E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2023; 11(5): 88-98

www.fisheriesjournal.com
Received: 20-05-2023
Accepted: 01-07-2023

Francis C
National Biotechnology Development Agency, Arochukwu, Abia State, Nigeria

Odo UU
Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nigeria

Ugwanyi KC
Micheal Okpara University of Agriculture, Umudike, Nigeria

Egbuji JV
Animal and Environmental Physiology Unit, University of Nigeria, Nigeria

Lemuel AS
National Biotechnology Development Agency, Arochukwu, Abia State, Nigeria

Okpechi NJ
National Biotechnology Development Agency, Arochukwu, Abia State, Nigeria

Ugwu IJA
Parasitology and Public Health Unit, University of Nigeria, Nigeria

Odo GE
Department of Zoology and Environmental Biology, University of Nigeria, Nigeria

Corresponding Author:
Francis C
National Biotechnology Development Agency, Arochukwu, Abia State, Nigeria

Abnormalities in behavioral responses and hematological parameters of African catfish *Clarias gariepinus* juveniles exposed to acute and sub-chronic effects of mancozeb

Francis C, Odo UU, Ugwanyi KC, Egbuji JV, Lemuel AS, Okpechi NJ, Ugwu IJA and Odo GE

DOI: https://doi.org/10.22271/fish.2023.v11.i5b.2853

Abstract
The present study investigated the effects of acute and sub-chronic exposure to Mancozeb on behavioral responses and haematological parameters of *Clarias gariepinus* juveniles. A total of 120 *Clarias gariepinus* with standard length and weight which ranged from 9.8 cm to 17.5 cm and 11g to 55 g respectively were used for the experiment. Prior to the experiment, determination of median lethal concentration. $LC_{24\text{-}96h}$ of Mancozeb, a total of 150 *Clarias gariepinus* juviniles were used. They were exposed to five treatments: 0.00, 150, 300, 450, 600 and 750 mg/L (A-E). While, during sub-lethal determination four treatments were exposed to 0.00. 20.55, 41.09 and 82.18 mg/L (A-D). Each group were replicated three times. Acute toxicity was carried out and the percentage mortality and survival were recorded. The 96 hours LC_{50} value of Mancozeb on *Clarias gariepinus* juveniles was estimated to be 410.90 mg/L. The physico-chemical parameters of water observed for five weeks showed changes and fluctuations in temperature range from 28 °C to 32 °C, pH levels ranged from 7.3 to12.3, Alkalinity range 0.88 to 4.10 mg/L, CO_2 values ranged from 3-12.5 mg/L. While dissolved oxygen from 6.6 to 7.8 mg/L. Behavioral changes observed after 24-96 hours of fish exposure to different concentrations of Mancozeb revealed loss of equilibrium status and respiratory difficulties. The estimated safe levels varied from 41.09– 4.109 $\times 10^{-3}$ mg/L. Erythrocytes were sampled to investigate haematological parameters. The results showed significant concentration and duration-dependent decrease in RBC count, PCV, Hb and increased in WBC while MCH was not significantly different while MCHC and MCV fluctuated. The activities of neutrophils and lymphocytes were not significantly different among treatment groups whereas Monocytes, Basophiles and Eosinophiles fluctuated.

Keywords: Mancozeb, *Clarias gariepinus*, behavioral response, haematological parameters, acute toxicity, sub-chronic effects

Introduction
Mancozeb is a synthetic ethylene (Bis) dithiocarbamite belonging to a subclass carbamate fungicide (Thiruchelvam, 2005; Srivastava and Singh, 2014) [81, 79]. It is a carbamate fungicide applied on agricultural crops such as Vegetables, Cereals and fruits for the purpose of controlling or eradicating various fungal diseases (Cycoń *et al*., 2010; Paro *et al*., 2012) [18, 65]. Mancozeb is a mixture of two compounds with active ingredients Maneb (Manganese) and Zineb (Zinc) at a ratio of 2:1 respectively (Morgan, 1982; Hayes and Laws, 1991; Thiruchelvam, 2005) [52, 33, 81].
Mancozeb is produced in several forms such as liquid, dust, wet table powders, Water dispersible granules and ready to use formulations (Thiruchelvam, 2005) [81]. Mancozeb exhibit some hydrophobic characteristics, that is why it does not pollute ground water while its metabolite ethylenethiourea has the capacity to contaminate groundwater (Srivastava and Singh, 2013) [80]. Market global statistic record indicate that Mancozeb- containing products generate almost 740 dollars interests after sales (Dow Agrosciences, 2008) [24]. Mancozeb has low to moderate toxcity and research findings reveals that it caused detrimental consequences to Man and was classified as a carcinogen with a prove of genotoxic effects on experimental animals (Cecconi *et al*., 2007) [17].

Approximately, 0.1% of pesticides applied destroys target organisms (pests) while the remaining 99.9% end-up in the aquatic ecosystem (Palanikumar *et al*., 2013) [62]. Inappropriate usage of pesticides results to changes in biological, physical and chemical characteristics of living organisms (Fish) and all these end-up in the aquatic environment (Yadav *et al*., 2018; Sharma *et al*., 2019; Ruba *et al*., 2023) [85, 74, 70]. Mancozeb as a carbamate fungicide might affect the nervous system of an organism. It inhibits the role of neurotransmitter acetylcholinesterase (AchE) in the central nervous system of an organism (Insect) by its primary metabolite and carbon sulfide resulting to death.

Clarias gariepinus is a hardy fish of high commercial importance, distributed in the tropical regions example Nigeria and mostly utilized aquaculture candidate for the purpose of eco-toxicological investigations (Nwani *et al*., 2013; Odo *et al*., 2017; Balogh *et al*., 2023) [56, 59, 7].

Sub-lethal concentrations of pollutants in the aquatic habitats alter behavioral, structural and functional changes in fish and other aquatic organisms which occurs than mortality (Srivastava and Singh, 2013c, Ullah *et al*., 2014) [80, 83].

Haematological Parameters are useful bio-indicator of harmful toxicants effects indicating physiological condition and internal homeostasis of exposed fish (Iheanacho *et al*., 2022; Khan *et al*., 2023) [37, 42]. Few reports emphasized on the effects of Mancozeb on the behavioral responses (Srivastava and Singh, 2013; Saha *et al*., 2016; Sharma *et al*., 2016; Simakani *et al*., 2018) [80, 71, 75, 77] and Haematogical parameters (Atamanalp and yanik, 2003; Hashim and Mona; 2005; Kotb *et al*., 2019; Bojarski and Witeska, 2020) [6, 31,, 6, 43, 14]. However, there is no research findings on the effects of Mancozeb on the behavioral responses and haematological parameters of *Clarias gariepinus* juveniles.

The present study was to evaluate the abnormalities in behavioral responses and haematological parameters of African Catfish *Clarias gariepinus* Juveniles exposed to Acute and sub-chronic effects of Mancozeb.

Materials and Methods

Procurement and acclimatization of Experimental Fish

300 live specimens of juvenile catfish *Clarias gariepinus* with standard length and weight that ranged from 9.8 cm to 17.5cm and 11g to 55 g were used in the present study. *Clarias gariepinus* juveniles were procured from Freedom Fisheries Limited, University market Road, Nsukka, Enugu, Nigeria and was transported to Fisheries Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Later, they were allowed to acclimatize in the laboratory conditions for 2weeks in plastic tanks of 300 Litre (L) Capacity. Fishes were fed daily with food (Aqua-feed commercial feed size 3mm) contaminating 40% crude protein twice daily at 2-3% body weight and feeding was endedin 24 hours preceding to the range finding and toxicity test to diminish ammonia content in water. The Fishes were disinfected with 0.05% potassium permanganate ($KMnO_4$) for 2minutes to avoid any dermal infection.

Water temperature was measured in the laboratory using mercury in glass thermometer graduated in degree Celsius (0-100 °C). pH of water was achieved by applying the protocol of APHA (1992) [3] in the Laboratory using Hanna pH (Hi-1922 model).

$$\text{Total alkalinity (mg/l)} = \frac{V \times M \times 1000}{Ml \text{ of sample used}}$$

Where
V = volume of acid used
M = Molarity of acid used

Free CO_2 were determined according to the method of APHA (1998) [4].

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{B.R. \times \ N \times 44 \times 100}{\text{Amount of sample (ml)}}$$

Where, B.R. = Burette reading (100 ml)
N=Normality of Sodium Hydroxide (0.05N).
44= Equivalent weight of CO_2

The dissolved Oxygen was analysed,the water samples were collected from each of the treatment group as well as the control using dissolved oxygen and Biological oxygen demand (BOD) bottles and small plastic bottles of 50 ml according to APHA (1992) [3].

Sources of the Test Compound

The commercial formulation of Mancozeb 80% WP(Z-FORCE®) weighing 50 g with batch number 01062018 marked by Jubaili Agrotec Limited Abuja, Nigeria were procured from Ogige Local Market Nsukka, Enugu State, Nigeria and stored at room temperature.

Mancozeb is distributed with the trade names Maneb, Nemispot, Policar, Dithane, Zimaneb.

Determination of lethal median concentration (LC_{50})

Acute toxicity bioassay to determine the 96h LC_{50} values of Mancozeb were conducted with a definitive test in a semi-static system in the laboratory after standard methods (APHA, 2005) [5]. The fishes were divided into six groups each containing twenty Juvenile *Clarias gariepinus* and at different concentration 0.0, 150, 300, 450, 600, 750 mg/L derived from range finding test using plastic tanks of 20 litre capacity each. Ten litres of water were poured into each tank. Another set of 10 fish were simultaneously maintained with equal amount of tap water but without the test compound and regarded as control. Fish were fed throughout the experiment and the lethality toxicity end point were observed. Fish were visually observed daily and considered dead when no sudden swimming in response to gentle touch was detected. Dead fish were removed with plastic forceps and the mortality were recorded at intervals 24, 48, 72 and 96h exposure time. The LC_{50} values (95% confidence limit) of different concentration of Mancozeb in *C. gariepinus* were1740.58 mg/L (95% CI, 14148.68 – 21088.18), 23044.02 mg/L (95% CI, 18333.49 - 28421.20), 6211.00 mg/L (95Cl, 5129.85 – 7419.65) for 24, 48, 72 and 96 h exposure time.

The $LC_{24\text{-}96}$ values of the test Mancozeb for the fish at 24, 48, 72 and 96 hours were determined by probit analysis (Finney, 1971) using SPSS version 17.0.

The 96 hLC_{50} of Mancozeb was calculated to be 410.90 mg/L. The safe level of the test compound was calculated by multiplying the 96 hLC_{50} with different application factors (AF) as indicated by the international Joint commission (IJC, 1977) was used to determine the concentration at which 50% mortality LC_{50} occurred using SPSS version 17.0. (See Table 5)

Determination of Sub-lethal exposure

The 96 h LC_{50}value of Mancozeb on *Clarias gariepinus* was established to be 410.90mg/L. The experimental design for sub-lethal exposure consist of 120 Fish assigned into four

groups 0.00, 21.74 mg/L, 43.48 mg/L, and 86.96 mg/L(A – D), each with three replicates summing up to 30 with three replicates(10 Fish a replicates) without regard to sex.
Fish in the first treatment group were exposed to tap water serve as control (A), while those in the second, third and Fourth were treated 21.74 mg/L, 43.48mg/L and 86.96 mg/L (A – D), each with three replicates summing up 30 Fish with three replicates (10 Fish in a replicate) without concern to sex.
Fish in the first treatment group were exposed to tap water serve as control(A), while those in the second, third and fourth were treated with 21.74 mg/L (B), 43.48 mg/L(C), 86.96 mg/L(D) of Mancozeb corresponding to 1/20th, 1/10th and 1/5th of the 96h LC_{50} value were derived after acute toxicity experiment. Each tank contained 10L dechlorinated tap water with 10 fish. The experiment lasted 28 days during which the fish were fed with small quantity of food approximately 1% of the total body weight about an hour before the Mancozeb were on daily basis. The feeding was to avoid mortality and catabolism.

Determination of Haematological parameters
The red blood cell(RBC) count were determined using modernized microscope Neubauer counter and Toison's solution as diluting fluid (Mgbenka *et al*., 2005) [49]. The white blood cell count wsas estimated with a Neubauer microscope counter using Turk's solution as the blood sample diluting fluid. According to Chinabut *et al*. (1991) different types of WBCs such as Neutrophils, monocytes, Lymphocytes, eosinophils and Basophils were identified and calculated as percentages Blaxhall and Daisley (1973) [11] the cyanmethane method was followed in estimating the haemoglobin(HB) of the blood. The packed cell volume (PCV) was determined by centrifugation of the blood for 5minutes at 1400 g in heparinzed glass capillaries using a micro haematocrit centrifuge (Hawkesley and sons, lancing, uk) at room temperature (Nelson and Morris, 1989) [53]. The Pcv, Hb, and RBC were used in formulating erythrocytic indices such as Mean cell volume (MCV), and mean cell haemoglobin concentrationas calculated in accordance to Sood (2006) [86]:

MCHC = Hb (g/dl) X 10/PCIV%
MCV = PCV X 10/RCV%
MCH were calculated according formula proposed by Dacie and Lewis (2001) [87].

MCH (pg cell^{-1}) = Hb (gdl- 1)X10/RBC count in millions mm^{-3}

Statistical Analysis
Data was analysed with Statistical Packages for Social Sciences (SPSS) version 20.0 (IBM Corp, Armork, USA) and Stat plus v5.9.8 (Analyst soft Inc., Walnut, Canada), probit regression analysis using Finney method (lognormal distribution) for (LC) was recorded. Two-way analysis of variance (ANOVA) was used to compare concentration of Mancozeb and duration of exposure dependent effects. The mean was partitioned using DMRT (Duncan Multiple Range Test). Level of significance was set at $P \leq 0.05$ respectively.

Results
Physico-Chemical Parameters of the Test water
The physico-chemical parameters of the test water used for sub-lethal concentration for 5 weeks were shown in table 1. The temperature ranged from 29.4 to 29.8 °C, pH ranged from 9.23 to 9.43, Alkalinity ranged from 1.95 to 2.43, CO_2 ranged from 6.5 to 8.88 mg/L, DO_2 ranged from 7.0 to 7.15 mg/L.

Table 1: Physico-chemical parameters of the water used for the experiment on *Clarias gariepinus* lethal concentration

Characteristics	Unit	Mean± SEM	Range (Min.-Max.)
Temperature	°C	29.57±0.12	0.40 (29.4-29.8)
pH	-	9.34±0.06	0.20 (9.23-9.43)
Alkalinity	mg l^{-1}	2.17±0.14	0.48 (1.95-2.43)
CO_2	mg l^{-1}	7.63±0.69	2.38 (6.50-8.88)
DO_2	mg l^{-1}	7.07±0.04	0.15 (7.0-7.15)

Table 2: Effect of Mancozeb on behavioural characteristics of *Clarias gariepinus* at different concentration levels

Duration	Concentration (mg/l)	Jumping	Equilibrium status	Opercula Movement	Fin movement	Air Gulping	Erratic swimming	Convulsion	Skin Colouration	Haemorrhage
24 h	A- Control	-	+++	+++	+++	-	-	-	-	-
	B- 150	-	+++	+++	+++	+	+	-	-	-
	C- 300	-	+++	+++	+++	+	-	-	+	-
	D- 450	+	+++	++	++	-	-	-	-	-
	E- 600	++	++	++	++	+	-	-	+	-
	F- 750	+++	+	+	+	-	-	+	+	-
48 h	A-Control	-	+++	+++	+++	-	-	-	-	-
	B-150	-	+++	++	+++	+	+	-	+	-
	C-300	-	++	++	++	+	-	-	-	-
	D-450	++	++	++	++	+	+	+	+	-
	E-600	+++	++	+	+	++	+	++	++	-
	F- 750	+++	+	+	+	++	-	++	++	-
72 h	A-Control	-	+++	+++	+++	-	-	-	-	-
	B-150	-	++	++	++	++	++	-	++	+
	C-300	-	+	++	++	+	+	++	++	+
	D-450	++	+	+	+	++	+	++	++	+
	E-600	+++	-	+	+	++	+	++	++	+
	F-750	+++	-	+	+	++	+	+++	++	+
96 h	A-Control	-	+++	+++	+++	-	-	-	-	-
	B-150	-	+	++	+	+	+	-	+	+
	C-300	-	+	+	+	+	+	++	+	+
	D-450	++	+	+	+	++	+	++	+	+
	E-600	++	-	+	-	+++	++	+++	+++	++
	F-750	++	-	+	-	+++	+++	+++	+++	+++

Notes: None=-, Mild=+, Moderate=++, Strong=+++.

Table 3: Results of various toxicity investigations of mancozeb on some Fish species

Mancozeb	Assay	Fish species	Result	Reference
	96h LC_{50}	Punctuation	12.95 mg/L	Sharma *et al.* (2018)
	96h LC_{50}	*Cyprinus carpio*	8.03 mg/L	Simakani *et al*. (2018) [77]
	96h LC_{50}	*Oreochromis niloticus*	11.68 mg/L	Saha *et al* (2016) [71]
	96h LC_{50}	*Onchohynchus mykiss*	0.092 mg/L	Atamanalp and Yanik (2003) [6]
	96h LC_{50}	*Clarias batrachus* Adult	14.36 mg/L	Srivastava and Singh (2013) [80]
	96h LC_{50}	*Clarias batrachus* fingerlings	14.04 mg/L	Srivastava and Singh (2013) [80]
	96h LC_{50}	*Clarias gariepinus* Juveniles	410.90 mg/L	This study

Table 4: Mortality of *Clarias gariepinus* exposed to different concentration of Mancozeb

Groups	Concentration (mg/l)	Sample size (n-20)	Mortality (%age mortality)			
			24 h	48 h	72 h	96 h Survival
Control	0	20	0 (0)	0 (0)	0 (0)	0 (0) 100
A	150	20	0 (0)	1 (5)	0 (0)	1 (5) 95
B	300	20	0 (0)	0 (0)	1 (5)	4 (20) 80
C	450	20	3 (15)	1 (5)	2 (10)	11 (55) 45
D	600	20	5 (25)	4 (20)	3 (15)	14 (70) 30
E	750	20	6 (30)	4 (20)	6 (30)	17 (85) 15

Table 5: Lethal concentration of Mancozeb on *Clarias gariepinus*

Percentile	Concentration (CI = 95%)			
	24 h	48 h	72 h	96 h
5	803.31 (370-1225.09)	790.56 (334.84-1247.46)	359.58 (176.48-533.98)	50.10 (30.75-68.88)
10	1845.00 (1432.16-2288.01)	1958.92 (1480.91-2477.65)	779.03 (616.94-951.47)	90.48 (76.52-104.91)
20	3649.59 (2495.38-4609.45)	4148.07 (2725.40-5731.63)	4163.01 (1030.96-1927.60)	142.79 (111.22-175.45)
25	5860.12 (5171.78-6593.88)	6964.68 (6068.94-7925.13)	226986 (2022.66-2532.12)	197.52 (181.72-214.02)
30	7729.28 (6946.22-8554.17)	9439.06 (8391.53-10548.21)	2932.20 (2656.99-3221.17)	238.05 (221.66-255.03)
40	11080.56 (8856.24-13666.09)	14029.29 (10958.10-17647.73)	4089.51 (3326.22-4967.84)	303.01 (261.07-349.67)
50	17409.58 (14148.68-21088.18)	23044.02 (18333.49-28421.20)	6211.00 (5129.85-7419.65)	410.90 (357.94-468.34)
60	27093.76 (22215.10-32541.24)	37458.67 (30093.79-45771.59)	9349.57 (7785.55-11081.52)	553.52 (485.05-627.28)
70	42672.61 (34280.05-51987.69)	61706 (48465-76578.34)	14229.27 (11628.01-17090.42)	751.44 (649.67-860.04)
75	60939.49 (54765.61-67443.09)	91207.05 (81084.98-101924.20)	19791.39 (17933.23-21741.14)	956.62 (890.73-1024.85)
80	81047.39 (72290.40-90582.85)	124765.82 (109999.50-140931.03)	25761.92 (23182.31-28559.40)	1159.05 (1073.9-1250.13
90	137965.60 (97093.27-194366.29)	224343.28 (152096.49-326020.38)	42068.93 (30452.55-57858.79)	1650.58 (1309.96-2090.76)
95	281553.07 (215692.84-363002.46)	490702.94 (365522.32-647527.59)	81413.07 (63706.12-103095.67)	2673.79 (2242.65-3184.53)
99	732795.95 (439342.60-1201930.70)	1412203 (798578.99-2412405.62)	196378.52 (122997.27-311944.01)	5028.27 (3621.46-7133.45)

CI= confidence interval

Table 6: Estimation of safe level for *Clarias gariepinus* after 96 h Exposure

Pesticide	96hrLC_{50} (mg/l)	Method	Application factor	Safe level (mg/L)
Mancozeb	410.90	Hart *et al*. (1948)*	-	1211.21
		Sprague (1971)	0.1	41.09
		CWQC (1972)	0.01	4.109
		NAS/NAE	0.1 - 0.00001	41.09 - 4.109 × 10^{-3}
		CCREM (1991)	0.05	20.595
		IJC (1977)	5% LC_{50}	20.595

***** C= 48h $LC_{50} \times 0.03S^2$ Where C is the presumed harmless concentration and S=24 h LC_{50}

Behavioral changes and physiological abnormalities of *Clarias gariepinus* exposed to Mancozeb at different concentration levels for both acute and sub-lethal toxicity (See table 2). In the control duration of exposure, normal behavioral responses and no mortality were observed. Treatment groups with Mancozeb exhibited physiological and behavioral abnormalities based on increase in duration and concentration. The tanks with higher concentration of the test chemical fish displayed faster opercular movement, jerky movement, erratic swimming, skin coloration, convulsion, hyperactivity, gulping of air, hemorrhage and loss of equilibrium status were also observed. *Clarias gariepinus* lost equilibrium balance, became exhausted owing to respiratory complications and finally settled down at the bottom and mortality occurred.

In sub- lethal concentration no mortality was observed and fish displayed abnormal behavioral changes throughout the experimental period. Result findings on different investigations of Mancozeb on some fish species showed variations in LC_{50} based on the pesticide type, duration of exposure and stage of maturity (See Table 3).

Percentage mortality of *Clarias gariepinus* Juveniles exposed to graded concentration of Mancozeb at 96h increase in toxicant concentration (See Table 4). Fishes exposed to 150 mg/L, 300 mg/L, 450 mg/L, 600 mg/L and 700 mg/L had 5%, 20%, 55%, 70% and 85% which recorded the highest mortality compared to other concentrations. While no mortality was recorded at the control group. Throughout the exposure duration no absolute (100%) mortality occurred (See Table 4).

The toxicant concentration in all the groups exposed to Mancozeb decreased as time progressed. LC_{50} values of

Mancozeb with 95% confidence limit of various concentration in *Clarias gariepinus* for the duration of 24, 48, 72 and 96 h were 1740.58 mg/L (95% cl, 14148.68 – 21088.18), 23044.02 mg/L (95% cl, 1833.49–2842.20), 6211.00 mg/L (95% cl, 5129.85 – 7419.65) in Table 5. The estimated safe level of Mancozeb values in *Clarias gariepinus* varied from 41.09 to 4.109 x 10^{-3} mg/L respectively (See Table 6).

Haematological Parameters

The Red blood cell count (RBC) in the experimental group were significantly different from those of their control (P <0.05) throughout the duration of the exposure except on day 1 where there was no significant difference (p>0.05). There was no significant difference (p>0.05). There was significant different in packed cell volume (PCV) values between the control and exposed fish throughout the experiment except day 1, but PCV was significantly decreased (p<0.05) from day 7, 14, 21 and 28 days of exposure. The increase was dosage dependent as concentration of Mancozeb increased the WBC level significantly vary (p<0.05). The increase was dosage dependent as concentration of Mancozeb increased the HB level significantly declined on day 7 onward (p<0.05) except day 1 which did not significantly vary (p>0.05). The values of erythrocytic indices (MCH, MCHC and MCV) showed significant variation throughout the exposure duration in Research laboratory conditions (See Table 7).

Table 7: Effect of Mancozeb exposure on Haematology of *Clarias gariepinus*

Parameters	Concentration (mg/L)	Duration 1 Day	7 Day	14 Day	21 Day	7 Days recovery
RBC (x10^6 Cell/mm)	Control	10.41±0.14^{a2}	10.63±0.12^{a2}	10.82±0.06^{a1}	10.60±0.16^{a2}	10.68±0.19^{a2}
	20.55	10.39±0.13^{a1}	8.71±0.08^{b3}	7.63±0.11^{b3}	5.98±0.39^{b4}	9.74±0.06^{b2}
	41.09	10.30±0.16^{a1}	7.65±0.27^{c2}	7.34±0.47^{c2}	5.60±0.14^{b3}	9.18±0.29^{c2}
	82.18	10.48±0.11^{a1}	7.36±0.22^{d3}	7.22±0.40^{c3}	5.71±0.26^{b4}	9.37±0.11^{c2}
PCV (%)	Control	32.00±1.15^{a1}	31.00±0.58^{a1}	30.33±0.88^{a1}	30.00±0.58^{a1}	33.00±1.53^{a1}
	20.55	30.67±1.20^{a1}	21.67±1.20^{b3}	20.67±1.76^{b34}	19.00±0.58^{b4}	27.67±0.88^{b2}
	41.09	32.00±1.15^{a1}	23.00±0.58^{b3}	20.33±1.20^{b4}	18.00±1.15^{b4}	26.33±0.88^{b2}
	82.18	30.33±1.45^{a1}	19.00±0.58^{c3}	18.33±1.20^{c3}	18.00±1.15^{b3}	26.00±1.15^{b2}
WBC (x10^4 cells/mm)	Control	8933.33±240.37^{a1}	8766.67±88.19^{a1}	8866.67±176.38^{a1}	9633.33±120.19^{b1}	9433.33±88.19^{a1}
	20.55	9200.00±404.15^{a2}	11266.67±352.77^{a2}	11466.67±480.74^{a12}	46100.00±325.61^{a1}	9700.00±635.09^{a2}
	41.09	9433.33±523.87^{a1}	11133.33±405.52^{a1}	12833.33±317.98^{a1}	13133.33±463.08^{b1}	10100.00±208.17^{a1}
	82.18	9633.33±120.19^{a1}	11433.33±523.87^{a1}	12766.67±88.19^{a1}	13533.33±656.59^{b1}	10366.67±260.34^{a1}
Hb (g/dl)	Control	8.40±0.12^{a,4}	8.53±0.24^{a,4}	9.20±0.12^{a,3}	10.00±0.17^{a,2}	10.57±0.13^{a1}
	20.55	8.03±0.09^{a2}	6.17±0.12^{b3}	6.33±0.47^{b3}	6.27±0.07^{b3}	9.90±0.42^{b1}
	41.09	8.27±0.07^{a2}	6.30±0.25^{b3}	6.03±0.09^{b3}	5.57±0.13^{c4}	9.00±0.30^{c1}
	82.18	8.20±0.12^{a2}	6.10±0.06^{b3}	5.33±0.07^{c4}	5.47±0.23^{c4}	8.13±0.07^{d1}
MCH (pg/cell)	Control	7.96±0.13^{a2}	8.03±0.25^{a2}	8.49±0.10^{b2}	33.32±1.17^{a1}	9.89±0.08^{a2}
	20.55	7.72±0.14^{a2}	7.08±0.19^{a2}	8.29±0.50 ^{b2}	33.06±1.33^{a1}	10.16±0.36^{a2}
	41.09	8.03±0.06^{a2}	8.28±0.56^{a2}	8.29±0.54^{b2}	31.19±2.24^{a1}	9.80±0.11^{a2}
	82.18	7.82±0.04^{a3}	8.30±0.20^{a3}	16.12±8.40^{a2}	30.64±2.51^{a1}	8.69±0.17^{a3}
MCHC (g/dl)	Control	26.33±1.15^{a3}	28.01±1.66^{b23}	30.37±0.70^{a12}	9.43±0.20^{a4}	32.12±1.13^{bc1}
	20.55	26.25±0.72^{a3}	28.63±1.61^{b23}	30.78±1.68^{a2}	10.59±0.8 ^{a4}	35.94±2.61^{a1}
	41.09	25.89±0.77^{a3}	27.38±0.67^{b23}	29.90±2.23^{a2}	9.95±0.37^{a4}	34.32±2.20^{ab1}
	82.18	27.18±1.51^{a3}	32.17±1.18^{a1}	29.37±2.19^{a23}	9.61±0.60^{a4}	31.41±1.45^{c12}
MCV (fl cell)	Control	30.90±1.05^{a1}	29.07±0.82^{ab1}	28.01±0.67^{a1}	28.31±0.74^{b1}	30.86±0.92^{a1}
	20.55	29.55±1.39^{a12}	24.89±1.54^{ab3}	27.03±1.92^{a23}	32.07±1.25^{b1}	28.37±0.99^{a12}
	41.09	31.05±0.80^{a12}	30.16±1.37^{a12}	28.14±3.41^{a2}	32.08±1.24^{b1}	28.78±1.82^{a12}
	82.18	29.91±1.68^{a2}	26.27±1.11^{b23}	25.72±3.00^{a3}	36.88±5.85^{a1}	27.76±1.25^{a23}
Neutrophils	Control	25.33±3.71^{a1}	20.67±1.76^{a1}	20.67±2.33^{a1}	17.67±1.45^{a1}	20.00±5.03^{a1}
	20.55	25.00±2.89^{a1}	21.67±4.41^{a1}	17.00±1.53^{a1}	23.33±4.06^{a1}	18.33±1.67^{a1}
	41.09	20.67±1.76^{a1}	18.33±1.20^{a1}	123.00±98.04^{a1}	21.67±4.41^{a1}	25.00±2.87^{a1}
	82.18	18.00±3.51^{a1}	17.33±1.45^{a1}	22.00±1.53^{a1}	20.00±0.58^{a1}	23.67±3.18^{a1}
Leucocytes	Control	67.67±1.45^{a1}	77.67±1.86^{a2}	75.00±2.89^{a12}	78.00±2.00^{a2}	75.33±3.71^{a12}
	20.55	74.67±2.91^{ab1}	78.00±4.16^{a1}	81.67±1.67^{a1}	75.00±2.89^{a1}	79.67±2.60^{a1}
	41.09	78.33±1.67^{b1}	78.33±0.88^{a1}	76.00±4.00^{a1}	74.67±3.53^{a1}	74.00±3.46^{a1}
	82.18	74.33±2.96^{ab1}	75.67±4.33^{a1}	76.00±3.06^{a1}	75.33±3.71^{a1}	75.00±2.89^{a1}
Monocytes	Control	3.33±0.88^{a1}	1.67±0.88^{a1}	3.00±1.00^{a1}	2.00±0.58^{a1}	2.67±0.67^{b1}
	20.55	0.33±0.33^{a1}	0.33±0.33^{a1}	0.67±0.67^{a1}	1.00±0.58^{a1}	1.00±0.58^{ab1}
	41.09	0.67±0.33^{a1}	1.67±1.20^{a1}	0.67±0.33^{a1}	2.00±1.53^{a1}	0.67±0.33^{a1}
	82.18	1.67±1.67^{a1}	2.33±1.20^{a1}	1.67±1.20^{a1}	3.33±2.33^{a1}	1.33±0.33^{ab1}
Basophils	Control	2.33±1.33^{a2}	0.00±0.00^{a1}	0.67±0.33^{a12}	0.67±0.33^{a12}	0.67±0.33^{a12}
	20.55	0.00±0.00^{a1}	0.00±0.00^{a1}	0.33±0.33^{a1}	0.33±0.33^{a1}	0.33±0.33^{a1}
	41.09	0.33±0.33^{a1}	0.67±0.33^{a1}	0.00±0.00^{a1}	0.67±0.67^{a1}	0.67±0.33^{a1}
	82.18	1.67±1.67^{a1}	0.67±0.33^{a1}	0.33±0.33^{a1}	0.67±0.67^{a1}	1.33±0.33^{a1}
Eosinophils	Control	1.33±0.33^{a1}	0.00±0.00^{a1}	0.67±0.67^{a1}	1.33±0.33^{a1}	0.67±0.33^{a1}
	20.55	0.00±0.00^{a1}	0.00±0.00^{a1}	0.33±0.33^{a1}	0.33±0.33^{a1}	0.67±0.67^{a1}
	41.09	0.00±0.00^{a1}	1.00±0.58^{a1}	0.33±0.33^{a1}	1.00±0.58^{a1}	0.00±0.00^{a1}
	82.18	1.67±1.67^{a1}	0.67±0.67^{a1}	0.00±0.00^{a1}	0.67±0.67^{a1}	0.00±0.00^{a1}

Data were presented with mean± standard deviation. Means with different alphabet superscripts along each column represent significant differences for the concentrations while Means with different number superscripts along each row represent significant differences for the exposure duration

Differential white blood cell count (See Table 7) Neutrophils and Lymphocytes values were not significantly different from the control. Monocytes significantly varied across treatment group and duration except day 21 period where there was no significant different (p>0.05). Basophils significantly varied (p<0.05) across treatment day 1while on day 7, 14, 21 and 28days.Esonophils significantly varied on day 1 (p<0.05) except day 7 onward there was no significant different from the control.

Differential white blood cell count (Figure 1a & 1b), Neutrophils and Lymphocytes values were not significantly different from the control. Monocytes significantly varied across treatment group and duration except day 21 period where there was no significant different (p>0.05). Basophils significantly varied (p<0.05) across treatment day 1while on day 7, 14, 21 and 28 days. Esonophils significantly varied on day 1 (p<0.05) except day 7 onward there was no significant different from the control.

Differential White Blood Cell

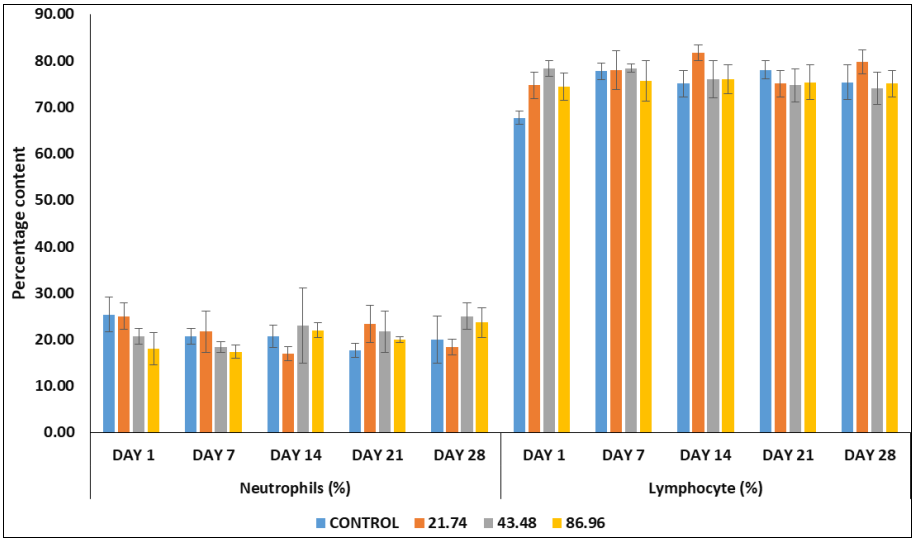

Fig 1a: Effects of exposure to various sub-lethal levels of Mancozeb on differential white blood cell (neutrophils and lymphocytes) in *Clarias gariepinus*

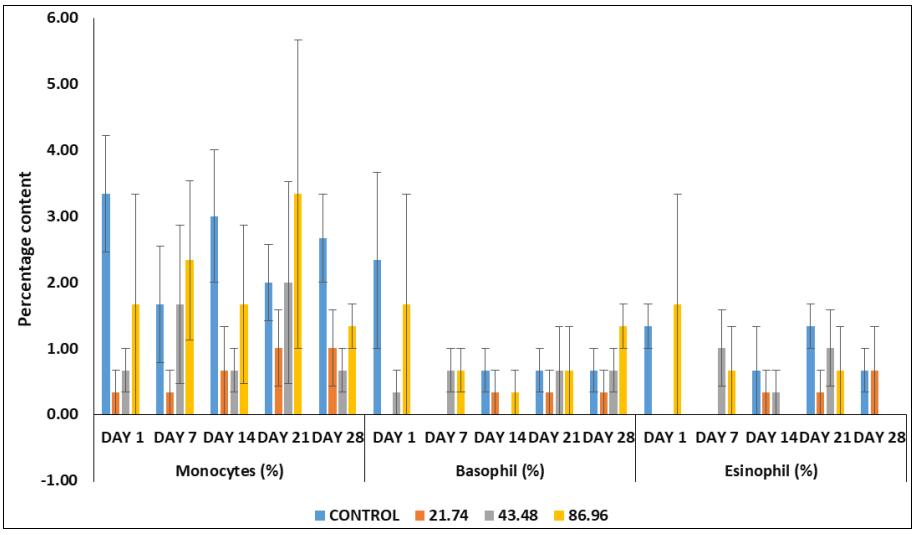

Fig 1b: Effects of exposure to various sub-lethal levels of Mancozeb on differential white blood cell (Momocytes, basophil and eosinophil) in *Clarias gariepinus*

Discussion

The abnormal behavioral alterations in Mancozeb exposed fish may show disruption in the internal structure of the fish which could be ascribed to the neurotoxic property of the toxicant. The present study verifies the toxic effect of Mancozeb on the Juveniles of freshwater fish *Clarias gariepinus.* Several abnormal behaviors such as loss of equilibrium status, incessant jumping, mucus secretion, restlessness, increased opercula activity, gulping of air and mortality occurred. The findings of the present study were in line with the abnormal behavioral alterations observed in *Clarias gariepinus* to Cyperdicot reported by Odo *et al*. (2017) and *Tilapia zillii* exposed to Glyphosateas reported by Nwani *et al*. (2013) [56]. Similar behavioural responses on loss of equilibrium on *Clarias batrachus* exposed to higher concentration of Mancozeb were reported by Srivastava and Singh (2013) [80]. It was possibly due to the dysfunction of the Central nervous system (Sikka and Gurbuz, 2006) [76].

Sluggish movement of fish and alteration of their swimming ability makes them more vulnerable to the predators, mucus secretions, diminish their feeding capacity, loss of equilibrium status due to damage to the gills and ability not to protect their territories (Ullah *et al*., 2014) [83].

Monitored the relation between behavioural and physiological indicators of fish toxicity. Behavioural changes are mainly related to Alcetyl cholinesterase (AchE) inhibition, alteration of brain transmitter levels sensory deficiency and thyroid hormone levels and eventually death of the fish (Payne *et al*., 1996; Barata *et al*., 2004; Adedeji *et al*., 2008; Becker *et al*., 2013; de Assis *et al*., 2014; de Araujo *et al*., 2016; Bodnar *et al*., 2022) [66, 9, 1, 10, 21, 20, 13]. According to Srivastava and Singh (2013) [80] the Acetyl cholinesterase action of pesticides disturbed the survival and initiated mortality of *Clarias batrachus* when exposed to Mancozeb and Propinacole thereby delaying Cholenesterase action by disturbing the regular movement of fish. Behavioural changes are among the most sensitive pointers of prospective lethal effects of toxicants (Banaee *et al*., 2011; Qiu *et al*., 2017; Qu and Wang, 2020) [8, 68, 69]. The pollutant behaviours can be monitored at three levels namely column, sediments and biomass of aquatic organisms. The abnormal behavioural changes and mortality observed may show internal disruption of the physiology of the body characteristic to the neurotoxic effect of the pesticides (De camposventura, de angelis and Marin-Morales, 2008; Nwani *et al*., 2011) [22, 57]. The observed behavioral alterations on *Clarias gariepinus* exposed to Mancozeb in the present study might have affected respiratory system, thyroid dysfunction, Central nervous system (brain), weak carcinogenic effect, feeding habits, equilibrium status, cannibalism, haematological profile, reproduction and growth parameters. Research findings states that Mancozeb is moderate to highly toxic to fish and other aquatic organisms (Grande *et al*., 1994; Marques *et al*., 2016) [30, 46].

In this investigation, the toxicity level of Mancozeb on *Clarias gariepinus* was found to be 410.90 mg/L based on the 96hr LC_{50}which revealed decrease in value over time. From the present result of 96hr LC_{50}value was however higher than the 211.80 mg/L of Glyphosate as reported by Nwani *et al*. (2013) [57] for Juvenile *Tilapia zillii*. Messaad and AL Zailaie (2017) [48] reported LC_{50} value of 115.25 mg/L in Arabian Killifish *Apanius dispar* exposed to Glyphosate and Ogah (2021) [60] reported that 96hr LC_{50} was 151.36 mg/L in *Clarias gariepinus* juveniles were lower than the value obtained in the present study. EL-Harbawi. (2014) [25] was estimated 96 hr LC_{50} to be 374.11 mg/L when *Lates calcarifer to* exposed to Imidazolium ionic liquids and Omitoyin *et al*. (2022) [61] the 96 hr LC_{50} of Copper and Zinc were 15.03 mg/L and 324 mg/L for *Heterobranchus bidorsalis* was both lower than the present value result. While, Gaur and Mathur (2019) [29] results showed that the 96hr LC_{50} of Phenolic compounds (Phenol and Mcresol) for *Labeo rohita* was 3212 mg/L and 2957 mg/L thus higher than the 96 hr LC_{50} in the present study. Carneiro *et al*. (2023) [16] result revealed that ZN-Al layered double hydroxides was estimated to be 559.9 mg/L after 96 hr LC_{50}on embryo *Danio rerio* and it is higher than the present result value. However, the LC_{50} values of the present study decreased as the exposure time increased from 24 h to 96 h due to Mancozeb pesticide toxicity. Acute toxicity assessments are vital parameter for regulatory purposes and evaluation of toxicants on concentration, sex, developmental stages and exposure period on organisms (Hayasaka *et al*., 2012; Pandey *et al*., 2011) [32, 63]. Consequently, acute and chronic toxicity tests are mostly employed to assess the toxicity of pesticides on non-target organisms (Santos *et al*., 2010) [72]. Similarly, Nwani *et al*. (2015) [55] reported that acute toxicity derived from 96 hr LC_{50} is one of the most vital parameter for estimating toxicity of chemicals in fish Eco-toxicological investigations.

Carbamate based fungicides research results reveal that toxicity varies from specie to specie and also strains of the same species. Toxicity data evidently reveal that the fingerlings are generally prone than adult fish species because of the dependence of age and body size (Srivastava and Singh, 2014; Kanu *et al*., 2021) [79, 40]. The estimated safe levels of Mancozeb in the present study for *Clarias gariepinus* juviniles varied from 41.09-4.109 $\times 10^{-3}$ mg/L. This is similar to Nwani *et al*. (2013) [56] for Glyphosate safe level which varied from 2.118 – 2.118 $\times 10^{-3}$ mg/L. However, the result is in contrast to the findings of Soni and Verma (2018) [78] safe levels range from 0.05-0.58 mg/L estimates of herbicide Pretilachlor to *Clarivate* was lower. The variation in safe level indicate that the difference obtained were all dependent on concentration and duration to Mancozeb exposure. However, due to high variation in safe levels as determined by different methods, the estimates of safe levels cannot be guaranteed (Pandey *et al*., 2005; Soni and Verma, 2018) [64, 78]. Application factors are used to establish acceptable toxicant concentration ranges depending on water quality, species and life stage (Tiwari *et al*., 2011) [82]. Elmegaard *et al*. (2000) [26] and Pandey *et al*. (2005) [64] reported that Laboratory data extrapolation to field is not always meaningful value and Categorically it is challenging to make decision on the acceptable concentration that may be considered "safe" in the field studies and laboratory experiments. In another report by Kenaga (1979) [41] the major weakness in calculation of the application factor (AF) is its dependence on LC_{50} value.

Exposure of *Clarias gariepinus* to sub-lethal concentrations of Macozeb resulted to numerous changes in some haematological parameters. The RBC, PCV and Hb values were significantly declined at higher concentrations of Mancozeb. The reduction in these parameters is caused by haemolyse due to Mancozeb effect on the fish. The decrease may also be caused by limited erythrocyte synthesis due to impairment of osmoregulation across the gill epithelium and build-up of toxicants in the gill histological section (Saravanan *et al*., 2011; Pereira *et al*., 2013) [73, 67]. These revealed a noticeable condition of anaemia, that pesticides can induce anaemia and affect the general condition of fish (Min and Kang, 2008) [50]. This anaemia may be due to ROS induced oxidative damage through oxidation of haemoglobin or additional cellular components (Bloom and Brandt, 2008) [12]. Furthermore, haematological parameters are subjected to environmental factors and variations due to stress (Hlavova, 1993) [35]. The WBC significantly increased at higher concentration of Mancozeb. During toxic exposure the WBC counts were enhanced. The observed Leukocytosis on day 21 of exposure showed an immune protective response against the toxicant (Odo *et al*., 2017) [59]. This means that fish can develop a defensive mechanism to tackle Mancozeb toxic effect. White blood cells carryout several works such as defense against infections, attack of foreign substances, production, transportation and channelling of antibodies in immune response (Ajima *et al*., 2017; Burgo-Aceves *et al*., 2019) [2, 15]. The increase in WBC is an adaptive mechanism measure. This may be caused as a result of stimulation of

immunological defence mechanism to fight stress (Henry *et al*., 1978) [34]. Variations and changes in WBC affect the upkeep of functional immune system of Fish (Liu *et al*., 2017) [45]. In our study with MCH treatment with Mancozeb, there was variations on the values of MCH. The present study revealed that the values of MCH in the experimental fish were not significantly different from those of the treatment group except day 14. While in exposure duration there was significant difference. This may have adverse effect on the oxygen carrying capacity of the fish blood resulting to hypoxia (Islam *et al*., 2015) [39]. In our study on MCHC, variations in the haematological indices indicated that MCHC were significantly different on day 7 and 28 for treatment groups except day 1, 14, 21. While, across exposure duration there was no significant difference. MCHC revealed fluctuating pattern. MCHC fluctuating pattern could be caused by Mancozebpesticidal effect on fish haematology which Stimulate environmental factors and stress. In our study on MCV, variations in the haematological indices indicated that values of MCV were significantly different on day 7 and 21 except day 1, 14, 28. While, across exposure duration there was significant different except control. MCV is a useful parameter for detecting anemia. The study showed significant variation in MCH, MCHC and MCV of *Clarias gariepinus* exposed to different concentrations of Mancozeb at different exposure duration in the Research laboratory conditions. This could be ascribed to rise in the supply of RBC indices by Mancozeb outside the production capacity of the bone marrow of the fish body iron content (Holy *et al*., 2015) [36]. Neutrophils and Lymphocytes activity in the exposed fish were not significantly different from the control (p>0.05) throughout the experiment and there was variation. However, this show no exposure to the pesticide and it has no effect on those parameters. Changes in WBC differential counts are signs to environmental stress in living organisms (Dogan and Can, 2011) [23]. The revealed mixed trend in the values on Neutrophils, Lymphocytes, monocytes, Basophils and Eosinophils in the Mancozeb exposed to *Clarias gariepinus* juveniles resulted to physiological stress response (Nwani *et al*., 2016) [58].

In the present study, the effect of Mancozeb on the Monocytes, Basophils and Eosinophils depended on the interaction between treatment and duration (p>0.05) compared to control resulted to variation. White blood cell counts such as monocytes, basophils and eosinophils were comparable to the control throughout the experimental duration. Similarly, observations have also revealed it in several fish species treated with different pesticides (Velisek *et al*., 2009; Mohammed *et al*., 2012) [84, 51].

Conclusion

The present finding revealed several evidences that Mancozeb can cause detrimental effects to behavioural responses and haematological parameters of *Clarias gariepinus* at both acute and sub-lethal exposure. Further research investigations should focus on how to use application factors for estimating safe level concentration of several carbamate fungicides on other Ichthyofaunal species.

References

1. Adedeji OB, Adedeji AO, Adeyemo OK, Agbede SA. Acute toxicity of Diazinon to the African catfish *Clarias gariepinus*. African Journal of Biotechnology. 2008;7(5):651-654.
2. Ajima MN, Pandey PK, Kumar K, Poojary N. Neurotoxic effects, molecular responses and oxidative stress biomarkers in Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) exposed to verapamil. Comparative Biochemistry and Physiology Part C: Toxicology Pharmacology. 2017;196:44-52. doi:10.1016/j.cbpc.2017.03.009.
3. American Public Health Association (APHA). Standard Methods for the Examination of Water and Waste Water. 22nd Edition. American Public Health Association, Washington, DC; c1992.
4. Apha A. Standard methods for the examination of water and wastewater. American Public Health Association. Inc., Washington, DC; c1998.
5. APHA, AWWA, WPCF Standard Methods for the Examination of Water and Waste Water. (21st Edition). American public health Association, New York; c2005.
6. Atamanalp M, Yanik T. Alterations in haematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. Turkish Journal of Veterinary and Animal Sciences. 2003;27(5):1213-1217.
7. Balogh RE, Csorbai B, Guti C, Keszte S, Urbányi B, Orbán L, *et al*. Validation of a male-specific DNA marker confirms XX/XY-type sex determination in several Hungarian strains of African catfish (*Clarias gariepinus*). Theriogenology, 2023;205:106 – 113.doi: https://doi.org/10.1101/2022.10.12.511891
8. Banaee M, Sureda A, Mirvaghefi AR, Ahmadi K. Effects of Diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). Pesticide Biochemistry and Physiology. 2011;99(1):1-6. doi.org/10.1016/j.pestbp.2010.09.001
9. Barata C, Solayan A, Porte C. Role of B- esterase in assessing toxic organophosphorus (Chlorpyrifos, Malathion) and carbamate (Carbofuran) pesticides to Daphnia magna. Aquatic Toxicology. 2004;66(2):125-139. doi.org/10.1016/j.aquatox.2003.07.004
10. Becker AG, Parodi TV, Goncalves JF, Bagatini MD, Spanevello RM, Morsch VM, *et al*. Ectonucleotidase and acetylcholinesterase activities in silver catfish (*Rhamdia quelen*) exposed to different salinities. Biochemical Systematics and Ecology. 2013;46:44-49. doi.org/10.1016/j.bse.2012.09.016
11. Blaxhall PC, Daisley KW. Routine hematological methods for use with fish blood. Journal of Fish Biology. 1973;5:771-781. doi.org/10.1111/j.1095-8649.1973.tb04510.x
12. Bloom JC, Brandt JT. Toxic responses of the blood. In: Casarett, L.J., Klaassen, C.D. (Eds.), Casarett and Doull's, Toxicology: The Basic Science of Poisons., 7th ed. McGraw-Hill Medical, New York; c2008. p. 455-484.
13. Bodnar OI, Horyn OI, Soroka OV, Nimko KV, Falfushynska HI. Pesticide pollution of aquatic ecosystems: Environmental risks and mechanisms of impact on aquatic organisms (A review). Hydrobiological Journal. 2022;58(2):62-78. DOI: 10.1615/HydrobJ.v58.i2.60
14. Bojarski B, Witeska M. Blood biomarkers of herbicide, insecticide and fungicide toxicity to fish-a review. Environmental Science and Pollution Research. 2020;27:19236-19250. doi: 10.1007/s11356-020-08248-8
15. Burgos-Aceves MA, Lionetti L, Faggio C. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Science of the Total Environment. 2019;670:1170-

1183. doi: 10.1016/j.scitotenv.2019.03.275
16. Carneiro D, Damasceno EP, Ferreira V, Charlie-Silvia I, Tedim J, Maia F, *et al*. Zn-Al layered double hydroxides induce embryo malformation and impair locomotor behavior in Deniorerio. Nanoimpact. 2023;30:1-12. Doi:10.1016/j.impact.2023.100457
17. Cecconi SR, Paro R, Rossi G, Macchiarelli G. The effects of the endocrine disruptors dithiocarbamates on the mammalian ovary with particular regard to mancozeb. Current Pharmaceutical Design. 2007;13(29):2989-3004. doi: 10.2174/138161207782110516.
18. Cycoń M, Piotrowska-Seget Z, Kozdrój J. Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. International Biodeterioration and Biodegradation. 2010;64(4):316. – 323.doi.org/10.1016/j.ibiod.2010.03.006
19. Davies AK, Maney DL, Maerz JC. The use of leukocyte profiles to measeure stress in vertebrates: A review for ecologists. Functional Ecology. 2008;22(5):760-772. doi.org/10.1111/j.1365-2435.2008.01467.x
20. Jacob LT, Auta J, Habila JD, Yerima R. Acute toxicity of Cadmium (Cd2+) to the developmental stages of freshwater fish, *Clarias gariepinus* (Burchell, 1822). Int. J. Biol. Sci. 2021;3(2):45-52. DOI: 10.33545/26649926.2021.v3.i2a.134
21. De Assis CRD, Linhares AG, Oliveira VM, Franca RCP, Santos JF, Marcuschi M, *et al*. Characterization of catalytic efficiency parameters of brain cholinesterase in tropical fish. Fish Physiology and Biochemistry. 2014;4:1659-1668. doi: 10.1007/s10695-014-9956-1
22. De Campos Ventura BC, De Angelis DF, Marin- Morales MA. Mutagenic and genotoxic effects of the Atrazine herbicide in *Oreochromis niloticus* (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay. Pesticide Biochemistry Physiology. 2008;90:42-51. doi:10.1016/j.pestbp.2007.07.009
23. Dogan D, Can C. Hematological, biochemical, and behavioral responses of *Oncorhynchus mykiss* to dimethoate. Fish Physiology and Biochemistry. 2011;37:951-958. doi: 10.1007/s10695-011-9492-1
24. Dow Agro Sciences. Internal market research based on panel data supplied by Agrobase-Logigram, Archamps, France; c2008.
25. EL-Harbawi M. Toxicity measurement of Imidazolium liquids using acute toxicity test. Procedia Chemistry. 2014;9:40-52. doi.org/10.1016/j.proche.2014.05.006
26. Elmegaard N, Jagers GAM, Akkerhuis OP. Ministry of environment and energy, National Research Institute, Safety factors in pesticide risk assessment. Different species sensitivity and acute-chronic relations. NERI Technical Report; c2000. p. 325.
27. Finney DJ. Probit analysis. Cambridge: Cambridge University Press; c1971.
28. Finney YT. Probit Analysis. Cambridge University Press, London; c1971.
29. Gaur V, Mathur A. Determination of LC50 of phenolic compounds (Phenol and m-cresol) for a fish. Labeorohita. International Journal of Medical Laboratory Research. 2019;4(1):55-63.
30. Grande M, Anderson S, Bers D. Effects of pesticides on fish. Norwegian Journal of Agricultural Science. 1994;13:195-206.
31. Hashim EF, Zaki MS. Assessment of the hazardous effect of fungicide dithane on *Clarias lazera* (Catfish) including haematological, biochemical and immunological parameters. Journal of Agricultural Science. 2005;13(3):1005-1018. DOI:10.21608/ajs.2005.15347
32. Hayasaka D, Korenaga T, Suzuki K, Saito F, Sânchez-Bayo F, Goka K, *et al*. Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. Ecotoxicology and Environmental safety. 2012;80:355-362. DOI:10.1016/j.econv.2012.04.004
33. Hayes WJ, Laws ER. Handbook of pesticide toxicity, Classes of pestricides. New York: Academic press Inc. 1991;3:1451.
34. Henry E, Donald H, Moroan P, Simmons A. A pesticide dieldrin induced immune haemolytic anaemia. Environmental Research. 1978;17:155.
35. Hlavova V. Reference values of the haematological indices in grayling (*Thymallus thymallus*). Comparative Biochemistry and Physiology. 1993;105:525-532.
36. Holy B, Kenanagha B, Onwuli DO. Haemato-pathological effect of dichlorvos on blood picture and liver cells of albino rats. Journal of Toxicology and Environmental Health Sciences. 2015;7(2):18-23. doi:10.5897/JTEHS2015.0327
37. Iheanacho A, Ekpenyong J, Nwose R, Adeolu AI, Offu P, Amadi-Eke A, *et al*. Effects of burnt tire-ash on Na+/K+, Ca2+ATPase, serum immunoglobin and brain acetylecholinesteras activities in *Clarias gariepinus* (Burchell, 1822). Drug Chemistry and Toxicology; c2022. p. 1-7. https://doi.org/10.1080/01480545.2022.2061987
38. International Joint Commission. New and revised Great lake water quality objectives. Windsor: Ontario; c1977.
39. Islam M, Islam S, Howlader MR, Lucky NS. Comparative efficacy of Albendazole, Fenbendazole, and Levamisole against gastrointestinal nematodiasis in cattle of Bangladesh. International Journal of Biological Research. 2015;3(1):25-35. DOI:10.14419/ijbrbr.v3i1.4301
40. Kanu KC, Otitoloju AA, Amaeze NH. Assessment of the risk of death of *Clarias gariepinus* and *Oreochromis niloticus* pulse-exposed to selected agricultural pesticides. Scientific Reports. 2021;11(1):1-12. DOI:10.1O38/s41598-021-94262-w
41. Kenaga EE. Acute and chronic toxicity of 75 pesticides to various animal species rats, honeybees, fish. Down to Earth, USA. 1979;35:25-31.
42. Khan MIR, Sharma M, Sonkar P, Komre P, Kumre S, Barik P. Documentation of phytochemicals, bio-toxicity and haemato-phytochemicals responses at sub-lethal concentrations of *Parthenium hysterophorus* hydroethanolic extract in *Cyprinus carpio*: A concept of waste valorization as phyto-chthyotoxin. Research Gate, 2023, 1-27.
43. Kotb GA, Farag AAG, Manal EA. Subacute influence of mancozeb (80% WP) on haematological and biomarkers of male rats. International Journal of Advanced Biological Sciences. 2019;6(12):73-80. DOI:10.22192/ijarbs.2019.06.12.010
44. Li Z, Velisek J, Zlabek V, Grabic R, Machova J, Kolarova J, *et al*. Chronic toxicity of verapamil on juvenile rainbow trout (*Oncorhynchus mykiss*). Effects on the morphological indices, haematological parameters and antioxidant responses. Journal of Hazardous

Materials. 2011;185(2-3):870-880. DOI:10.1016/j.jhazmat.2010.09.102
45. Liu Y, Xiao Q, Yang S, Zhao L, Fu H, Du J, *et al*. Characterization of hematopoiesis in Dabry's sturgeon (*Acipenser dabryanus*). Aquaculture and fisheries. 2017;2(6):262- 268. https://doi.org/10.1016/j.aaf.2017.10.007
46. Marques A, Rego A Guilherme S, Gaivão I, Santos MA, Pacheco M. Evidences of DNA and chromosomal damage induced by the Mancozeb-based fungicide mancozen (®) in fish (*Anguilla anguilla* L). Pesticide Biochemistry and Physiology. 2016;133:52-58. https://doi.org/10.1016/j.pestbp.2016.03.004
47. Maršálek P, Mikuliková I, Modrá H, Svobodová Z. Effect of prochloraz fungicide on neopterin and biopterin concentrations in blood plasma of common carp. Acta Veterinaria Brno. 2014;83(2):101-105. DOI:10.2754/avb201483020101
48. Messaad IA, Al Zailaie KA. Evaluation of glyphosate toxicity on Arabian killifish, *Aphanius dispar* collected from South western Saudi Arabia. Global Journal. Science. Frontier. 2017;17(3):43-50.
49. Mgbenka BO, Oluah NS, Arungwa AA. Erythropoietic response and haematological parameters in the Catfish *Clarias albopunctatus* exposed to sub-lethal concentrations of acetellic. Ecotoxicology and Environmental Safety. 2005;62:436-440.
50. Min EY, Kang JC. Effect of waterborne benomyl on the hematological and antioxidant parameters of the Nile tilapia, *Oreochromis niloticus*. Pesticide biochemistry and physiology. 2008;92(3):138- 143. DOI:10.1016/j.ec0env.2005.03.031
51. Mohammad Nejad Shamoushaki M, Soltani M, Kamali A, Imanpoor MR, Sharifpour I, *et al*. Effects of organophosphate, diazinon on some haematological and biochemical changes in *Rutilus frisii* kutum. Iranian Journal of Fisheries Sciences. 2012;11(1):105-117.
52. Morgan DP. Pesticide studied in man. Journal of American Medical Association. 1982;247(22):3140-3140.
53. Nelson DA, Moris MW. Basic methodology. Haematology and coagulation: Part IV. In. Nelson D A, Henry J B (eds), Clinical diagnosis and Management by Laboratory Methods. 17thedn. Philidaphia: W B Saunder; c1989. p. 578-625.
54. Nishida Y. The chemical process of oxidative stress by copper (II) and iron (III) ions in several neurodegenerative disorders. Monatsheftefür Chemie-Chemical Monthly. 2011;142:375-384. DOI:10.1007/s00706-010-0444-8
55. Nwani CD, Ekwueme HI, Ejere VC, Onyeke CC, Chukwuka CO, Peace OS, *et al*. Physiological effects of paraquat in juvenile African catfish *Clarias gariepinus* (Burchfiel 1822). Journal of Coastal Life Medicine. 2015;3(1):35-43. DOI: 10.12980/JCLM.3.2015JCLM-2014-0113
56. Nwani CD, Ibiam UA, Ibiam OU, Nworie O, Onyishi G, Attama C. Investigation of acute toxicity and behavioural changes in *Tilapia zillii* due to based glyphosate based herbicide forceup. Journal of Plant and Animal Sciences. 2013;23(3):888-892.
57. Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Kumar P, Lakra WS. Mutagenic and genotoxic assessment of atrazine-based herbicide to freshwater fish *Channa punctata* (Bloch) micronucleus test and single cell gel electrophoresis. Environmental Toxicology and Physiology. 2011;31:314-322. doi:10.1016/j.etap.2010.12.001
58. Nwani CD, Odo GE, Nwadinigwe AO, Onyeke CC, Atama CI, Ngwu G, *et al*. Short-term effects of albendazole on the oxidative stress markers and hematological parameters in tissues of African Catfish *Clarias gariepinus*. Journal of Aquatic Animal Health. 2016;28(4):222-228. doi:10.1080/08997659.2016.1194908
59. Odo GE, Agwu JE, Ivoke N, Ejere VC, Atama CI, Ezea CO, *et al*. Effect of short term exposure to cyperdicot on behavioral and haematological responses in African Catfish *Clarias gariepinus*. Turkish Journal of Fisheries and Aquatic Sciences. 2017;17:61-70. DOI: 10.4194/1303-2712-v17_1_08
60. Ogah J. Toxic effects of glyphosate on the behaviour, haematology, histopathology and growth of african catfish, *Clarias gariepinus* (Burchell 1822) Juviniles. Ph.D. Dissertation, Federal University of Technolog, Minna. 2021.
61. Omitoyin B, Ajani EK, Osho F, Olaoluwa M, Orisasona OO. Toxicity of Zinc and copper on *Heterobranchus bidorsalis* Geoffroy Saint-Halaire, 1809 juveniles. Renewable. 2022;1(1):1-17.
62. Palanikumar L, Kumaraguru AK, Ramakritinan CM. Toxicity, biochemical and clastogenic response of chlorpyrifos and carbendazim in milkfish *Chanos chanos*. International Journal of Environmental Science and Technology. 2013;11:765-774. DOI:10.1007/s13762-0264-6
63. Pandey AK, Nagpure NS, Trivedi SP, Kumar R, Kushwaha B, Lakra WS, *et al*. Investigation on acute toxicity and behavioral changes in *Channa punctata* (Bloch) due to organophosphate pesticide profenofos. Drug and chemical toxicology. 2011;34(4):424-428. DOI: 10.3109/01480545.2011.585650
64. Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Verma MS, *et al*. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish *Channa punctata* (Bloch). Ecotoxicology and environmental safety. 2005;61(1):114-120. DOI:10.1016/j.ecoenv.2004.08.004
65. Paro R, Tiboni GM, Buccione R, Rossi G, Cellini V, Canipari R, *et al*. The fungicide mancozeb induces toxic effects on mammalian granulosa cells. Toxicology and Applied Pharmacology. 2012;260(2):155-161. https://doi.org/10.1016/j.taap.2012.02.005
66. Payne J, Mathieu A, Melvin W, Fancey LL. Acetylcholinesterase an old biomarker with a new future? Field trails in association with two urban rivers and a paper mill in Newfoundland. Marine Pollution Bulletin. 1996;32(2):222-231.
67. Pereira L, Fernandes MN, Martinez CB. Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone. Environmental Toxicology and Pharmacology. 2013;36(1):1-8. DOI:10.1016/j.etap.2013.02.019
68. Qiu XC, Nomichi S, Chen K, Honda M, Kang IJ, Shimasaki Y, *et al*. Short-termand persistent impacts on behavior related to locomotion, anxiety and startle responses of Japanese medaka (*Oryzias latipes*) induced by acute, sublethal exposure to chlorpyros. Aquatic

Toxicology. 2017;192:148-154. doi:10.1016/j.bbr.2019.111917
69. Qu M, Wang DY. Toxicity comparison between pristine and sulfonate modified nano-polystyrene particles in affecting locomotion behavior, sensory perception and neuronal development in *Caenorhabditis elegans*. Science of the Total Environment. 2020;703:1-8. doi:10.1016/j.scitotenv.2019.134817
70. Ruba UB, Chakma K, Ali MP, Khan AU, Samiul M, Talucder. Environmetal pollution from injudicious application of pesticides: Bangladesh context. Journal of Agroforestry and Environment. 2023;16(1):40-49.
71. Saha NC, Giri SK, Chatterjee N, Biswas SJ, Bej S. Acute toxic effects of mancozeb to fish (*Oreochromis mossambicus*) and their behaviour. International Journal of Advance Research in Biological Sciences. 2016;3:40-44.
72. Santos LH, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MCBSM. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. Journal of hazardous materials. 2010;175(1-3):45-95. https://doi.org/10.1016/j.jhazmat.2009.10.100
73. Saravanan M, Kumar KP, Ramesh M. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pesticide Biochemistry and physiology. 2011;100(3):206-211. https://doi.org/10.1016/j.pestbp.2011.04.002
74. Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, *et al*. Worldwide pesticide usage and its impacts on ecosystem. SN Applied Sciences. 2019;1(11):1-16.
75. Sharma MR, Mushtaq R, Allayie SA, Vardhan H. Assessment of lethal toxicity of mancozeb and its consequences on the behavior of fresh water fish, *Puntius ticto*. Journal of International Academic Research for Multidisciplinary. 2016;4(2):132-138.
76. Sikka SC, Gurbuz N. Reproductive toxicity of organophosphate and carbamate pesticides. In Toxicology of organophosphate and carbamate compounds; c2006. p. 447-462. Academic Press. DOI:10.1016/B978-012088523-7/50033-8
77. Simakani P, Abolhasani MH, Hoseini SM, Determination of mancozeb toxicity and biochemical effects in common carp (*Cyprinus carpio*). International Journal of Aquatic Biology. 2018;6(3):157-161. https//doi.org/10.22034/ijab.v613.494
78. Soni R, Verma SK. Acute toxicity and behavior responses in *Clarias batrachus* (Linnaeus) exposed to herbicide pretilachlor. Heliyon, 2018, 4(2). https://doi.org/10.1016/.heliyon.2018.e01090
79. Srivastava P, Singh A. Potential effects of agricultural fungicide (Mancozeb) on fish *Clarias batrachus*. Research Journal of Biological Sciences. 2014;9(4):129-134. DOI:10.36478/rjbsci.2014.129.134
80. Srivastava P, Singh A. *In vivo* study of effects of dithiocarbamates fungicide (mancozeb) and its metabolite ethylene thiourea (ETU) on fresh water fish *Clarias batrachus*. Journal of Biology and Earth Sciences. 2013;3(2):228-235.
81. Thiruchelvam M. Mancozeb. Elselvier; c2005. p. 5-8.
82. Tiwari M, Nagpure NS, Saksena DN, Kumar R, Singh SP, Kushwaha B, *et al*. Evaluation of acute toxicity levels and ethological responses under heavy metal cadmium exposure in freshwater teleost, *Channa punctata* (Bloch). International Journal of Aquatic Sciences. 2011;2(1):36 – 47.
83. Ullah R, Zuberi M, Tariq M, Ullah S. Acute toxic effects of cypermethrin on haematology and morphology of liver, brain and gills of mahseer (Torputitora). International Journal of Agriculture and Biology. 2014;17(1):199-204. https://doi.org/10.2131/jts.39.829
84. Velisek J, Stastna K, Sudova E, Turek J, Svobodova Z. Effects of subchronic simazine exposure on some biometric, biochemical, hematological and histopathological parameters of common carp (*Cyprinus carpio* L.). Neuroendocrinology Letters. 2009;30(1):236-241.
85. Yadav KK, Gupta N, Kumar V, Choudhary P, Khan SA. GIS-based evaluation of groundwater geochemistry and statistical determination of the fate of contaminants in shallow aquifers from different functional areas of Agra city, India: levels and spatial distributions. RSC Advances. 2018;8(29):15876-15889. https://doi.org/10.1039/C8RA00577J
86. Sood VK. HVDC and FACTS controllers: applications of static converters in power systems. Springer Science & Business Media; c2006.
87. Bain BJ, Bates I, Laffan MA, Lewis SM. Dacie and Lewis practical haematology: expert consult: online and print. Elsevier Health Sciences; c2001.
88. De Araujo MC, Assis CRD, Silva LC, Machado DC, Silva KCC, Lima AVA, *et al*. Brain acetylcholinesterase of jaguar cichlid (*Parachromis managuensis*): From physicochemical and kinetic properties to its potential as biomarker of pesticides and metal ions. Aquatic Toxicology. 2016;177:182-189. doi.org/10.1016/j.aquatox.2016.05.019