range of aquatic organisms, including fish and crustaceans (Forget, 1991). Exposure to Dichlorvos can lead to acute toxicity, behavioural abnormalities, reduced feeding activity, and altered swimming behaviour (Gupta et al., 2021). Its toxicity to aquatic life is influenced by environmental factors such as water temperature and pH (Johnson & Finley, 1980). In addition, Dichlorvos can disrupt fish reproduction through effects on gonadal development and endocrine function (Ahmad et al., 2018). Its widespread agricultural application raises concerns regarding its

impact on aquatic ecosystems, thereby emphasizing the need for further investigation and regulation.

Fish and shellfish absorb contaminants from their aquatic environment through the gills and other permeable membranes (Sharma et al., 2012). Once accumulated in their tissues, pesticides may interfere with physiological and biochemical processes, negatively affecting growth, survival, and reproduction (Scott et al., 2004; Miranda-Ferreira et al., 2017). Prolonged exposure to pesticides increases stress levels in juvenile aquatic organisms, making them more vulnerable to predation (Clements et al., 2019). Oxidative stress is a common response to pesticide exposure in aquatic organisms and results from an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense mechanisms (Livingstone, 2001). Excess ROS can damage cellular components and result in cellular dysfunction (Halliwell & Gutteridge, 2007). Antioxidant enzymes therefore play a critical role in protecting aquatic organisms from oxidative damage (Van der Oost et al., 2003).

Furthermore, pesticides possess bioaccumulative properties and may gradually accumulate within the tissues of aquatic organisms over time (Clasen et al., 2018). Agricultural activities contribute significantly to water pollution, with a considerable proportion of surface water contamination originating from agricultural sources (Albou et al., 2024). Pesticides induce biochemical alterations at the molecular and cellular levels, making them valuable biomarkers in toxicological studies (Opute and Oboh, 2021). These biochemical responses assist in assessing the effects of environmental contaminants on fish by revealing physiological and metabolic disturbances (Loto et al., 2021).

Important biomarkers of liver toxicity include alanine transaminase (ALT) and aspartate transaminase (AST), both of which increase following liver damage caused by toxicant exposure (Ajibare and Loto, 2023). Previous studies have reported elevated ALT and AST levels as indicators of hepatocellular injury, while increased alkaline phosphatase (ALP) levels suggest impaired liver function (Zheng et al., 2018; Kumar et al., 2019). Other biomarkers are associated with kidney and liver function, where elevated creatinine (CREAT) indicates renal toxicity and reduced albumin (ALB) levels reflect liver dysfunction (Gupta et al., 2021). Antioxidant enzyme activities are also reduced under pesticide exposure, leading to oxidative stress and cellular damage (Semren et al., 2018).

Monitoring these biochemical markers is important for evaluating pesticide toxicity and protecting aquatic ecosystems (Esenowo et al., 2021; Loto et al., 2022). Aquatic ecosystems

support biodiversity and fisheries but are increasingly threatened by pesticide contamination (Lu et al., 2010).

Depuration kinetics refers to the elimination of toxic substances from the body of an organism (Newman & Unger, 2003). Understanding the depuration kinetics of Dichlorvos in aquatic organisms is essential for assessing the risks associated with pesticide exposure. Knowledge of the depuration kinetics of Dichlorvos in *Clarias gariepinus* can provide valuable insight into the risks linked to the consumption of contaminated fish and support the development of strategies for minimizing these risks. Studies have shown that the depuration rate of Dichlorvos is influenced by factors such as water temperature, pH, and the presence of other pollutants (Katagi, 2010). Depuration involves the biotransformation, excretion, and metabolic detoxification of xenobiotics, and its kinetics vary depending on species, exposure concentration, exposure duration, and environmental conditions (Rand, 1995).

African catfish (*Clarias gariepinus*) is an important aquaculture species that is highly susceptible to pesticide exposure, which may adversely affect its growth and health (Dedeke et al., 2014). *Clarias gariepinus* is an air-breathing catfish characterized by a scaleless, elongated bony body, long dorsal and anal fins, and a helmet-like head. According to Skelton (2001), it is probably the most widely distributed fish species in Africa. It occurs abundantly in rivers, streams, reservoirs, canals, ponds, dams, and lakes across Africa (Adeyemi, 2014). The species is known for its rapid growth in both length and weight under favourable environmental conditions (Britz and Pienaar, 1992). It is also widely consumed in Nigeria because it serves as an affordable source of animal protein for low-income populations.

Clarias gariepinus adapts easily to laboratory conditions due to its accessory respiratory structures, making it an excellent model organism for toxicological studies (Nwani et al., 2017). However, studies investigating the chronic and sublethal effects of pesticides on catfish remain limited. Existing research has focused mainly on acute toxicity while neglecting long-term biochemical and physiological effects (Ahmad et al., 2018). Considering species-specific variations in pesticide sensitivity, more comprehensive studies are required for accurate ecological risk assessment and conservation planning (Yu et al., 2020). Aquatic ecosystems are essential for biodiversity and human well-being, yet they continue to face serious threats from pesticide contamination (UNEP, 2018).

According to Clements et al. (2019), Dichlorvos disrupts aquatic food chains and adversely affects species such as *Clarias gariepinus*. Given the increasing occurrence of Dichlorvos in aquatic ecosystems and its potential to induce oxidative damage in fish, it is necessary to evaluate both the immediate physiological stress responses and the recovery capacity of

Clarias gariepinus following exposure. Such information is essential for environmental monitoring, formulation of regulatory guidelines, and development of mitigation strategies aimed at protecting aquatic life and public health.

2.0 MATERIALS AND METHODS

2.1 Experimental Fish Collection and Acclimatization

A total of 100 healthy *Clarias gariepinus* with an average weight of 150 ± 10 g and mean length of 25 ± 2 cm were sourced from a private fish farm located in Otuoke, Bayelsa State, Nigeria. The specimens were carefully transported to the laboratory in aerated containers and subsequently acclimatized for a period of 14 days in 300 L plastic tanks containing dechlorinated and continuously aerated water. Throughout the acclimatization period, water quality conditions were maintained at a temperature of $26 \pm 1^\circ\text{C}$, pH of 7.2 ± 0.3 , and dissolved oxygen concentration of 6.5 ± 0.5 mg/L. The fish were fed commercial pellet feed twice daily at 3% of their body weight, while feeding was suspended 24 hours before the commencement of toxicity exposure experiments.

2.2 Acute Toxicity Test

After the acclimation period, the experimental fish were carefully allotted into separate tanks containing varying concentrations of Dichlorovos, which were prepared based on preliminary range-determination trials. An additional group not exposed to the toxicant served as the control. The exposure experiment lasted for 96 hours under a static-renewal system. Observations for mortality were conducted after 24, 48, 72, and 96 hours of exposure. Any dead fish observed during the experiment were immediately removed to avoid deterioration of water quality and additional stress to the remaining fish.

The mortality records generated from the acute toxicity experiment were subjected to Probit statistical analysis in order to establish the concentration–response relationship of Dichlorovos. Mortality percentages obtained at the different exposure levels were transformed into Probit values and correlated with the logarithmic concentrations of the toxicant. A best-fit regression model was then generated, from which the median lethal concentration (LC_{50}) corresponding to Probit 5 was estimated. The derived LC_{50} value was subsequently adopted as the reference concentration for the sublethal exposure studies.

2.3 Sublethal Toxicity Test

Upon completion of the acute toxicity experiment, sublethal exposure levels of Dichlorovos were established using selected proportions of the 96-hour LC_{50} value, commonly 1/10, 1/5,

and 1/2 of the median lethal concentration. The experimental fish were subsequently subjected to these reduced concentrations over a prolonged exposure period in order to evaluate chronic toxicological and biochemical responses. Unlike acute toxicity tests that primarily assess mortality, the sublethal exposure experiment was designed to investigate physiological and metabolic disturbances that may occur without causing immediate death.

2.4 Experimental Design

The experimental fish were randomly assigned into four treatment groups comprising 15 fish per group. Group 1 served as the control and was maintained in clean dechlorinated water without Dichlorovos exposure. Group 2 was exposed to 0.10 mg/L Dichlorovos, while Groups 3 and 4 were exposed to 0.20 mg/L and 0.40 mg/L Dichlorovos, respectively. Each treatment consisted of three replicates containing five fish each.

The exposure experiment was conducted over a period of 28 days under semi-static conditions, with 50% water renewal and re-application of the toxicant every 48 hours to maintain stable Dichlorovos concentrations. During the exposure period, the fish were fed twice daily and regularly monitored for behavioural abnormalities and mortality.

2.5 Sampling Procedure

Blood samples were collected from the experimental fish on days 7, 14, 21, and 28 of the exposure period. Prior to sampling, the fish were anaesthetized using tricaine methanesulfonate (MS-222) at a concentration of 100 mg/L. Blood was carefully withdrawn from the caudal vein using sterile insulin syringes and immediately transferred into heparinized sample bottles to prevent coagulation. The collected samples were preserved at 4°C pending biochemical analyses.

2.6 Recovery Assessment in *Clarias gariepinus* Following 28-Day Dichlorovos Exposure

At the end of the 28-day exposure period, surviving fish from each treatment group were transferred into clean pesticide-free water in order to evaluate their recovery potential. The fish were maintained under the same laboratory conditions used during exposure. Additional blood samples were collected 72 hours after transfer to clean water to assess recovery trends in the measured biochemical parameters. During the recovery phase, no Dichlorovos was introduced into the water, although feeding continued as previously maintained during the exposure period.

2.7 Biochemical Analyses

2.7.1 Acetylcholinesterase (AChE) Activity

Acetylcholinesterase activity was determined using the method described by Ellman et al. (1961), based on the hydrolysis of acetylthiocholine iodide. The reaction mixture consisted of phosphate buffer, acetylthiocholine iodide, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), and the test sample. The production of the yellow-colored 5-thio-2-nitrobenzoate anion was monitored spectrophotometrically at 412 nm. Enzyme activity was expressed as $\mu\text{mol}/\text{min}/\text{mg}$ protein.

2.7.2 Plasma Cortisol Determination

Plasma cortisol concentration was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit obtained from manufacturers such as Sigma-Aldrich or Cayman Chemical, following the manufacturer's protocol. Blood samples were centrifuged at 3000 rpm for 10 minutes to separate plasma from cellular components. Plasma samples and prepared standards were dispensed into ELISA plate wells and incubated with the required enzyme conjugates and substrates. Following the reaction process, absorbance values were measured at 450 nm using a microplate reader. Cortisol concentrations were calculated from a standard calibration curve and reported in ng/mL.

2.7.3 Recovery Phase

Following the 28-day exposure to sublethal concentrations of Dichlorovos, surviving *Clarias gariepinus* were transferred into clean, uncontaminated water for a 72-hour depuration period. This recovery phase was designed to evaluate the ability of the fish to gradually restore normal physiological conditions after exposure-induced oxidative stress. At the end of the depuration interval, selected biochemical parameters were re-evaluated in order to assess the early recovery responses of the fish.

2.8 Statistical Analysis

All experimental results were presented as mean \pm standard error of the mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify significant differences among treatment groups and sampling periods. Statistical significance was accepted at $p < 0.05$. In addition, LC_{50} values were determined using Probit analysis.

3.0 RESULTS

3.1 Physicochemical Parameters of Test Water

Table 1: Physicochemical Parameters of Test Water During Exposure to Dichlorvos.

Parameter	Day 7	Day 14	Day 21	Day 28
Temperature (°C)	26.3 ± 0.5	26.4 ± 0.4	26.2 ± 0.6	26.5 ± 0.5
Ph	7.2 ± 0.2	7.3 ± 0.2	7.1 ± 0.3	7.2 ± 0.2
Dissolved Oxygen (mg/L)	6.7 ± 0.4	6.5 ± 0.5	6.6 ± 0.4	6.4 ± 0.3
Ammonia (mg/L)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01

All through the exposure time, the test media's physicochemical characteristics were within acceptable ranges. Although there were some variations, all of the values were optimal for *C. gariepinus* to survive (Table 1).

3.2 Acetylcholinesterase Activity ($\mu\text{mol}/\text{min}/\text{mg}$ Protein)

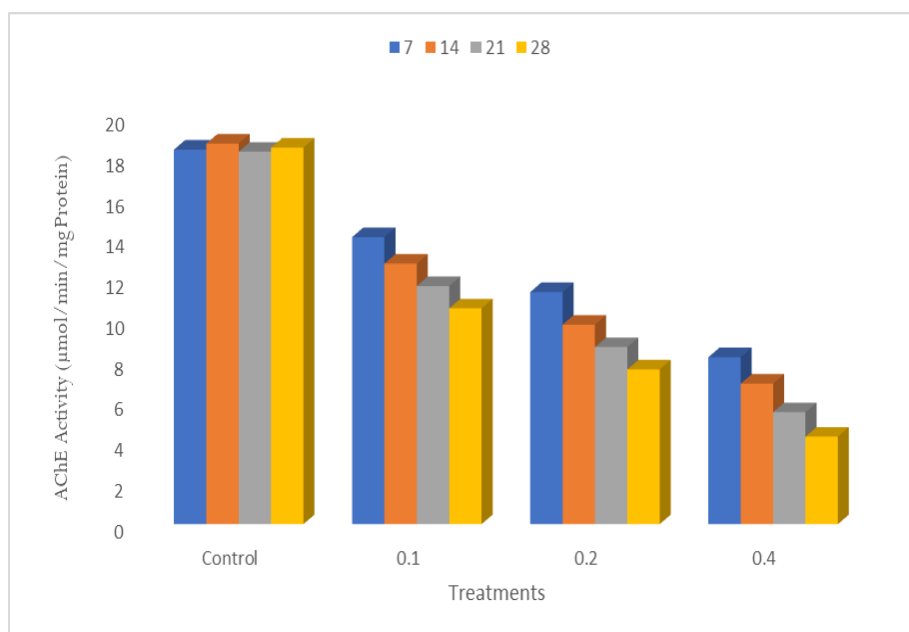


Figure 3: AChE Activity ($\mu\text{mol}/\text{min}/\text{mg}$ Protein) in the blood of *C. gariepinus* on days 7, 14, 21, and 28 of the exposure to Dichlorvos.

This study observed that AChE activity in *C. gariepinus* significantly decreased ($p < 0.05$) with increasing concentrations of Dichlorvos over 28 days, with significant inhibition ($p < 0.05$) observed in all Dichlorvos-treated groups. The inhibition exhibited dependence on both time and concentration. A notable decrease in AChE activity was observed by day 7 across all exposure groups. The inhibition intensified with prolonged exposure, suggesting persistent neurotoxic effects. The highest level of AChE inhibition occurred in the 0.40 mg/L Dichlorvos group, indicating a distinct concentration-response relationship.

3.3 Plasma Cortisol Levels (ng/mL)

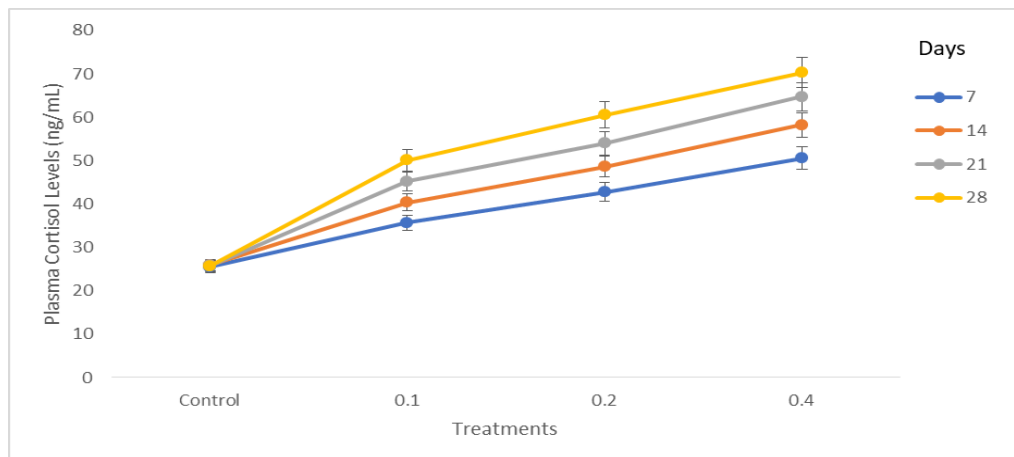


Figure 4: Plasma Cortisol Levels (ng/mL) in *C.gariepinus* on days 7, 14, 21, and 28 of the exposure to Dichlorvos.

This study observed a significant ($p < 0.05$) increase in plasma cortisol levels in *C. gariepinus* exposed to Dichlorvos over 28 days, demonstrating a clear trend that is both time- and concentration-dependent (Figure 4). On day 7, all groups exposed to Dichlorvos exhibited a significant increase in cortisol levels relative to the control group. Cortisol levels exhibited a consistent increase during the exposure period, reaching a maximum on day 28. Cortisol elevation was more pronounced at higher concentrations of Dichlorvos, particularly in the 0.40 mg/L group, indicating intensified cortisol secretion with increased chemical stress.

3.4 Recovery Trends in *Clarias gariepinus* following 28-Day Dichlorvos Exposure

The findings from the 72-hour recovery phase of biomarkers in *C. gariepinus* subjected to dichlorvos-free dechlorinated tap water reveal disparate recovery levels among the biomarkers (Table 3).

Table 3: 72-Hour Recovery Response of *C.gariepinus* in Dichlorvos free dechlorinated tap water.

Concentration	Biomarker	Control	72 hours recover response	Status at 72-hour
0.10mg/l	AChE(nmol/mg protein)	18.50±0.30	17.80±0.40	Similar to control
0.20mg/l	AChE(nmol/mg protein)	18.50±0.30	16.70±0.10	Moderately diminished
0.40mg/l	AChE(nmol/mg protein)	18.50±0.30	11.20±1.10	Substantially diminished
0.10mg/l	Cortisol(ng/ml)	26.20±0.50	27.90±0.20	Similar to control

0.20mg/l	Cortisol(ng/ml)	26.20±0.50	28.30±1.40	Similar to control
0.40mg/l	Cortisol(ng/ml)	26.20±0.50	28.90±2.19	Marginally increase still comparable

Table 4 WHO / INTERNATIONAL COMPARISON TABLE.

Parameter	Your Study Value	WHO / International Reference	Interpretation
Temperature	26.2–26.5°C	FAO/WHO fish culture range: 24–30°C	Within optimal range for <i>Clarias gariepinus</i> survival
pH	7.1–7.3	WHO freshwater range: 6.5–8.5	Neutral and suitable for aquatic life
Dissolved Oxygen	6.4–6.7 mg/L	Minimum recommended: >5 mg/L	Adequate oxygen, no hypoxic stress
Ammonia	0.02–0.04 mg/L	Safe level for fish: <0.05 mg/L	Within safe tolerance limits
AChE Activity	↓ from 18.50 to 11.20 nmol/mg (72h recovery)	WHO organophosphate effect: >50% inhibition = neurotoxicity	Strong neurotoxic inhibition at 0.40 mg/L
Cortisol (Exposure)	Elevated above control (max at day 28)	No WHO limit (stress biomarker baseline varies 5–25 ng/mL)	Indicates physiological stress response
Cortisol (Recovery)	27.9–28.9 ng/mL vs control 26.2 ng/mL	Elevated levels = incomplete recovery	Partial recovery at low dose, stress persists at high dose

3.5 Acetylcholinesterase Activity: The activity of AChE exhibits a notable decline at 0.40 mg/L, representing a substantial drop relative to the control group. At 0.10 mg/L, the reduction is negligible, suggesting nearly total recuperation of the neurological system at diminished dosages. At elevated concentrations, specifically at 0.40 mg/L, the recovery is inadequate, indicating persistent neurotoxic effects and compromised cholinergic regulation in the fish.

3.6 Cortisol Concentrations (ng/mL): Control: 26.20; 0.10 mg/L: 27.90 (similar to control); 0.20 mg/L: 28.30 (similar to control); 0.40 mg/L: 28.90 (marginally increased still comparable). Cortisol levels exhibit a small elevation across all treatments, signifying that the fish continue to endure stress. Nevertheless, the levels are nearly equivalent to the control at 0.10 mg/L and 0.20 mg/L, indicating that recovery from stress is comparatively efficient at lower doses of dichlorvos. At 0.40 mg/L, cortisol levels remain marginally raised, indicating

a limited recovery and suggesting that the fish continue to manage stress from exposure to this higher dose.

4.0 DISCUSSION

This study assessed oxidative stress responses, neurotoxicity, and endocrine disruption in *C. gariepinus* after exposure to different sublethal concentrations of Dichlorvos over 28 days. The evaluated biomarkers; Acetylcholinesterase and plasma cortisol offered a thorough insight into the toxicodynamic impacts of Dichlorvos on the physiological and biochemical systems of fish.

4.1 Acetylcholinesterase Activity

Acetylcholinesterase is an essential enzyme that hydrolyses the neurotransmitter acetylcholine into choline and acetate, thus concluding synaptic transmission. The inhibition of acetylcholinesterase serves as a well-established indicator of organophosphate pesticide toxicity and is commonly utilized as a biomarker for neurotoxicity (Fulton & Key, 2001). Dichlorvos, similar to other organophosphate pesticides, irreversibly inhibits acetylcholinesterase activity through the phosphorylation of the enzyme's active site. The accumulation of acetylcholine in synaptic clefts results in continuous nerve stimulation, neuromuscular disruption, and potential behavioural alterations (Lionetto et al., 2013). The inhibition of acetylcholinesterase by organophosphate pesticides is extensively documented in fish species. Rao (2006) and Lionetto *et al.* (2013) observed comparable dose- and time-dependent inhibition of AChE in fish subjected to organophosphate pesticides, underscoring AChE activity as a sensitive and reliable biomarker for neurotoxicity induced by pesticides. Extended inhibition of AChE, as demonstrated in this study, may result in loss of motor coordination, impaired feeding behaviour, respiratory distress, and ultimately mortality if exposure persists.

The neurotoxic effect, as evidenced by decreased AChE activity, aligns with the oxidative stress reflected in other biomarkers (SOD, CAT, GPx), indicating that Dichlorvos induces both oxidative and neurological toxicity in *C. gariepinus*

4.2 Plasma Cortisol

Cortisol functions as the principal stress hormone in fish and acts as a vital biomarker for physiological stress responses to environmental disruptions, such as chemical exposure. The persistent increase in cortisol levels indicates a prolonged stress response resulting from Dichlorvos toxicity. Cortisol in fish facilitates energy mobilization and the maintenance of

homeostasis during stressful conditions. Chronic elevation of cortisol can result in negative physiological effects, including immune system suppression, impaired growth and reproductive functions, and disruptions in osmoregulation and metabolism.

Increased cortisol levels generally signify the activation of the hypothalamic-pituitary-interrenal (HPI) axis as a response to stressors (Barton, 2002). The observed trend of elevated cortisol levels corresponds with the finding of Ramesh et al. (2009), who documented significant increases in plasma cortisol in fish subjected to environmental stressors and pesticides. The studies demonstrate that cortisol serves as a sensitive and reliable indicator of sublethal stress in fish exposed to chemical agents.

The observed increase in cortisol levels in this study, along with neurotoxic biomarkers, suggests that Dichlorvos exposure initiated a systemic stress response that exceeded the fish's compensatory mechanisms.

4.3 72- Hour Depuration

The 72-hour depuration research indicated initial signs of physiological and biochemical recovery in *C. gariepinus* following 28 days of sublethal exposure to Dichlorvos. The assessed biomarkers which are neurotoxicity marker (AChE), and an endocrine stress marker (plasma cortisol) function as precise instruments for evaluating detoxification, mitigation of oxidative stress, and physiological resilience following exposure.

Acetylcholinesterase: Acetylcholinesterase activity exhibited preliminary indications of reactivation following exposure, especially in the 0.10 mg/L group. Dichlorvos, similar to other organophosphates, inhibits acetylcholinesterase by covalently adhering to its active site, hence disrupting cholinergic neurotransmission (Fulton & Key, 2001). The observed partial reactivation substantiates the hypothesis that enzyme inhibition may be reversible following the elimination of the toxicant, but complete recovery generally necessitates additional time (Lionetto et al., 2013).

4.4 Plasma cortisol:

A decrease in plasma cortisol was observed in all treatment groups, with the most pronounced reduction in the 0.10 mg/L group. Cortisol, the primary stress hormone in fish, increases in reaction to environmental stresses and reverts to baseline levels once equilibrium is reestablished (Barton, 2002). The noted decrease signifies a mitigation of acute stress, although it implies insufficient endocrine normalization in the higher-dose cohorts. The initial phases of biochemical recovery were most pronounced in the lowest exposure

group, demonstrating a dose-dependent detoxification response. Nonetheless, the inadequate restoration of the biomarkers after 72 hours, particularly at elevated Dichlorvos concentrations, indicates that short-term depuration is insufficient for complete physiological normalization. These results align with other research indicating a gradual restoration of enzymatic functions during extended detoxification periods (Velisek et al., 2011; Zhang et al., 2017).

4.5 Discussion of Findings in Relation to WHO/International Standards

The physicochemical parameters of the experimental water remained within acceptable international limits for freshwater fish throughout the exposure period. Temperature values (26.2–26.5°C) were within the FAO/WHO recommended range of 24–30°C for tropical aquatic organisms, indicating that thermal stress did not influence the observed biological responses. Similarly, pH values (7.1–7.3) were within the WHO acceptable freshwater range of 6.5–8.5, confirming stable water chemistry throughout the experiment.

Dissolved oxygen levels (6.4–6.7 mg/L) exceeded the minimum threshold (>5 mg/L) recommended for fish survival, suggesting that oxygen limitation was not a confounding stress factor. Ammonia concentrations (0.02–0.04 mg/L) were also below the toxic threshold of 0.05 mg/L for freshwater fish, indicating that water quality remained suitable for aquatic life during the experiment. Therefore, the physiological and biochemical alterations observed in *Clarias gariepinus* can be attributed primarily to Dichlorvos exposure rather than environmental deterioration.

The marked reduction in acetylcholinesterase (AChE) activity observed across exposure groups, particularly the severe inhibition recorded at 0.40 mg/L (11.20 nmol/mg protein), is consistent with WHO-recognized mechanisms of organophosphate toxicity. According to WHO toxicological classification, organophosphates such as Dichlorvos act as potent cholinesterase inhibitors, leading to neurotoxicity through accumulation of acetylcholine at synaptic junctions. The concentration-dependent inhibition observed in this study confirms progressive neurotoxic stress, with incomplete recovery at higher exposure levels.

Plasma cortisol levels increased significantly during exposure, peaking at day 28, indicating activation of the hypothalamic–pituitary–interrenal (HPI) stress axis. Although WHO does not define specific cortisol limits for fish, elevated cortisol is widely accepted as an indicator of physiological stress. The persistence of slightly elevated cortisol levels during the recovery phase (27.9–28.9 ng/mL compared to control 26.2 ng/mL) suggests that full physiological recovery was not achieved, particularly at higher exposure concentrations.

The recovery data further revealed that AChE activity showed near-complete recovery at 0.10 mg/L but remained substantially suppressed at 0.40 mg/L. This indicates dose-dependent recovery potential, where lower exposure levels allow partial restoration of neurological function, while higher concentrations cause persistent enzymatic inhibition. This pattern aligns with established WHO/FAO toxicological principles that prolonged or high-dose exposure to organophosphates can result in sustained biochemical impairment.

Overall, comparison with WHO-relevant environmental and toxicological standards confirms that while water quality conditions remained suitable for fish survival, Dichlorvos exposure induced significant neurotoxic and endocrine disruption in *Clarias gariepinus*. The findings highlight the ecological risk posed by organophosphate contamination in aquatic systems and reinforce the need for stricter regulation and monitoring of pesticide discharge into freshwater environments.

5.0 CONCLUSION

This study demonstrates that exposure to Dichlorvos induces oxidative stress, neurotoxicity, and physiological stress in *Clarias gariepinus*, with effects that are clearly dependent on both concentration and duration of exposure. The observed inhibition of acetylcholinesterase activity, together with elevated plasma cortisol levels, indicates significant disruption of neurological function and endocrine balance, reflecting both cellular and systemic toxicity.

The results further show that the toxic effects of Dichlorvos, particularly neurotoxic and stress-related responses, may persist beyond the removal of the contaminant from the aquatic environment. The limited recovery observed within the 72-hour depuration period suggests that complete physiological restoration requires a longer recovery time, especially at higher exposure concentrations. This highlights the potential need for extended depuration periods or supportive mitigation strategies, such as antioxidant supplementation, to enhance recovery and detoxification processes.

Overall, the findings emphasize the ecological risk posed by Dichlorvos in aquatic systems and underscore the need for stricter regulation of its usage and discharge into the environment. Such measures are essential to protect fish health, preserve aquatic biodiversity, and maintain ecological integrity in freshwater ecosystems.

5.1 Recommendations

1. Implementation of more stringent environmental regulations regarding the application and disposal of Dichlorvos and analogous organophosphate pesticides in proximity to aquatic ecosystems.
2. Continuous monitoring of aquatic environments for pesticide residues and systematic evaluation of fish health through oxidative stress and neurotoxicity biomarkers.
3. Education and awareness initiatives for farmers and pesticide users regarding the safe handling, application, and potential risks of Dichlorvos to aquatic ecosystems.
4. Further research should examine the recovery potential of *C. gariepinus* following exposure and assess protective interventions, including dietary antioxidants.
5. Promotion of safer, less toxic, and environmentally friendly alternatives to Dichlorvos for pest control.

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