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ANTIOXIDANT RECOVERY DYNAMICS AND OXIDATIVE STRESS IN CLARIAS GARIEPINUS: POST DICHLORVOS EXPOSURE

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ABSTRACT

Agricultural intensification and vector control efforts have led to the widespread contamination of aquatic ecosystems with organophosphate pesticides, often ignoring the physiological toll on non-target organisms. This study investigated the oxidative stress profile and subsequent recovery dynamics in the African sharptooth catfish, *Clarias gariepinus*, following exposure to Dichlorvos. A total of 100 juveniles were acclimated for 14 days, followed by acute toxicity testing to establish a 96-hour LC_{50}. Subsequently, fish were subjected to chronic exposure at three sublethal concentrations (0.10, 0.20, and 0.40 mg/L) for 28 days. To evaluate recovery potential, a 72-hour depuration phase in pesticide-free water was conducted. Biochemical analysis revealed that Dichlorvos induced significant ($p < 0.05$) concentration- and duration- dependent oxidative disturbances. Exposure resulted in a marked elevation of Malondialdehyde (MDA), signaling extensive lipid peroxidation, alongside the significant suppression of the antioxidant enzymes Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx). Following the depuration phase, partial restoration of enzyme activity was observed only in the lowest exposure group (0.10 mg/L). In contrast, biomarkers in the higher dosage groups remained significantly altered, indicating that 72 hours is insufficient for complete physiological remediation. These findings demonstrate that Dichlorvos-induced oxidative damage persists beyond the initial exposure period, highlighting the limited recovery dynamics of *C. gariepinus*. This research underscores the ecological risks of organophosphate pollution and advocates for extended depuration periods and stricter regulatory frameworks to protect aquatic biodiversity.

KEYWORDS: *Clarias gariepinus*, Dichlorvos, Oxidative Stress, Antioxidant Recovery,

Depuration, and Biomarkers.

1. INTRODUCTION

Aquatic environments are increasingly impacted by pesticide contamination resulting from agricultural runoff, improper application, and discharge into water bodies. Among these pesticides, organophosphates are widely used due to their effectiveness against a broad range of pests. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is a commonly used organophosphate insecticide; however, its high toxicity to non-target organisms, particularly fish, has raised significant environmental concerns (United States Environmental Protection Agency, 1996). Once introduced into aquatic systems, dichlorvos can adversely affect fish through multiple toxicological pathways.

The primary mechanism of dichlorvos toxicity involves the inhibition of acetylcholinesterase (AChE), an essential enzyme responsible for the breakdown of acetylcholine at neural synapses. Inhibition of AChE leads to the accumulation of acetylcholine, resulting in continuous nerve stimulation, neuromuscular dysfunction, and potentially death (Fulton & Key, 2001). Beyond neurotoxicity, increasing evidence indicates that organophosphate pesticides, including dichlorvos, also induce oxidative stress by promoting the excessive production of reactive oxygen species (ROS) (Lushchak, 2011).

Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species and the antioxidant defense system of the organism. Reactive oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals can damage cellular macromolecules, including lipids, proteins, and DNA, leading to impaired physiological functions (Lushchak, 2011). One of the major consequences of oxidative stress is lipid peroxidation, which disrupts membrane integrity and produces secondary metabolites such as malondialdehyde (MDA), a widely used biomarker for oxidative damage.

Fish possess well-developed antioxidant defense systems to counteract oxidative stress. These include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which work synergistically to neutralize reactive oxygen species and maintain cellular redox balance (Livingstone, 2001). Changes in the activities of these enzymes, alongside levels of lipid peroxidation, are commonly used as sensitive biomarkers in ecotoxicological studies to evaluate the impact of environmental pollutants on aquatic organisms.

The African catfish, *Clarias gariepinus*, is widely distributed across Africa and is of significant economic importance in aquaculture. Its tolerance to a wide range of environmental conditions and sensitivity to pollutants make it a suitable model organism for toxicological studies (Adewolu, Adeniji, & Adejobi, 2008). Previous studies have shown that exposure to pesticides and other contaminants can induce oxidative stress in *Clarias gariepinus*, resulting in altered antioxidant enzyme activities and increased lipid peroxidation (Nwani, Lakra, Nagpure, Kumar, Kushwaha, & Srivastava, 2010).

While numerous studies have focused on the toxic effects of pesticides during exposure, relatively few have examined the post-exposure recovery phase, which is essential for understanding the resilience of aquatic organisms. Recovery studies provide insight into whether antioxidant systems can return to baseline levels after the removal of a toxicant or whether oxidative damage persists. Evidence suggests that recovery may be incomplete or delayed, indicating prolonged physiological stress even after exposure has ceased (Lushchak, 2011).

Despite growing interest in oxidative stress biomarkers, there remains limited information on the antioxidant recovery dynamics following dichlorvos exposure in *Clarias gariepinus*. Most existing studies emphasize acute toxicity and biomarker responses during exposure, with less attention given to the temporal patterns of recovery. This gap limits a comprehensive understanding of the long-term ecological effects of pesticide contamination in aquatic environments.

Therefore, this study aims to investigate the antioxidant recovery dynamics in *Clarias gariepinus* following dichlorvos exposure, focusing on key biomarkers such as superoxide dismutase, catalase, glutathione peroxidase, and lipid peroxidation. The study seeks to evaluate the extent and rate of recovery of the antioxidant defense system after exposure, thereby providing insight into the resilience of fish to pesticide-induced oxidative stress and contributing to improved environmental risk assessment.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

1 Test species: *Clarias gariepinus* (African catfish)

2 Quantity: 100 juveniles (mean weight: 150 ± 10 g; mean length: 25 ± 2 cm)

3 Source: Private fish farm, Otuoke, Bayelsa State, Nigeria

3.0.1 Chemicals and Reagents

a. Toxicant

i. Dichlorvos (DDVP)

ii. Source: Commercial-grade organophosphate insecticide (e.g., Sniper®)

iii. Purity: ≥99%

iv. Solvent: Dechlorinated water

b. Biochemical Reagents

i. Antioxidant Enzyme Assays Superoxide Dismutase (SOD)

- Sodium pyrophosphate buffer
- Phenazine methosulphate (PMS)
- Nitroblue tetrazolium (NBT)
- NADH solution

Catalase (CAT)

- Hydrogen peroxide (H_2O_2)
- Phosphate buffer (pH 7.0)
- Ammonium molybdate

Glutathione Peroxidase (GPx)

- Reduced glutathione (GSH)
- Hydrogen peroxide (H_2O_2)
- Phosphate buffer
- 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB)

ii. Lipid Peroxidation (MDA)

- Thiobarbituric acid (TBA)
- Acetic acid
- Sodium dodecyl sulfate (SDS)
- Butanol–pyridine mixture

2.2. METHODS

2.2.1. Experimental Fish Collection and Acclimatisation

A total of 100 healthy *C. gariepinus* (mean weight: 150 ± 10 g; mean length: 25 ± 2 cm) were

collected from a private fish farm in Otuoke, Bayelsa State, Nigeria. The fish were transported in well-aerated containers to the laboratory and acclimatized for 14 days in aerated plastic tanks (300 L capacity) containing dechlorinated water. Water quality parameters were maintained as follows: temperature, 26 ± 1°C; pH, 7.2 ± 0.3; dissolved oxygen, 6.5 ± 0.5 mg/L; Fish were fed commercial fish pellets at 3% of their body weight twice daily during acclimatisation, but were not fed 24 hours before toxicity testing.

2.2.2. Acute and Sublethal Toxicity Tests of Dichlorvos on c.gariepinus Using Probit Analysis to Determine LC50

In a controlled laboratory experiment, an acute toxicity test was conducted to evaluate the effects of Dichlorovos, an organochlorine pesticide, on *C. gariepinus*. The primary aim of the study was to determine the median lethal concentration (LC_{50}) of Dichlorovos, which represents the concentration capable of causing 50% mortality in the test population within a specific exposure period.

2.2.3. Acute Toxicity Test Procedure

Healthy juvenile *C. gariepinus* were procured from a fish farm in Otuoke, acclimatized under laboratory conditions for 14 days, and maintained in well-aerated aquaria with optimal water quality parameters. Following acclimation, the fish were randomly distributed into tanks containing graded concentrations of Dichlorovos based on preliminary range-finding tests. A control group without Dichlorovos exposure was also maintained.

The fish were exposed to the toxicant for 96 hours under static renewal conditions. Mortalities were recorded at 24, 48, 72, and 96 hours of exposure. Dead fish were promptly removed to prevent water contamination and stress to surviving individuals.

2.2.4. Determination of LC_{50} Using Probit Analysis

The mortality data obtained from the acute exposure were analyzed using Probit analysis to establish the concentration-response relationship. The percentage mortalities at different concentrations were converted into Probit units and plotted against the logarithmic values of Dichlorovos concentrations. A regression line was fitted, and the LC_{50} value was extrapolated from the concentration corresponding to the Probit 5 value. The LC_{50} determined from this experiment served as a reference point for subsequent sublethal testing.

2.2.5. Sublethal Toxicity Test

Following the acute study**,** sublethal concentrations of Dichlorovos were selected, typically as

fractions of the 96-hour LC_{50} (e.g., 1/10th, 1/5th, and 1/2 of LC_{50}). The fish were then exposed to these lower concentrations for an extended period to assess chronic and biochemical effects that do not necessarily result in mortality but may impact physiological functions.

2.2.6. Experimental Design

The fish were randomly divided into four groups (n = 15 per group) as follows:

i. **Group 1 (Control):** Exposed to clean, dechlorinated water with no Dichlorvos.
ii. **Group 2 (Low Dose):** Exposed to 0.10 mg/L of Dichlorvos.
iii. **Group 3 (Medium Dose):** Exposed to 0.20 mg/L of Dichlorvos.
iv. **Group 4 (High Dose):** Exposed to 0.40 mg/L of Dichlorvos.

Each treatment group have three replicates with five each. Exposure lasted for 28 days, with 50% water renewal and fresh dosing every 48 hours to maintain Dichlorvos concentration. Fish were fed twice daily throughout the exposure period while been observed for behavioural changes and mortality.

2.2.7. Sampling Procedure

Blood samples were collected from fish on days 7, 14, 21, and 28 of the exposure periods. Fish were anaesthetised using tricaine methanesulfonate (MS-222) at 100 mg/L. Blood was drawn from the caudal vein using sterile insulin syringes and transferred immediately into heparinized bottles to prevent coagulation. Samples were stored at 4°C before biochemical analyses.

2.2.8. Recovery Trends in *Clarias gariepinus* Following 28-Day Dichlorvos Exposure

At the end of the 28-day exposure, surviving fish from each treatment group were transferred to clean, pesticide-free water to evaluate recovery potential. Fish were maintained under the same laboratory conditions, and blood samples were further collected 72 hours post-exposure to assess recovery trends. During this phase, no Dichlorvos was present, and fish were continuously fed as during the exposure period.

2.2.9 Biochemical Analyses

The following biochemical markers were analyzed to assess oxidative stress responses, neurological stress, and endocrine disruption in *C. gariepinus* exposed to sublethal concentrations of Dichlorvos.

2.2.9.1. Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was determined using the method described by Misra and Fridovich (1972). This method is based on the ability of SOD to inhibit the autoxidation of epinephrine to adrenochrome at alkaline pH.

Tissue homogenates were prepared in phosphate buffer (pH 7.4). The reaction mixture contained carbonate buffer, epinephrine, and the sample. The increase in absorbance was monitored at 480 nm for 150 seconds. SOD activity was expressed in units per milligram of protein (U/mg protein).

2.2.9.2. Catalase (CAT) Activity

Catalase activity was measured following the protocol of Aebi (1984), which assesses the rate of decomposition of hydrogen peroxide (H_2O_2).

Sample homogenates were prepared in phosphate buffer. The reaction mixture consisted of phosphate buffer and hydrogen peroxide. The decomposition of hydrogen peroxide was monitored by the decrease in absorbance at 240 nm. Catalase activity was expressed in µmol H_2O_2 decomposed per minute per milligram of protein.

2.2.9.3. Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase activity was assayed based on the method of Paglia and Valentine (1967), which measures the rate of oxidation of reduced glutathione (GSH) by cumene hydroperoxide.

The reaction mixture contained phosphate buffer, sodium azide, reduced glutathione, NADPH, glutathione reductase, and the sample. The reaction was initiated by the addition of hydrogen peroxide. The decrease in absorbance due to the oxidation of NADPH was measured at 340 nm. GPx activity was expressed in units per milligram of protein (U/mg protein).

2.2.9.4. Malondialdehyde (MDA) Concentration

Malondialdehyde, a marker of lipid peroxidation, was quantified using the Thiobarbituric Acid Reactive Substances (TBARS) assay as described by Buege and Aust (1978).

Tissue homogenates were mixed with thiobarbituric acid (TBA) reagent and heated in a boiling water bath for 15 minutes. The pink chromogen formed by the reaction of MDA with TBA was extracted with n-butanol. Absorbance was measured at 532 nm. MDA levels were expressed in nmol MDA per milligram of protein.

2.2.10. Recovering Phase

After a 28-day exposure to sublethal levels of Dichlorvos, *C. gariepinus* were placed in uncontaminated, pesticide-free water for a 72-hour depuration interval. Biochemical indicators were reassessed after this period to evaluate the initial recovery response to oxidative, neurological, and endocrine stress.

2.2.11. Statistical Analysis

All results were expressed as mean ± standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences between treatments and sampling times. The level of statistical significance was set at $p < 0.05$. LC_{50} values were estimated 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)	27.3 ± 0.5	27.4 ± 0.4	27.2 ± 0.6	27.5 ± 0.5
Ph	7.2 ± 0.2	7.3 ± 0.2	7.1 ± 0.3	7.2 ± 0.2
Dissolved Oxygen (mg/L)	6.3 ± 0.4	6.2 ± 0.5	6.4± 0.4	6.5 ± 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 for water quality parameters in aquaculture: Temperature (28 ± 2°C), pH (7.5±0.5),DO(5.0±1.0mg/L),Ammonia(0.01 ± 0.01 mg/L) (Boyd,2017;FAO,2018;Hargreaves &Tucker,2004;WHO,2017). Although there were some variations, all of the values were optimal for *C. gariepinus* to survive (Table 1).

3.2 Oxidative Stress Biomarkers

3.2.1 Superoxide Dismutase Activity

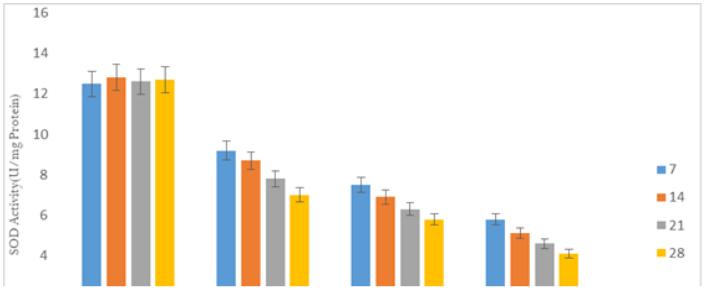

Figure 1: Superoxide Dismutase Activity (U/mg Protein) in the blood of *C.gariepinus* on days 7, 14, 21, and 28 of the exposure to Dichlorvos.

In contrast to the control group, *C. gariepinus* exposed to sublethal concentrations of Dichlorvos (0.10 mg/L, 0.20 mg/L, and 0.40 mg/L) over a 28-day period showed a significant decrease in SOD activity in a concentration- and time-dependent manner (Figure 1).

On day 7, fish subjected to the lowest concentration (0.10 mg/L) demonstrated a significant decrease in SOD activity relative to the control group. The activity of SOD diminished progressively with increased exposure duration and Dichlorvos concentration, signifying a gradual inhibition of antioxidant defense systems. The most significant reduction occurred at 0.40 mg/L, indicating that elevated pesticide levels impair the fish's antioxidant system.

3.2.2 :Catalase Activity (µmol H_2O_2/min/mg Protein)

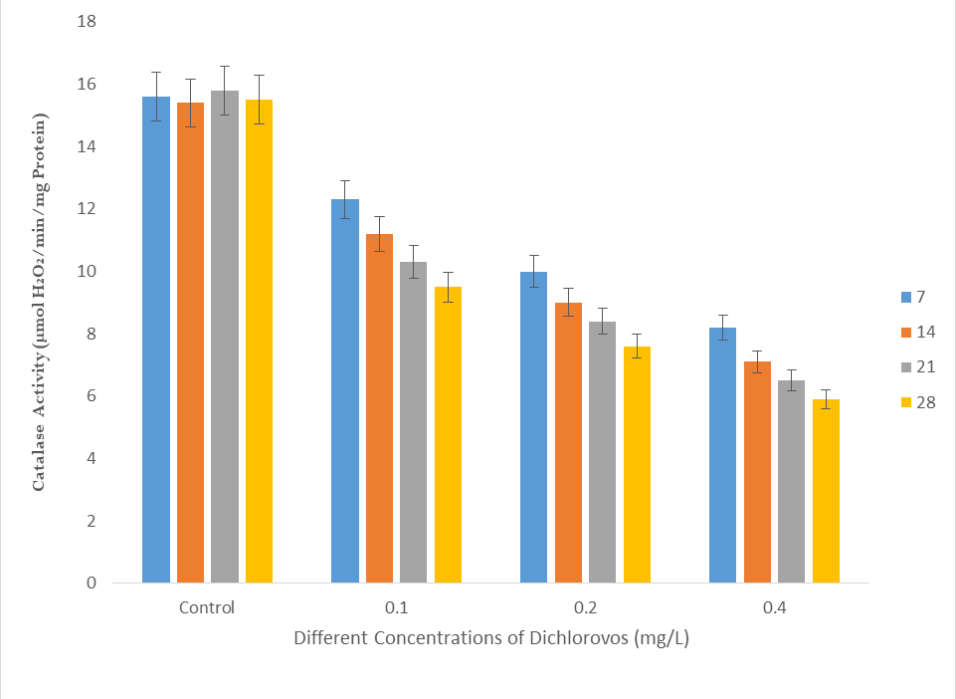

Figure 2: Catalase Activity (µmol H_2O_2/min/mg Protein) in the blood of *C.gariepinus* on days 7, 14, 21, and 28 of the exposure to Dichlorvos.

Over 28 days, CAT activity in *C. gariepinus* treated to Dichlorvos decreased progressively. The decline was concentration- and time-dependent, which was consistent with the trend seen with SOD activity (Figure 2).

On day 7, fish treated with 0.10 mg/L Dichlorvos showed a significant ($p< 0.05$) drop in CAT activity compared to the control, indicating an early response to oxidative stress. CAT activity continued to drop dramatically over the 28-day exposure period, particularly in the 0.20 mg/L and 0.40 mg/L groups. The highest inhibition of CAT activity was found at 0.40 mg/L Dichlorvos, indicating severe oxidative stress and inadequate antioxidant defence at

higher doses.

4.2.3. **Glutathione Peroxidase (GPx) Activity**

Table 2: Glutathione Peroxidase (GPx) Activity (U/mg Protein) in the blood of *C.gariepinus* on days 7, 14, 21, and 28 of the exposure to Dichlorvos.

Treatment	Day 7	Day 14	Day 21	Day 28
Control	8.5 ± 0.5	8.7 ± 0.4	8.6 ± 0.5	8.8 ± 0.4
0.10 mg/L	6.3 ± 0.4	5.7 ± 0.3	5.1 ± 0.3	4.7 ± 0.2
0.20 mg/L	5.1 ± 0.3	4.4 ± 0.2	3.8 ± 0.3	3.3 ± 0.2
0.40 mg/L	3.9 ± 0.2	3.1 ± 0.2	2.7 ± 0.2	2.1 ± 0.1

This study demonstrated a significant ($p < 0.05$) and progressive decline in GPx activity in C. gariepinus exposed to Dichlorvos at all tested concentrations over the 28-day exposure period. By day 7, GPx activity decreased in all groups exposed to Dichlorvos compared to the control, indicating a rapid onset of oxidative stress (Table 1). The decrease in GPx activity was more pronounced at elevated concentrations of Dichlorvos, indicating a dose-response relationship. On day 28, the 0.40 mg/L group exhibited the lowest GPx activity, suggesting cumulative oxidative damage and potential depletion of glutathione reserves. GPx activity was significantly ($p < 0.05$) suppressed in all treated groups.

3.2.4. Malondialdehyde (MDA) Levels

Table 3: Malondialdehyde (MDA) Levels (nmol/mg Protein) in the blood of *C.gariepinus* on days 7, 14, 21, and 28 of the exposure to Dichlorvos.

Treatment	Day 7	Day 14	Day 21	Day 28
Control	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
0.10 mg/L	2.8 ± 0.2	3.2 ± 0.2	3.5 ± 0.2	4.0 ± 0.3
0.20 mg/L	3.7 ± 0.2	4.2 ± 0.3	4.8 ± 0.3	5.3 ± 0.3
0.40 mg/L	4.6 ± 0.3	5.2 ± 0.3	5.9 ± 0.4	6.5 ± 0.4

This study demonstrates that MDA levels in *C. gariepinus* exposed to Dichlorvos increased progressively across all concentrations over the 28-day exposure period (Table 2). This trend contrasts with the declining levels of antioxidant enzymes (SOD, CAT, GPx), which showed significant increases ($p < 0.05$) with both exposure time and Dichlorvos concentration. By day 7, MDA levels were significantly elevated ($p < 0.05$) in fish exposed to Dichlorvos relative to the control group. MDA levels increased consistently across all exposure durations, signifying persistent oxidative damage. The highest concentrations of MDA were consistently observed in fish exposed to 0.40 mg/L Dichlorvos, indicating a dose-response relationship.

3.3 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 4).

Table 4: 72-Hour Recovery Response of C.gariepinus in Dichlorvos free dechlorinated tap.

Concentration	water.**Biomarker**	**Control**	**72 hours recovery response**	**Status at 72 hours**
0.10mg/l	SOD(U/mg protein)	12.50±0.40	12.10±1.30	Proximal to control
0.20mg/l	SOD(U/mg protein)	12.50±0.40	11.70±0.40	Marginally lower yet proximate
0.40mg/l	SOD(U/mg protein)	12.50±0.40	11.70±0.40	Marginally lower yet proximate
0.10mg/l	CAT(U/mg protein)	15.70±0.10	15.10±0.50	Similar to control
0.20mg/l	CAT(U/mg protein)	15.70±0.10	15.10±0.10	Similar to control
0.40mg/l	CAT(U/mg protein)	15.70±0.10	14.60±0.10	Similar to control
0.10mg/l	GPx(U/mg protein)	8.40±0.60	8.20±0.70	Similar to control
0.20mg/l	GPx(U/mg protein)	8.40±0.60	7.60±0.40	Marginally reduced
0.40mg/l	GPx(U/mg protein)	8.40±0.60	5.90±0.50	Significantly diminished
0.10mg/l	MDA(nmol/mg protein)	1.50±0.70	1.80±0.12	Approaching control,albeit somewhat elevated
0.20mg/l	MDA(nmol/mg protein)	1.50±0.70	2.40±0.70	Exceeding control
0.40mg/l	MDA(nmol/mg protein)	1.50±0.70	2.70±0.10	Further escalated

Superoxide Dismutase Activity: SOD activity exhibits negligible reductions across all treatments. The readings are comparable to the control, suggesting that the fish's antioxidant defense mechanism is nearly fully restored after 72 hours, even at the maximum dose of dichlorvos (0.40 mg/l). This indicates that the recovery from oxidative stress is efficiently sustained, allowing the fish to neutralise oxidative radicals proficiently, despite chemical exposure.(Table 4)

Catalase Activity:The CAT activity exhibits a little reduction across all treatment concentrations, however the activity values remain closely aligned with the control group. This signifies that the fish's capacity to neutralise hydrogen peroxide is almost fully restored,

demonstrating substantial recovery from oxidative stress after 72 hours. The recovery is nearly complete, with negligible effect on the enzyme's capacity to manage oxidative damage. (Table 4)

Glutathione Peroxidase Activity: GPx activity exhibits a decline, particularly at elevated concentrations (0.20 and 0.40 mg/L), suggesting that the recovery for this marker is less thorough. The value at 0.10 mg/L (8.20) remains proximate to the control (8.40), indicating some recovery at diminished doses. The efficacy of recovery diminishes with increasing concentrations of dichlorvos, signifying heightened oxidative stress at elevated treatment doses.(Table 4)

Malondialdehyde (MDA) Concentrations: MDA levels rise with treatment concentration, especially around 0.40 mg/l. This indicates that lipid peroxidation persists, signifying inadequate recovery from oxidative stress at elevated quantities. The elevated MDA levels compared to the control across all treatment concentrations indicate that recovery is impeded with increased dichlorvos exposure, resulting in incomplete lipid repair.(Table 4)

4.0. DISCUSSION

This study assessed Antioxidant recovery dynamics and oxidative stress responses in *C. gariepinus* after exposure to different sublethal concentrations of Dichlorvos over 28 days. The evaluated biomarkers; SOD, CAT, GPx, MDA—offered a thorough insight into the toxicodynamic impacts of Dichlorvos on the physiological and biochemical systems of fish.

4.1.1 Oxidative Stress Markers

The activities of antioxidant enzymes, Superoxide Dismutase, Catalase, and Glutathione Peroxidase exhibited significant reductions that were both time- and concentration-dependent in fish exposed to Dichlorvos, relative to the control group. The observed suppression indicates that antioxidant defence mechanisms were increasingly compromised by reactive oxygen species (ROS) produced due to Dichlorvos-induced toxicity. In contrast, Malondialdehyde levels, which serve as a biomarker for lipid peroxidation, exhibited a significant increase across all treatment groups in a time- and dose-dependent manner, suggesting an elevation in oxidative damage to membrane lipids.

4.1.1. Superoxide Dismutase Activity

The first line of defense against oxidative stress in biological systems is provided by

superoxide dismutase (SOD), a vital antioxidant enzyme that catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen (Misra and Fridovich, 1972).

The decrease in SOD activity suggests that *C. gariepinus'* antioxidant defense capacity was compromised, possibly due to the excess generation of reactive oxygen species (ROS) caused by Dichlorvos toxicity. When the rate of ROS generation exceeds SOD's ability to neutralize them, oxidative damage to lipids, proteins, and DNA is likely (Lushchak, 2011). Adeyemi *et al*. (2009) observed that exposure to organophosphate pesticides reduced antioxidant enzyme activity in fish species.Similarly, inhibitory effects on antioxidant enzymes in fish exposed to organophosphate pesticides have also been reported by Adeyemi et al. (2009), and Li et al. (2010). These findings confirm SOD suppression as a reliable biomarker of pesticide-induced oxidative stress in aquatic organisms. Reduced SOD activity may reflect enzyme inhibition or degradation caused by prolonged oxidative stress, which could impair normal cellular function and contribute to the observed increase in lipid peroxidation in this study.

4.1.2. Catalase Activity

Catalase is an essential antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen, thus safeguarding cells from oxidative damage resulting from the accumulation of hydrogen peroxide (Aebi, 1984). The reduction in CAT activity may be attributed to enzyme inactivation caused by the excessive production of reactive oxygen species (ROS), especially hydrogen peroxide, which is typically detoxified by the enzyme (Lushchak, 2011). Ramesh et al. (2009) and Velisek et al. (2011) have documented comparable reductions in CAT activity after exposure to organophosphate pesticides in various fish species, thereby reinforcing the validity of CAT as a biomarker for oxidative stress in ecotoxicological research.

When CAT is overwhelmed or inactivated, hydrogen peroxide may accumulate, resulting in cellular damage through the Fenton reaction, which generates highly reactive hydroxyl radicals (Ramesh et al.2009). The decrease in CAT activity corresponds with heightened lipid peroxidation, as evidenced by increased MDA levels, indicating that the fish's antioxidant system was inadequate to mitigate oxidative damage resulting from Dichlorvos exposure.

4.1.3. Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) functions as a crucial antioxidant enzyme that catalyzes the reduction of hydrogen peroxide and lipid peroxides to non-toxic substances, utilizing reduced

glutathione as a substrate. This enzyme is crucial for safeguarding cell membranes against oxidative damage (Flohé and Günzler, 1984).

The reduction in GPx activity suggests a diminished capacity to neutralize hydrogen peroxide and lipid peroxides, potentially resulting in the accumulation of these toxic compounds and heightened lipid peroxidation, as indicated by increased MDA levels in this study. The inhibition of GPx, in conjunction with SOD and CAT, indicates a significant impairment of the antioxidant defence system in *Clarias gariepinus* under oxidative stress induced by Dichlorvos. Li et al. (2010) and Zhang et al. (2017) have reported analogous trends, indicating that organophosphate pesticides result in reduced GPx activity in fish, thereby confirming the enzyme's susceptibility to oxidative stress induced by pesticides.

Chronic exposure to oxidative agents such as Dichlorvos likely depleted the glutathione pool necessary for GPx to execute its protective role, thereby rendering the fish susceptible to cellular and tissue damage.

4.1.4. Malondialdehyde (MDA) Levels

Malondialdehyde (MDA) serves as a recognized biomarker for lipid peroxidation and oxidative stress. Increased MDA levels signify membrane lipid damage resulting from the overproduction of reactive oxygen species (ROS) (Del Rio *et al.,* 2005).

By day 7, MDA levels were significantly elevated ($p < 0.05$) in fish exposed to Dichlorvos compared to the control group. MDA levels consistently increased with all exposure durations, signifying persistent oxidative damage. The observed elevation of MDA aligns with findings by Ramesh and Saravanan (2008) and Li *et al.* (2010), which indicated significant increases in lipid peroxidation in fish subjected to organophosphate pesticides, thereby supporting MDA as a sensitive biomarker for oxidative damage.

The increase in MDA levels indicates lipid membrane degradation resulting from significant oxidative stress. The reduced activity of antioxidant enzymes (SOD, CAT, and GPx) likely resulted in inadequate neutralization of reactive oxygen species (ROS), which in turn led to uncontrolled lipid peroxidation.

This suggests that the antioxidant defence system was impaired, facilitating the progression of oxidative damage. The accumulation of MDA is harmful as it can compromise membrane integrity, hinder cellular functions, and induce apoptosis or necrosis (Ayala et al., 2014).

4.1.5. 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 comprise antioxidant enzymes (SOD, CAT, GPx) and a lipid peroxidation marker (MDA). These indicators function as precise instruments for evaluating detoxification, mitigation of oxidative stress, and physiological resilience following exposure.

4.1.5.1. Superoxide Dismutase:A slight enhancement in SOD activity was noted, especially in the 0.10 mg/L group, suggesting that the antioxidant defense mechanism commenced recovery following the elimination of the toxicant. SOD facilitates the dismutation of superoxide radicals into hydrogen peroxide, hence safeguarding cells from oxidative harm (Lushchak, 2011). Despite an enhancement in SOD activity within 72 hours, levels in greater exposure groups remained inferior to control, indicating a persistent oxidative burden or a protracted recovery.

4.1.5.2. Catalase: Catalase activity, which facilitates the decomposition of hydrogen peroxide into water and oxygen, exhibited a modest increase following depuration, particularly in the low-dose group. . Studies have shown that exposure to low concentrations of toxicants, like pesticides, may cause minimal oxidative stress, allowing the antioxidant defense system to recover or remain functiona(Nawaz et al., 2020). The small changes observed in the enzyme activities, as seen in the findings, support the idea that strong recovery occurs at lower concentrations.

The partial restoration of CAT aligns with the observations of Ramesh and Saravanan (2008), who noted that CAT necessitates an extended duration to revert to baseline following pesticide-induced suppression.

4.1.5.3. Glutathione Peroxidase: GPx activity showed a little increase in the 0.10 and 0.20 mg/L groups. Partial recovery at lower concentrations of pollutants is often seen, while higher concentrations may overwhelm the antioxidant system, leading to more significant impairment (Zhou et al., 2017). This aligns with the findings, where GPx showed partial recovery at 0.10mg/L but impairment at 0.40 mg/L. GPx, a selenium-dependent enzyme, is essential for cellular detoxification by reducing lipid hydroperoxides and hydrogen peroxide. The slower recovery indicates that glutathione-dependent pathways may exhibit heightened sensitivity to oxidative damage or necessitate a longer duration for regeneration (Li et al., 2010).

4.1.5.4. Malondialdehyde(MDA): Malondialdehyde levels exhibited a small reduction during the 72- hour depuration period, particularly in the 0.10 mg/L group. MDA is a result of the peroxidation of polyunsaturated fatty acids and functions as a marker for membrane lipid degradation (Ayala et al., 2014). Longer-term studies have examined the recuperative capacity of *C. gariepinus* following oxidative stress. Aderemi *et al*. (2018), for example, tracked antioxidant responses during and after extended exposure to Dichlorvos. While antioxidant enzyme levels showed some improvement during depuration, elevated MDA remained in hepatic tissues, indicating incomplete resolution of oxidative damage.

The sustained elevation of MDA in higher concentration groups signifies ongoing oxidative stress and membrane deterioration, presumably resulting from residual pesticide effects. An increase in MDA levels with increasing pesticide concentrations suggests that higher levels of the chemical cause impaired recovery from oxidative damage (Ahmad et al., 2020). The findings, where MDA levels increase with treatment concentrations, particularly at 0.40 mg/L, align with these observations.

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study demonstrated that exposure of *Clarias gariepinus* to dichlorvos induces significant oxidative stress and physiological disturbance, with effects that are dependent on both concentration and duration of exposure. The observed alterations in antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—together with elevated malondialdehyde (MDA) levels, indicate a disruption of the fish's antioxidant defense system and increased lipid peroxidation.

The findings further reveal that oxidative damage may persist even after cessation of exposure, suggesting delayed or incomplete recovery during the depuration period. This indicates that the antioxidant system of *C. gariepinus* requires extended time to restore homeostasis following dichlorvos exposure.

Overall, the study highlights the need for adequate recovery (depuration) periods and potential supportive interventions, such as antioxidant-based strategies, to enhance physiological recovery. It also emphasizes the importance of regulating the use of dichlorvos in aquatic environments to minimize oxidative damage and protect fish health and ecosystem stability.

5.2 Recommendations

Based on the findings of this study, the following recommendations are made:

1 Regulation of Dichlorvos Use: Regulatory authorities should enforce stricter control on the use and disposal of dichlorvos, especially in areas close to aquatic environments, to reduce contamination of water bodies.

2 Environmental Monitoring: Routine monitoring of pesticide residues and oxidative stress biomarkers in aquatic organisms should be implemented to detect early signs of ecological stress and pollution.

3 Adequate Depuration Periods: Fish exposed to dichlorvos or similar organophosphate pesticides should be allowed sufficient depuration time before consumption or further experimental use to ensure partial recovery of physiological and biochemical functions.

4 Public Awareness: Farmers and pesticide users should be educated on the ecological risks associated with indiscriminate use of dichlorvos and encouraged to adopt safer pest control alternatives.

5 Further Research: Additional studies should be conducted to investigate long-term recovery patterns and the potential use of antioxidant supplements or natural detoxifying agents in improving recovery of *Clarias gariepinus* following pesticide exposure.

REFERENCES

1 Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

2 Adewolu, M. A., Adeniji, C. A., & Adejobi, A. B. (2008). Feed utilization, growth and survival of *Clarias gariepinus* fed different dietary protein levels. *Journal of Fisheries and Aquatic Science, 3*(5), 292–297.

3 Adeyemi JA (2014) Oxidative stress and antioxidant enzyme activities in the Africana catfish Clarias gariepinus,experimentally challenged with Escherichia coli and Vibrio fishcheri. *Fish Physiology and Biochemistry*. 40: 347-354

4 Ahmad, I., Hamid, T., Fatima, M., Chand, H. S., & Jain, G. (2018). Toxicity, oxidative stress, and biochemical responses in fish and prawn exposed to dichlorvos and cypermethrin. *Environmental Science and Pollution Research*, 25(23), 23041-23052.

5 Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014*, 360438.

6 Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology, 52*, 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6

7 Del Rio, D., Stewart, A. J., & Pellegrini, N. (2005). A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism and Cardiovascular Diseases, 15*(4), 316–328.

8 Fulton, M. H., & Key, P. B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. *Environmental Toxicology and Chemistry, 20*(1), 37–45.

9 Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin, 42*(8), 656–666.

10 Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology, 101*(1), 13–30.

11 Li, Z. H., Li, P., Shi, Z. C., & Gao, R. T. (2010). Effects of acute exposure of herbicide on the antioxidant defense system in the liver of common carp (*Cyprinus carpio* L.). *Pesticide Biochemistry and Physiology, 97*(2), 127–131.

12 Li, Z.-H., Velisek, J., Zlabek, V., Grabic, R., Machova, J., & Randak, T. (2010). Modulation of glutathione-related antioxidant defense system of fish chronically treated by the fungicide propiconazole. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 152(2), 92–98. https://doi.org/10.1016/j.cbpc.2010.03.006.

13 Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry, 247*(10), 3170–3175.

14 Nawaz, S., Javed, M., & Farooq, M. (2020). Biochemical and physiological responses of freshwater fish to pesticide exposure: oxidative stress and antioxidant enzyme activity. *Environmental Toxicology*, 35(5), 589-598.

15 Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B., & Srivastava, S. K. (2010). Toxicity of the herbicide atrazine: Effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). *International Journal of Environmental Research and Public Health*, 7(8), 3298–3312.

16 Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine, 70*(1), 158–169.

17 Ramesh, M., & Saravanan, M. (2009). Haematological and biochemical responses in a freshwater fish *Cyprinus carpio* exposed to chlorpyrifos. *International Journal of*

Integrative Biology, 5(1), 61–65.

18 United States Environmental Protection Agency. (1996). *Test methods for evaluating solid waste, physical/chemical methods (SW-846).* Washington, DC: Office of Solid Waste.

19 Velisek, J., Stara, A., Machova, J., & Svobodova, Z. (2011). Effects of long-term exposure to simazine in real environmental concentration on common carp (*Cyprinus carpio L.*). *Ecotoxicology and Environmental Safety, 74(*4), 972–976.

20 Zhang, C., Chen, X., & Ling, Y. (2018). Ecological risk assessment of typical pesticides in the Dongting Lake region, China. *Ecological Indicators*, 85, 811- 817.

21 Zhang, F. (2014). Effects of trichlorfon on oxidative stress and cortisol in common carp. *Aquatic Toxicology*, 150, 1–10.

22 Zhang, L., Li, J., Wang, Y., & Jiang, G. (2017). Time-dependent recovery of biomarkers in zebrafish (Danio rerio) after exposure to sublethal concentrations of trichlorfon. *Environmental Science and Pollution Research,* 24(6), 5847–5855. https://doi.org/10.1007/s11356-016-8327x

23 Zhang, Y., Dong, F., Gao, J., & Lin, K. (2017). Toxicity and oxidative stress responses in zebrafish (*Danio rerio*) induced by exposure to the herbicide simazine. *Environmental Pollution, 226*, 32–41.

24 Zhang, W., Wang, Z., & LI, J. (2021). Antioxidant defense in fish exposed to endocrine-disrupting chemicals: A review. *Fish and shellfish Immunology*, 117, 235 -243.

25 Zhou, J., Li, S., Wa Sharma, M., Sahni, Y. P., Mandloi, A. K., & Pandey, G. (2012). Toxicity in fish polluted with heavy metals, chemicals or drugs. *Jigyasa-I*, 6, 67-71.