HEMATOLOGICAL AND BIOCHEMICAL PROFILE OF *CHANNA PUNCTATUS* AFTER INTOXICATION WITH COMBINATION OF MANCOZEB AND MALATHION

A THESIS

Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

ZOOLOGY

By

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Under the Supervision

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~ 2021 ~

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SUPERVISOR'S CERTIFICATE

This is to certify that **Mrs. Ankita Singh** has completed the necessary academic turn and the swirl presented by her is a faithful record is a bonafide original work under my guidance and supervision. She has worked on the topic **"Hematological and Biochemical Profile of *Channa punctatus* after Intoxication with Combination of Mancozeb and Malathion"** under the School of Science, Maharishi University of Information Technology, Lucknow. No part of this thesis has been submitted by the candidate for the award of any other degree or diploma in this or any other University around the globe.

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DECLARATION BY THE SCHOLAR

I hereby declare that the work presented in this thesis entitled **"Hematological and Biochemical Profile of *Channa punctatus* after Intoxication with Combination of Mancozeb and Malathion"** in fulfillment of the requirements for the award of Degree of Doctor of Philosophy, submitted in the Maharishi School of Science, Maharishi University of Information Technology, Lucknow is an authentic record of my own research work carried out under the supervision of **Dr. Rakesh Babu**, Department of Zoology, Maharishi University of Information Technology, Lucknow. I also declare that the work embodied in the present thesis-

i. is my original work and has not been copied from any journal/ thesis/ book; and

ii. has not been submitted by me for any other Degree or Diploma of any University/ Institution.

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ACKNOWLEDGEMENT

The award of degree **"Doctor of Philosophy"** is one of the hardest deserving achievements which are not easily found. During the entire research work some valuable people conceived their enormous positions in my heart. In this regard, I am grateful to the University and express my deep sense of gratitude to its **Hon'ble Prof. (Dr.) Bhanu Pratap Singh**, Vice-Chancellor for delivering this great opportunity to me. I also want to convey Professor regards to the registrar of MUIT, who has always motivated me for this work.

I would like to express my special appreciation and thanks to my supervisor **Dr. Rakesh Babu**, Department of Zoology, Maharishi University of Information Technology, Lucknow you have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been priceless. I am heartily thankful to you for being a great mentor for greatly obliged cooperation, for having adequate belief in my abilities to help me incrementally in build up my understanding of the research area and for all your precious endeavours on behalf of me. I also thanks to you for your invaluable suggestions, glorious supervision, devoted encouragement, prosperous discussions, precious feedback and committed interest in the entire duration of my research work.

I would also extend my deepest gratitude towards **Prof. Akhand Pratap Singh,** Prof. & Registrar, Maharishi University of Information Technology Lucknow, for being an excellent administrator and teacher.

I also express my gratitude to **Mr. Girish Chhimwal**, Deputy Registrar, Maharishi University of Information Technology, Lucknow, Whose affectionate behavior has supported me through the course of this investigation.

Words are less to extend my highest gratitude of deep sense of thanks to **Dr. Anil Kumar Dixit,** Dean Research, Maharishi University of Information Technology Lucknow, for always extending her unconditional support and encouragement during my journey of doctoral research.

Most importantly none of this would have been possible without the love and patience of my family. I can't imagine my current position without the love and support from my family. I very much thank to my beloved husband **Mr. Yatendra Singh** for his patience, understanding and magnificent devotion to his family and my sweet ever smiling daughter **Yashashvi** and son **Ojas** for making everything worthwhile.

I would also like to thank all of my friends who supported me in writing, and incanted me to strive towards my goal. At the end I would like express appreciation to my beloved who spent sleepless nights with and was always my support in the moments when there was no one to answer my queries.

Last, but not the least, I thank to Invisible **God Almighty** by whose grace and blessings I find myself in a position of putting forth this presentation.

(Ankita Singh)

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Introduction

INTRODUCTION

Problem of pesticidal pollution is very severe in the era of excess use of pesticides in every field like houses, crops, industries etc. In every house there is some kind of mosquito repellent or insecticide for flies, mosquito and other harmful insects, even ratkill is very common for control of rats. In crops, it is very evident fact that the farmers use excess and excess of pesticides to increase crop yield by reducing damage by pests and fertilizers. In pesticide making industry, the waste was run off in water bodies.

The overall impact of above activities related to pesticides and insecticides is contamination of aquatic bodies adversely. This affect ecosystem at every trophic level the pesticide can accumulate in aquatic organisms or directly kill them and destroy balance of ecosystem. The past work on this phenomenon is done in vast aspects.

Water pollution is major issue form the last many decades. it's far more effective in Rivers and water bodies almost dense cities. The waste from houses and industries containing various pollutants like heavy metals, insecticides, sewage, detergents etc. These toxic components are very toxic to aquatic life. These metals

accumulate in body of fishes and affect their physiology and biochemistry. It's very necessary to guage impact of water quality to elucidate the harmful effects of pollution and evaluate pollution control strategies conducted by government. Bioaccumulation is main explanation for toxic effect in human after fish consumption. By this phenomenon the humans also are affected adversely. Water pollution by large varieties of pollutants has become a major source of apprehension in the last some decades; Pollution could be a major problem for the environment. A variety of contaminants have polluted numerous water resources, not only because of the danger to public water supplies, but because of the harm to organisms live in water also. The capability to estimate the impact of business municipal sewage discharge and waste water on a specific environment would definitely be extremely beneficial in increasing development rapidly.

Pesticides are substances that disrupt the delicate balance of species that marks a healthy ecosystem. Pesticides are a cost-effective method of pest management. Pesticides are frequently used to prevent pests from spreading in imports and exports, to control weeds in gardens, and to protect homes and furnishings from destruction. Pesticides are a diverse group of compounds with widely varying modes of action, metabolism and elimination from

the body and uptake by the body and toxicity to focus on and organisms non-targeted. The dangers of poisoning are proportional to the dose, duration of exposure and sensitivity and toxicity. Around the mid-twentieth century, insecticidal use in agriculture exploded. Fungicides are also used in agriculture to keep seed corn free of mycosis. These compounds are then released into neighbouring water bodies, where they are devoured by fish and other aquatic life. Bioaccumulation and biomagnification cause these fat-soluble pollutants to concentrate in fish fat.

Fishes are the most sensitive of all aquatic species to pollution, making them the best indicator of water body pollution. Because effluents are one of the most important components in organic phenomena, their accumulation becomes hazardous to aquatic organisms. The desperate and uncared use of fungicides in agriculture practices has further enhanced the matter to the worldwide importance. As fish are the simplest source of protein and mineral salts but they're facing the environmental contamination. The injurious effect of certain fungicide on various vitals and their accumulation within the white muscles of the inhabitants has attracted the eye of variety of workers. The fish selected is usually utilized in laboratory because it's hardy and simply available throughout year. This is often valued as its ability

to sustain long periods of time out of the water. Catfish that walks is frequently marketed and treated with ease, resulting in a rather fresh food product.

In the present study the effect of combination of pesticides viz. mancozeb and malathion has been observed in terms of biochemical and hematological parameters which are very evident of any kind of toxicity in aquatic organism which is fish *Channa punctatus* in present study. The hematological parameters are first evidence of toxicity which gone to biochemical level and alter protein profile and lipid profile.

Review of Literature

REVIEW OF LITERATURE

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Oil within the *Oreochromis mossambicus* fish. Diwakar and Pandey (2020) conducted histological Study of malathion sublethal toxicity in gills of Clarias batrachus. Diwakar and Pandey (2020) observed histological sublethal concentration effect on the liver of freshwater fish *Clarias batrachus* (Linn.) exposed to malathion. Sezgi Arman (2021) observed consequences of acute triclosan exposure on zebrafish gill and liver tissues (Danio rerio). Kakakhel *et al*. (2021) worked on Fish exposed to high-concentration silver nanoparticles for a long time developed toxicity, bioaccumulation, mortality and histological changes (*Cyprinus carpio*).

Materials and Methods

MATERIALS AND METHODS

3.1 SELECTION OF ANIMAL

A test animal was chosen: *Channa punctatus* (Bloch.*)*. It has a long, elongated body that is wider at the head and narrows as it gets closer to the tail. Fresh water is a good source of it. It is a hardy fish that adapts well to aquarium conditions.

3.2 FISH COLLECTION

Between September and October, the fish were caught, when the ambient temperature ranged between 250 and 300 degrees Celsius. Adult live *Channa punctatus* (Bloch.) specimens ranging in size from 16 to 18 cm and weighing 40 to 70 g were acquired locally sourced.

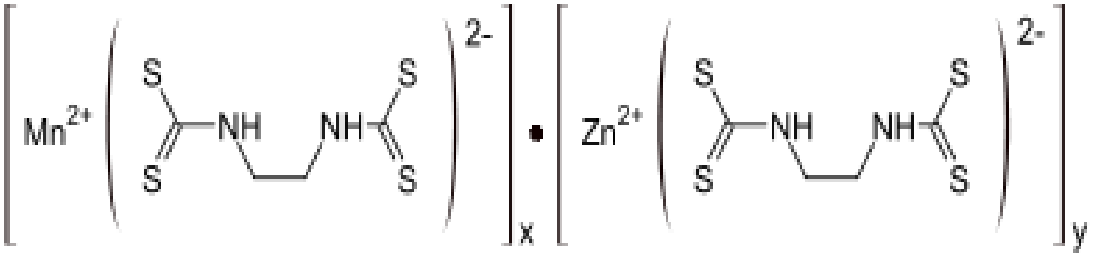
3.3 FISH FEEDING and MANAGEMENT

They were thoroughly examined for any injuries before being immersed for a few minutes in a 0.2 percent KMnO4 solution to clear any skin infections. Finally, they were kept in a huge glass aquarium for 15 days in a laboratory setting. Every other day, the dechlorinated water was utilised and changed. Temperature, pH, and hardness were among the physiochemical properties of test water that were recorded on a regular basis.

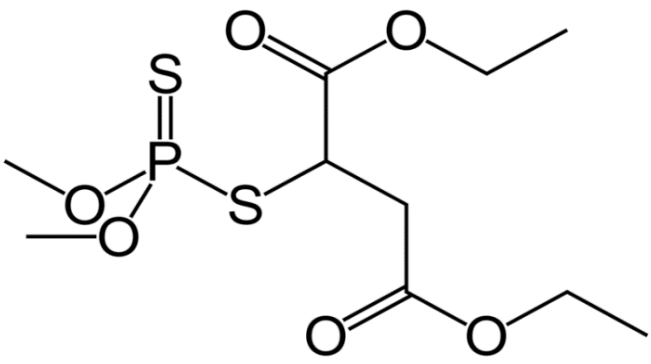
3.4 EXPERIMENT COMPOUNDS

1. Mancozeb

Mancozeb is a dithiocarbamate agricultural fungicide that is not systemic that has a many sites, contact-protective activity. It's made up of maneb and zineb, two more dithiocarbamates. Many fungal infections are controlled by the mixture in a variety of agricultural fruits, crops, nuts, ornamentals and vegetables. Trimanoc, Penncozeb, Dithane, Vondozeb, Manzeb, Manzane and Nemispot are some of the brand names for it. As early as 2008, a combination of zoxamide and mancozeb was approved in Canada for the control of the Gavel mildew (Mancozeb, 1993, Gowan, 2008).



2. Malathion



Properties	
Molecular formula (M.F.)	$C_{10}H_{19}O_6PS_2$
Molar mass	330.358021
Appearance	Colorless clear liquid
Density	1.23 g/cm^3
Boiling point (B.P.)	156°C, 157 °C (313°F to 315 °F; 429K to 430 K) at 0.7 mmHg
Melting point (M.P.)	2.9 °C (37.2 °F; 276.0 K)
Solubility inwater	145 mg/L at 20 °C[1]
Solubility	Dissolve in acetone and ethanol; much soluble in ethyl ether

3.5 LC50 VALUE DETERMINATION

After 96 hours of exposure, statistically the median lethal concentration is the calculated dose that causes the death of fifty percent (50%) of a specific organism's population under specific set of testing conditions (experimental). To investigate the value of LC_{50} of pesticidefor *Channa punctatus* (Bloch.), the experiment was created in this manner. During the experiment, there were also control groups. Four groups (A, B, C, and D) were created with varied pesticide concentrations to determine the LC50. Every group has 6 fishes. After 96 hours, for each dose, the fish mortality rate was recorded. The data were evaluate statically via Method of log

dose/probit regression line (Finney, 1971). On the basis of two variables, log dosages, a regression line was created and utilised to derive the predicted probit required for Calculation of the LC50.

3.6 EXPERIMENTATION

The test (experiment) was carried out in 5 aquariums, one of which was utilized as under control and the others as a pollution study. Each tank includes ten fish that were given sub-lethal doses of mancozeb and malathion in combination at various time interval (24, 48, 72 and 96 hr). The LC5o value was used to determine the sublethal concentration.

3.7 BLOOD COLLECTION

After 24, 48, 72, and 96 hours of exposure to a mixture of pesticides, five fish from each group of control and treated fish were slaughtered for the investigations. After severing the caudal peduncle of the living fish using a scissor, the blood was collected.

3.8 SEPARATION OF SERUM

Allowing the blood sample centrifuge tubes to stand in a slanting position was approved. For around an hour at room temperature before being centrifuged for 30 minutes at 2500rpm.

3.9 ANALYSIS OF THE HEMATOLOGY

Haematological examination was performed on blood samples that had been treated with the anticoagulant EDTA. Total

erythrocyte count is one of the blood measures (TEC) by Neubaur chamber haemocytometer, haemoglobin concentration by standard Sahli's haemoglobin meter, packed cell volume (PCV) by Wintrobe's method, total leukocyte count (TLC) by Neubaur chamber haemocytometer, mean corpuscular haemoglobin concentration (MCHC), Mean corpuscular volume (MCV), erythrocyte sedimentation rate (ESR) and mean corpuscular haemoglobin (MCH) by Wintrobe's method.

3.10 TOTAL ERYTHROCYTE COUNT (TEC)

Method

Dacie and Lewis described an enhanced Standard Neubaur haemocytometer for estimating total erythrocyte count (1975).

Principle

Blood was drawn into a RBC standard pipette and dilute two hundred times with Hayem's solution that was isotonic to blood, to determine the total erythrocyte count. To reduce the number of cells per unit volume, dilution was used. As a result, visual counting is much easier under the microscope.

Procedure

The pipette (RBC) was cleaned and dried with distilled water. The oxalated blood had to be sucked up and in the RBC pipette at the 0.5 mark, and in the pipette of RBC, up to the 100 mark

Hayem's solution was sucked up, resulting in a 1:200 ultimate dilution. The pipette by shaking horizontally, the contents were well combined. The counting chamber was covered with the cover slip and then charged with the diluting blood sample after a drop of diluent fluid was inserted along the edge of the cover slip. The long capillary tube of the RBC pipette contained 2-3 drops of the fluid is ejected before charging the counting chamber, as it is assumed to be clear of cells. For a few seconds, the cells in the charged counting chamber are allowed to settle and scatter uniformly across the whole surface of the counting chamber by being left undisturbed. Finally, a high magnification examination of the counting chamber is performed (40X). Five squares were used to count the cells. Count huge squares in the RBC counting chamber, four at each corner and one in the centre. Each square's RBCs on the lower and right sides were added to the total, while left and top sides were thrown out.

Calculation

Total erythrocyte count (TEC) (million/mm^3) = 10,000 × total Red Blood Cells counted in 5 squares

CONCENTRATION of HAEMOGLOBIN

Wintrobe *et al*., 1981 described the conventional Sahli's method for estimating haemoglobin concentration.

Principle

The blood acid haematin solution prepared in graded tubes is compared to the normal acid haematin solution in a sealed tube.

Procedure

The haemoglobinometer's tube with a graduation was washed with distilled water, then methylated spirit, and lastly dried. With the use of a glass dropper, fill the tube that is graduated with N/10 HCl up to the mark of 2g/dl. The oxalated blood was now sucked into the Hb (haemoglobin) pipette until it reached the 0.02ml mark, and then sincerelly placed into the graduated tube containing N/10 HCl. The tube had been thoroughly shaken. until the contents were mixed well and permitted to stand for five minutes to ensure total clearance of blood from the haemoglobic pipette. Then, with the glass rod, distilled water was added drop by drop until the contents of the graduated tube matched the colour of the regular glass tube. Following that, the reading was recorded. The concentration of haemoglobin in the blood is measured in g/dl.

TLC (TOTAL LEUCOCYTE COUNT)

The total leucocytes were counted using an enhanced standard Neubaur chamber haemocytometer, which Dacie and Lewis describe (1975).

Principle

WBC dilution fluid was used to dilute anticoagulated blood, which removed red cells through haemolysis and emphasised white cell nuclei. Counting was done using a microscope with a lower power 45X objective in order to determine fluid volume analyzed and blood dilution. In unadulterated whole blood, white blood cells numbers were determined.

Procedure

In the WBC pipette, well mixed oxalated blood was sucked up to the mark 0.5, then white blood cells (WBC) fluid that was diluting sucked up to 11 mark (1:20). The blood was then fully blended by vigorous shaking. Then discarding the few first drops of blood, the Neubaur haemocytometer and cover slip were charged with diluted blood. For 5 minutes, the counting chamber, which had been charged, was left undisturbed. Allow cells to settle and become evenly dispersed around the chamber's surface. Finally, a low magnification examination of the chamber was performed. In the counting chamber, the counting was done in four corner squares.

Calculation

Total Leucocyte Count (TLC) (cells/mm^3) = 100 X The total no. of White Blood Cells counted in 4 square.

VOLUME OF PACKED CELLS

Wintrobe's tube method, developed by Wintrobe *et al*., 1981, was used to determine the packed cell volume.

Principle

When blood that has been oxalated is spun at a typical speed, RBCs settle to the base, while the amount of space they occupy remains constant, and will only be little reduced following a series of centrifugations. This column of red cell is referred to as PCV (packed cell volume), which is represented as a percentage (%) of the total column of blood.

Procedure

To avoid air bubbles, Up to the 100 mm mark, Wintrobe's tube was full with oxalated blood using a fine glass dropper. For 30 minutes, the tube was spun at 3000 rpm. when centrifugation completed, the height of the cellular layer column was measured and the packed cell volume was calculated Packed Cell Volume.

Calculation

$$\text{Volume of Packed Cells (percent)} = \frac{\text{Length of RBC column}}{\text{Length of the entire blood column}} \times 100$$

RATE OF ERYTHROCYTE (RBCs) SEDIMENTATION (RES)

Wintrobe's approach was used to calculate the erythrocyte

sedimentation rate (1981).

With the use of a fine glass dropper, Up to the 100mm mark, the Wintrobe's tube was full with oxalated blood and set vertically in the stand. The column level to which the erythrocytes had dropped down was observed after one hour, and the results were represented in mm/hr.

CORPUSCULAR MEAN VOLUME (CMV)

The corpuscular mean volume was computed using the Wintrobe *et al*., 1981 formula using total erythrocyte count (TEC) and packed cell volume (PCV) and the corpuscular mean volume was represented in fl (Femto leter).

Calculation

$$\text{Corpuscular mean Volume (fl)} = \frac{\text{Packed cell volume (PVC)}}{\text{(Total RBCs count)}} \times 10$$

MCH (MEAN CORPUSCULAR HAEMOGLOBIN)

The following formula of Wintrobe *et al*., 1981 was used to compute the mean corpuscular haemoglobin (MCH) from total erythrocyte count and haemoglobin concentration picogram (pg).

Calculation

$$\text{Mean Corpuscular Haemoglobin (pg)} = \frac{\text{Hb concentration}}{\text{Total RBCs count}} \times 10$$

Hb CONCENTRATION IN THE CORPUSCULAR MEAN

Wintrobe *et al*., 1981 derived the mean corpuscular haemoglobin concentration (MCHC) from total erythrocyte count (TEC) and haemoglobin concentration using the formula given below: mean corpuscular haemoglobin concentration expressed in g/dl.

Calculation

$$\text{MCHC (g/dl)} = \frac{\text{Hb concentration}}{\text{PVC (Packed Cell Volume)}} \times 100$$

SERUM TOTAL PROTEINS ESTIMATION

The total serum proteins were calculated by the modified Biuret and Dumas (1971) method.

Principle

Serum protein reacts with Cu (copper) in Biuret Reagent in an alkaline media to form a blue purple complex with absorbance maxima at 550 nm.

Procedure

There were 3 test tubes utilised, each labelled "Blank," "Standard," and "Test." Each test tube received 3ml of biuret reagent. In a labelled test tube "Standard," 0.05 mL of protein standard was introduced. After that, 0.05ml sample of serum was

transferred to a labelled test tube "Test," thoroughly mixed, and allowed to stand for 5 minutes at room temperature. On a colorimeter set to 550nm, the optical density of “Standard” and “Test” were calculated against a blank.

Calculation

$$\text{Total Serum proteins (g/100ml)} = \frac{\text{Optical Density of Test}}{\text{Optical Density of standard}} \times 7.2$$

SERUM ALBUMIN, GLOBULIN AND ALBUMIN-GLOBULIN ESTIMATION

RATIO

Serum albumin was calculated using a modified Biuret & Dumas (1971) technique.

Principle

At pH 3.68, albumin in serum interacts with the dye Bromocresol green to generate a green coloured complex with a 600 nm absorption.

Procedure

Three test tubes with the labels "blank," "standard," and "Test" were taken. Each test tube received 3.0ml of Buffered Dye Reagent. In a test tube labelled ‘Standard,' 0.02ml of protein standard was used. In a test tube labelled ‘Test,' 0.02ml of serum

was used. The test tubes were thoroughly mixed before being left at room temperature for one minute. A red filter was used to measure the O.D. (optical density) of the test (T) and standard (S) against a blank (B).

Calculation

$$\text{Albumin Serum (g/100ml)} = \frac{\text{Optical density of Test}}{\text{Standard's Optical Density.}} \times 50$$

Serum globulin (g/100ml) = Total serum proteins – serum albumin

$$\text{Albumin/Globulin Ratio} = \frac{\text{Serum Albumin}}{\text{Serum Globulin}}$$

SERUM CHOLESTEROL ESTIMATION

The cholesterol of serum was calculated by the CHOD-PAP kit method used by Roeschlau *et al*. (1974).

PRINCIPLE

The following enzyme-catalyzed processes are used to calculate cholesterol.

$$\text{Cholesterol's ester} \xrightarrow{\text{CE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } o_2 \xrightarrow{\text{CHOD}} \text{Cholesterol-4-en-3 + } H_2O_2$$

POD

$$4AAP + Phenol + 2H_2O_2 \longrightarrow + Quinoneimine + 4H_2O$$

REAGENTS

- Cholesterol-determination reagent
- Working cholesterol standard reagent (200mg%)

PROCEDURE

'Test,' 'Standard,' and 'Blank' were written on three test tubes..

Test

In a test tube labelled 'Test,' 1ml of cholesterol reagent and 0.02ml of serum sample (vide supra) were combined.

Standard

1ml of cholesterol reagent and 0.02ml of cholesterol standard solution were poured in a test tube labelled as 'Standard'.

Blank

In a test tube labelled 'Blank,' cholesterol reagent of 1 ml and 0.02ml of distilled water were combined.

For 10 minutes it incubate at 370°C after thoroughly mixing. A lavender color developed in tubes marked as 'Test' and 'Standard'. Optical density of Test and Standard was measured by

photoelectric colorimeter at 505nm after setting the zero with ‘Blank’.

CALCULATION

$$\text{Serum Cholesterol (mg/dl)} = \frac{\text{Optical density of ‘Test’}}{\text{Optical Density of ‘Standard’}} \times 200$$

SERUM TRIGLYCERIDE ESTIMATION

The serum triglyceride was calculated by method GPO-PAP described by Schettler and Nussel (1975).

REAGENTS

- Triglyceride monoreagent
- Standard 200mg%

PROCEDURE

The words ‘Test,' ‘Standard,' and ‘Blank' were written on three test tubes.

Test

In a test tube labelled ‘Test,' 1ml of triglyceride monoreagent and 0.02ml of serum sample were combined.

Standard

1ml of triglyceride monoreagent and 0.02ml of standard solution were poured in a test tube pointed as ‘Standard’.

Blank

In a test tube labelled Blank, 1ml of triglyceride monoreagent and 0.02ml of distilled water were combined. Incubate for 10 minutes at 370°C after thoroughly mixing. Optical density of 'Test' and 'Standard' was measured by photoelectric colorimeter at 505nm after setting the zero with 'Blank'.

CALCULATION

$$\text{Triglyceride inserum (mg/dl)} = \frac{\text{Opticaldensity of 'Test'}}{\text{Optical Density of 'Standard'}} \times 200$$

HIGH DENSITY LIPOPROTEIN ESTIMATION (HDL)

High density lipoprotein was estimated by the Wybenga and Pileggi method (1970).

PRINCIPLE

In the presence of divalent cations such as magnesium, phosphotungstate precipitates LDL, VLDL (low and very low density lipoproteins) and chylomicrons from serum. The HDL cholesterol in the supernatant is unaffected and is measured using the cholesterol reagent ERBA.

$$\text{Plasma/serum} \xrightarrow[Mg^{2+}]{\text{Phosphotungstate}} \underset{\text{(supernatent)}}{\text{HDL}} + \underset{\text{(Precipitate)}}{\text{VLDL + Chylomicrons}}$$

REAGENTS

- Reagent for Cholesterol
- Standard solution
- Reagent for precipitation

PROCEDURE

HDL Cholesterol Separation-

0.25ml of serum sample and 0.5ml of precipitating reagent were taken into centrifuge tubes. Allow 10 minutes for the reaction mixture to sit at room temperature after mixing thoroughly. To obtain a clear supernatent, the contents were centrifuged at 4000rpm for 10 minutes. Determine the cholesterol concentration of HDL in the sample using the supernatent.

There were 3 test tubes were taken as ‘Test’, ‘Standard’ and ‘Blank’.

Test

In a test tube to be labelled ‘Test,' 1ml of cholesterol reagent and 0.05ml of supernatant (vide supra) were combined.

Standard

1ml of cholesterol reagent and 0.05ml of standard solution were taken in a test tube marked as ‘Standard’.

Blank

In a test tube labelled "Blank," 1 mL cholesterol reagent and 0.05 mL distilled water were put’.

Mixed well and incubated for 10minutes at 37^0C. Optical density of 'Test' and 'Standard' was measured by photoelectric colorimeter at 505nm after setting the zero with 'Blank'.

CALCULATION

$$\text{Serum HDL (mg/dl)} = \frac{\text{Optical density of 'Test'}}{\text{Optical density of 'Standard'}} \times 75$$

LDL (LOW DENSITY LIPOPROTEIN) ESTIMATION

LDL (Low density lipoprotein) was calculated from the values of cholesterol (serum), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) by using following formula given by Friedwald *et al*. (1972).

LDL = CHOLESTEROL – (VLDL + HDL)

ESTIMATION OF VERY LOW DENSITY LIPOPROTEIN (VLDL)

Very low density lipoprotein was calculated by the following formula given by Friedwald *et al*. (1972).

$$\text{VLDL} = \frac{\text{Triglyceride (TG)}}{5}$$

STATISTICAL CALCULATIONS

For each biochemical parameters a minimum of 50 replicates were done and the data was statistically examined using the student's t test.

Mean (x)

The following formula was used to compute the mean:-

$$\overline{X} = \frac{\Sigma x}{N}$$

Where,

$\sum x$ = Addition of individual observation

N = Number of observations.

Standard Deviation (S.D.)

The S.D. was computed using the given formula-

$$S.D. = \sqrt{\frac{\Sigma(x - \overline{x})^2}{N - 1}}$$

Where,

$\Sigma(x-\overline{x})^2$ = The sum of all deviations' squares.

Standard Error of Mean (S.Em)

The following formula was used to compute the S.E. (standard error) of the mean:-

$$S.Em. = \frac{S.D.}{\sqrt{N}}$$

Where,

N = No. of observations.

S.D. = Standard deviation

Student ‘t’test

The value of ‘S', firstly was determined using the formula given below*:* **-**

$$'S' = \sqrt{\frac{\Sigma(x-\bar{x})^2 - \Sigma(y-\bar{y})^2}{N_1 + N_2 - 2}}$$

Where,
S = the difference between two samples' standard deviation,

That are variables X and Y.

Two variables mean is X and Y.

The observations N1 and N2 for the 2 variables x and y respectively.

So, The following formula was used to determine the ‘t':-

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

Degree of freedom (d.f.)

The following formula was used to calculate the degree of freedom:-

$$d.f. = N_1 + N_2 - 2$$

The Fisher's formula significance test was used to determine the t value. For a given (d.f.) degree of freedom, the probability ‘p' of obtaining ‘t' value was determined.

Analysis of Variance (ANOVA)

ANOVA was calculated by the following sequential steps.

(i) Sum of Squares (S.S.) -

Total S.S. = ? $(X - X)^2$

If zero in taken as the arbitrary mean, the deviations of the variates from zero will be the variates themselves.

Total S.S. = $(X_1^2 + X_2^2 + ...X_n^2) - \frac{(\Sigma \bar{X})^2}{n}$

Where,

ΣX = Grand total of Variates

n = Number of variates

Between groups sum of squares -

S.S. = $\Sigma(T_a^2 + T_b^2 + ...T_n^2) - \frac{(\Sigma \bar{X})^2}{n}$

Where = $T_a T_b$ ---------- T_n = Group total of Variates.

Within group S.S. = Total S.S. - B/W group S.S.

(ii) Degree of freedom:

Total Degree of freedom = N - 1

N = No. of observation

Degree of freedom b/w groups = K-1

K = No. of groups

Degree of freedom within groups = N - K

(iii) Variance =

$$\text{Variance} = \frac{\text{Sum of squares}}{\text{Degree of freedom}}$$

Level of confidence

The statistical tests of the chance of making a type I error at a given level, referred to as the degree of confidence, reduce the risks of making a type II error. The degree of certainty about the chances of making a type I error. The p value in our study was 0.05.

According to our calculations, the chances of committing a type II error are roughly 5% that is 95% of the observations will be correct, hence in this test, the supposed p value was 0.05.

The values of ‘p' were denoted as follows:-

P> 0.05
OR
P= 0.05
→ Non significant

P<0.05
OR
P= 0.02
→ significant*

P<0.01
OR
P= 0.01
→ Highly significant**

P= 0.001
OR
P<0.001
→ Very highly significant ***

Observation

OBSERVATION

In the present study, the haematotoxic and biochemical effects of mancozeb+malathion pesticide have been observed in control and exposed fish, *Channa punctatus* (Bloch.). The parameters have been analysed after 24, 48, 72 and 96 hours after the intoxication of mancozeb+malathion pesticide. From the data obtained, the mean ($\bar{X}$), standard error of mean (S.Em.), standard deviation (S.D.) and test of significance student's "t" test have been calculated by statistical software (stat pac version 3.0).

Determination of LC_{50} VALUE

In order to estimate the LC_{50}, the fishes were treated with different doses of mancozeb+malathion. In an equal proportion, four doses of 10, 20, 30, and 40 g/L were chosen and the mortality number & mortality percentage of fishes for each dose has been noted after 96 hours (Table- 1a). The mortality percentage showed a corresponding increase with increase dose of mancozeb+malathion.

LC_{50} has been calculated by the method log dose/probit regression (Finney, 1971). The test dose has been converted to their logarithms (Table-1b). Empirical probit values corresponding to the

percentage mortality have been obtained from standard table (Finney, 1971) and tabulation in the appropriate columns of the respective tables. Following that, the empirical probit was plotted versus log-dose on conventional graph paper with provisional line filling and the points were drawn (Fig. 1). Expected probit values ‘Y' for the value of ‘X' are read from this line (Table- 1b). The working probit (y) was determined using the formula below:

$$y = y_0 + kp$$

Where y0 and k are the expected probit (y) values from the table, and the mortality percentage is p.

The weighing coefficient for each point is also read from the table (Finney, 1971). Each weighted coefficient has been multiplied by the number of fish used, and the results have been referred to as ‘w' (Table-1b). The products of wx, wy, wxy, wx2, wy2 have been calculated and added as ∑wx, ∑wy, ∑wxy, ∑wx2, ∑wy2 accordingly for each row, and the mean has been determined using the formula below:

$$\overline{X} = \sum wx / \sum w$$

$$\overline{Y} = \sum wy / \sum w$$

The following formula was used to calculate the value of ‘b'

$$b = (\sum wxy - \overline{X} \sum wy) / (\sum wy^2 - \overline{X} \sum wy)$$

Regression equation

$$Y = \overline{Y} + b\,(x - \overline{X})$$

The regression line has been generated with the value of 'Y' corresponding to the original values of 'X'.

For the given value of LC_{50}, variance is calculated as

$$\text{Variance (V)} = \frac{1}{b^2}\left(\frac{1}{\sum w} + \frac{(m-\overline{X}^2)}{\sum wx^2 - \frac{(\sum wx)^2}{\sum w}}\right)$$

Following that, the 95 percent confidence fiducial limits m1 and m2 were computed using the methods below (Table-1c).

$$m_1 = m - 1.96\ V$$

$$m_2 = m + 1.96\ V$$

LC_{50} value of mancozeb+malathion was 26.50mg/25L with 0.0004 variance, 1.4421(+) and 1.4348(-)fiducial limits and Y = 4.55+4.87 (X-1.33) regression equation for the fish *Channa punctatus* (Bloch.) (Table-1a-1c and Fig.1).

All Experiments have been done under following steps-

(A) Hematological Studies

1. Total Erythrocyte Count (TEC)
2. Total Leucocyte Count (TLC)
3. Hemoglobin Concentration (Hb. Conc.)

4. Packed Cell Volume (PCV)
5. Erythrocyte Sedimentation Rate (ESR)
6. Mean Corpuscular Volume (MCV)
7. Mean Corpuscular Hemoglobin (MCH)
8. Mean Corpuscular Hemoglobin Concentration (MCHC)

(B) Protein Profile

1. Total Protein
2. Albumin
3. Globulin
4. Albumin-globulin Ratio

(C) Lipid Profile

1. Cholesterol
2. Triglyceride
3. High Density Lipoprotein (HDL)
4. Low Density Lipoprotein (LDL)
5. Very Low Density Lipoprotein (VLDL)

(A) HEMATOLOGICAL STUDIES

TOTAL ERYTHROCYTE COUNT (TEC)

Control Set

Total erythrocyte count of control set have an average of 3.65 million/mm^3. (Table-2, Fig. 2).

Treated group

Total erythrocyte count after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 3.20 million/mm^3, while after 48 hours an average of 2.55 million/mm^3, while after 72 hours an average of 2.35 million/mm^3 and after 96 hours an average of 2.10 million/mm^3.

The decrease in total erythrocyte count with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-2, Fig. 2).

TOTAL LEUCOCYTE COUNT (TLC)

Control Set

Total leucocyte count of control set have an average of 8500 cells/mm^3. (Table-3, Fig. 3).

Treated group

Total leucocyte count after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 8800 cells/mm^3, while after 48 hours an average of 9500 cells/mm^3, while after 72 hours an average of 9810 cells/mm^3 and after 96 hours 9980 cells/mm3 on average.

The increase in total leucocyte count with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-4, Fig. 3).

HEMOGLOBIN CONCENTRATION (Hb. Conc)

Control Set

Haemoglobin concentrations of control set have an average of 12.8 mg/dl. (Table-5, Fig. 4).

Treated group

Haemoglobin concentration after intoxication of mancozeb+malathion pesticide at 24 hours have 11.50 mg/dl on average, while after 48 hours an average of 10.20 mg/dl, while after 72 hours an average of 9.35 mg/dl and after 96 hours an average of 8.80 mg/dl.

After treating with mancozeb+malathion at various dosages, the decrease in haemoglobin concentration is considerable, as indicated in the table. (Table-4, Fig. 4).

PACKED CELL VOLUME (PCV)

Control Set

Packed cell volume of control set have an average of 45.45%. (Table-5, Fig. 5).

Treated group

Packed cell volume after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 40.50 %, while after 48 hours an average of 34.40 %, while after 72 hours an average of 30.10 % and after 96 hours an average of 28.50 %.

The decrease in packed cell volume with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-5, Fig. 5).

ERYTHROCYTE SEDIMENTATION RATE (ESR)

Control Set

Erythrocyte sedimentation rate of control set have an average of 2.66 mm/hr (Table-6, Fig. 6).

Treated group

Erythrocyte sedimentation rate after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 2.77 mm/hr, while after 48 hours an average of 3.35 mm/hr while after 72 hours an average of 3.90 mm/hr and after 96 hours an average of 4.38 mm/hr. The increase in erythrocyte sedimentation rate with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-6, Fig. 6).

MEAN CORPUSCULAR VOLUME (MCV)

Control Set

Mean corpuscular volume of control set have an average of 30.35 fl. (Table-7, Fig. 7).

Treated group

Mean corpuscular volume after intoxication of mancozeb+malathion pesticide at 24 hours have an average of

28.35 fl, while after 48 hours an average of 25.20 fl, while after 72 hours an average of 21.35 fl and after 96 hours an average of 19.28 fl. The decrease in mean corpuscular volume with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-7, Fig. 7).

MCH (MEAN CORPUSCULAR HAEMOGLOBIN)

Control Set

MCH (Mean corpuscular haemoglobin) of control set have an average of 25.50 pg. (Table-8, Fig. 8).

Treated group

Mean corpuscular haemoglobin after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 22.50 pg, while after 48 hours an average of 20.67 pg, while after 72 hours an average of 19.33 pg and after 96 hours an average of 17.24 pg. The decrease in mean corpuscular haemoglobin with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-8, Fig. 8).

CONCENTRATION OF MEAN CORPUSCULAR HAEMOGLOBIN (CMCH)

Control Set

The average corpuscular concentration of haemoglobin in the control group was 20.50 mg/dl. (Table-9, Fig. 9).

Treated group

Mean corpuscular haemoglobin concentration after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 18.33 mg/dl, while after 48 hours an average of 15.35 mg/dl, while after 72 hours an average of 12.10 mg/dl and after 96 hours 10.18 mg/dl on average.

The decline in mean corpuscular haemoglobin concentration with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-9, Fig. 9).

(B) PROTEIN PROFILE

TOTAL PROTEIN

Control Set

Total protein of control set have an average of 84.50 mg/dl. (Table-10, Fig. 10).

Treated group

Total protein after intoxication of mancozeb+malathion pesticide at 24 hours have 72.50 mg/dl on average, while after 48 hours an average of 65.70 mg/dl, while after 72 hours an average of 60.10 mg/dl and after 96 hours 57.75 mg/dl on average.

With exposure to mancozeb+malathion, there is a substantial decrease in total protein after treatment at various levels shown in respective table. (Table-10, Fig. 10).

ALBUMIN

Control Set

Albumin of control set has an average of 26.67 mg/dl. (Table-11, Fig. 11).

Treated group

Albumin after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 20.13 mg/dl, while after 48 hours an average of 17.15 mg/dl, while after 72 hours an average of 15.50 mg/dl and after 96 hours 13.70 mg/dl on average.

The decrease in albumin with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-11, Fig. 11).

GLOBULIN

Control Set

Globulin of control set has an average of 14.25 mg/dl. (Table-12, Fig. 12).

Treated group

Globulin after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 12.20 mg/dl, while after 48 hours an average of 10.10 mg/dl, while after 72 hours an average of 9.85 mg/dl and after 96 hours 8.50 mg/dl on average.

The decline in globulin with exposure to mancozeb+malathion is significant following treatment at various levels shown in respective table. (Table-12, Fig. 12).

ALBUMIN-GLOBULIN RATIO (A/G)

Control Set

Albumin-Globulin ratio of control set have an average of 1.87. (Table-13, Fig. 13).

Treated group

Albumin-Globulin ratio after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 1.64, while after 48 hours an average of 1.69, while after 72 hours an average of 1.57and after 96 hours an average of 1.61.

The decrease in Albumin-Globulin ratio with exposure to mancozeb+malathion is significant after treatment at various levels

(C) LIPID PROFILE

CHOLESTEROL

Control Set

Cholesterol of control set have an average of 155.50 mg/dl. (Table-14, Fig. 14).

Treated group

Cholesterol after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 162.40 mg/dl, while after

48 hours an average of 168.70 mg/dl, while after 72 hours an average of 175.80 mg/dl and after 96 hours 182.50 mg/dl on average.

The cholesterol increases with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-14, Fig. 14).

TRIGLYCERIDE

Control Set

Triglyceride of control set has an average of 112.50 mg/dl. (Table-15, Fig. 15).

Treated group

Triglyceride after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 120.67 mg/dl, while after 48 hours 128.50 mg/dl on average, while after 72 hours an average of 135.45 mg/dl and after 96 hours an average of 142.50 mg/dl.

The increase in triglyceride with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-15, Fig. 15).

HIGH DENSITY LIPOPROTEIN

Control Set

High density lipoprotein of control set has an average of 55.67 mg/dl. (Table-16, Fig. 16).

Treated group

High density lipoprotein after intoxication of mancozeb+malathion pesticide at 24 hours have 52.50 mg/dl on average, while after 48 hours an average of 46.67 mg/dl, while after 72 hours an average of 42.30 mg/dl and after 96 hours an average of 38.50 mg/dl.

The decline in high density lipoprotein with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-16, Fig. 16).

LOW DENSITY LIPOPROTEIN

Control Set

Low density lipoprotein of control set have 72.40 mg/dl on average. (Table-17, Fig. 17).

Treated group

Low density lipoprotein after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 75.37 mg/dl, while after 48 hours an average of 81.50 mg/dl, while after 72 hours an average of 86.70 mg/dl and after 96 hours 91.57 mg/dl on average.

The increase in low density lipoprotein with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-17, Fig. 17).

VLDLP (VERY LOW DENSITY LIPOPROTEIN)

Control Set

VLDLP (Very low density lipoprotein) of control set have 30.66 mg/dl on average. (Table-18, Fig. 18).

Treated group

Very low density lipoprotein after intoxication of mancozeb+malathion pesticide at 24 hours have an average of 35.33 mg/dl, while after 48 hours an average of 38.65 mg/dl, while after 72 hours an average of 41.30 mg/dl and after 96 hours an average of 44.50 mg/dl.

The increase in very low density lipoprotein with exposure to mancozeb+malathion is significant after treatment at various levels shown in respective table. (Table-18, Fig. 18).

Table 1a: *Channa punctatus* mortality percentage after 96 hours of treatment with mancozeb+malathion

Set	Concentration (µg/L)	Fishes Numbers	Time of Exposure (hrs)	Number of Mortality	mortality percentage
1	10	6	96	1	16.66%
2	20	6	96	3	50.00%
3	30	6	96	4	66.66%
4	40	6	96	5	83.33%

Table 1b: Determination of LC_{50} of for *Channa punctatus* using probit analysis with different concentrations mancozeb+malathion

Sets	**Conc. (µg/L)**	**No. of fishes 'N'**	**% mor.**	**Log conc. 'x'**	**Empirical probit**	**Expected probit 'Y'**	**Working probit 'y'**	**Weighting coefficient 'n'**	**Weight w = nxN**	**wx**	**wy**	**wxy**	**wx^2**	**wy^2**
1	10	6	16.66	1.00	4.05	4.08	3.010	0.471	2.82	2.82	8.4882	8.4882	2.82	25.54
2	20	6	50.00	1.30	5.44	4.55	4.151	0.581	3.48	4.524	14.44548	18.77	5.88	59.96
3	30	6	66.66	1.47	5.75	5.49	5.125	0.581	3.48	5.1156	17.835	26.21	7.51	91.40
4	40	6	83.33	1.60	5.95	5.90	5.953	0.471	2.82	4.512	16.78746	26.85	7.21	99.93
									∑W = 12.60	∑WX=16.97	∑Wy= 57.55	∑WXy= 80.34	$\sum WX^2$=23.44	$\sum Wy^2$= 276.85

Table 1c: LC_{50} value and regression equation for LC_{50} of mancozeb+malathion to *Channa punctatus*

Fish to be experimented	**Compound**	**Regression equation**	**LC_{50}µg/L**	**Variance**	**Fiducial limits**
Channa punctatus	Mancozeb + malathion	Y = 4.55+4.86 (X-1.33)	26.50	0.0004	m_1 = (+) 1.4421 m_2 = (-) 1.4348

Fig. 1: Regression line for LC_{50} of mancozeb+malathion to *Channa punctatus*

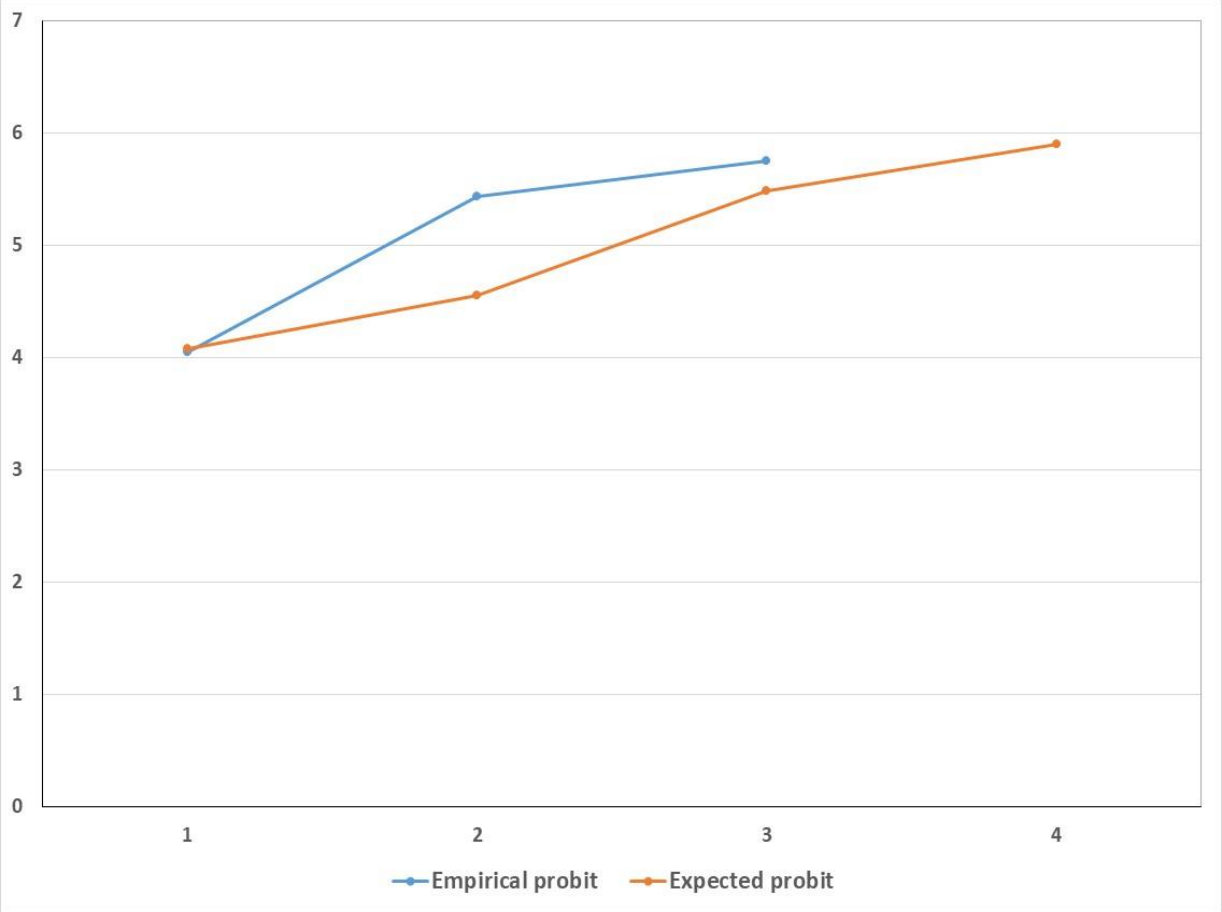


Table 2: Total erythrocyte count (million/mm^3) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

TEC	Control	Exposure Hours			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	3.65	3.20	2.55	2.35	2.10
±S.Em.	±0.10	±0.11	±0.12	±0.15	±0.18
Level of significance l	-	p< 0.05	p< 0.01	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Fig. 2: Total erythrocyte count (million/mm^3) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

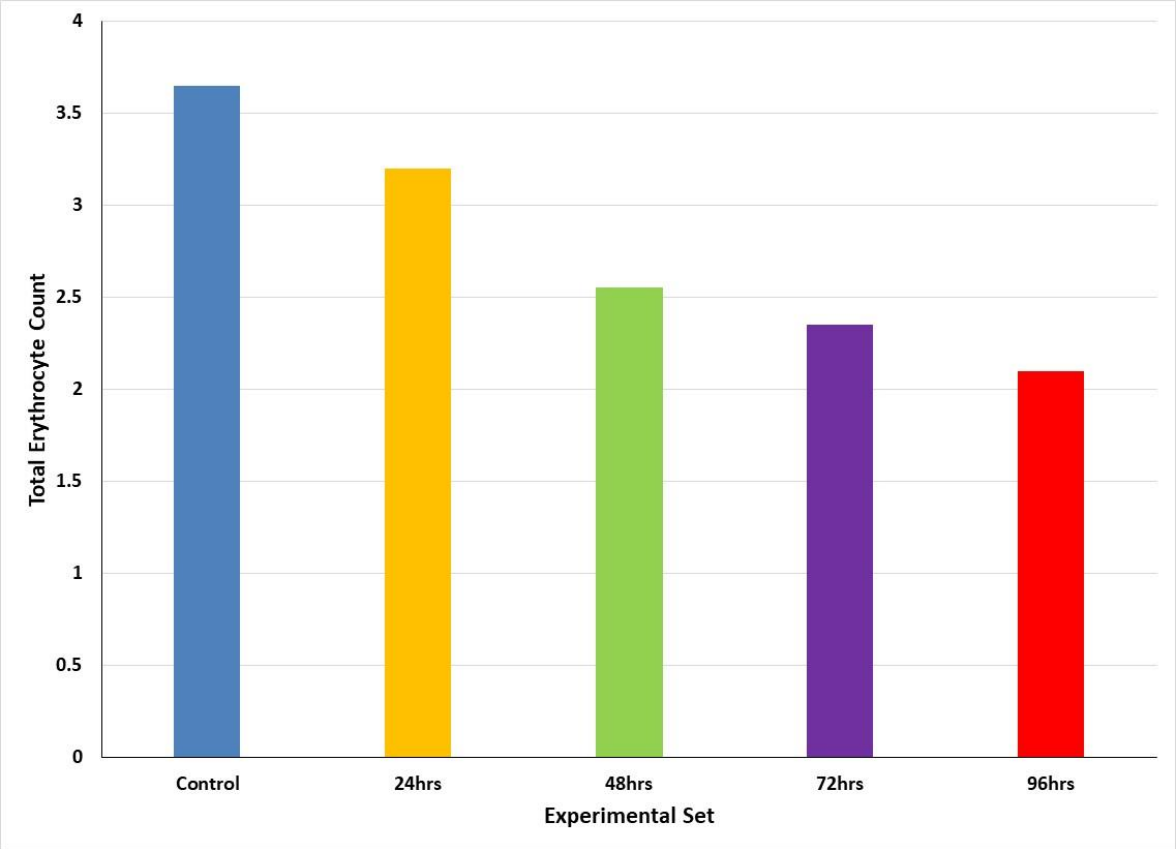


Table 3: Total leucocyte count (TLC) (cells/mm^3) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

TLC	Control	Exposure Hours			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	8500	8800	9500	9810	9980
±S.Em.	±32.10	±55.50	±50.15	±58.90	±55.20
Level of significance	-	p> 0.05	p< 0.05	p< 0.01	p< 0.01

S.Em. = Standard error of mean

Fig. 3: Total leucocyte count (TLC) (cells/mm^3) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

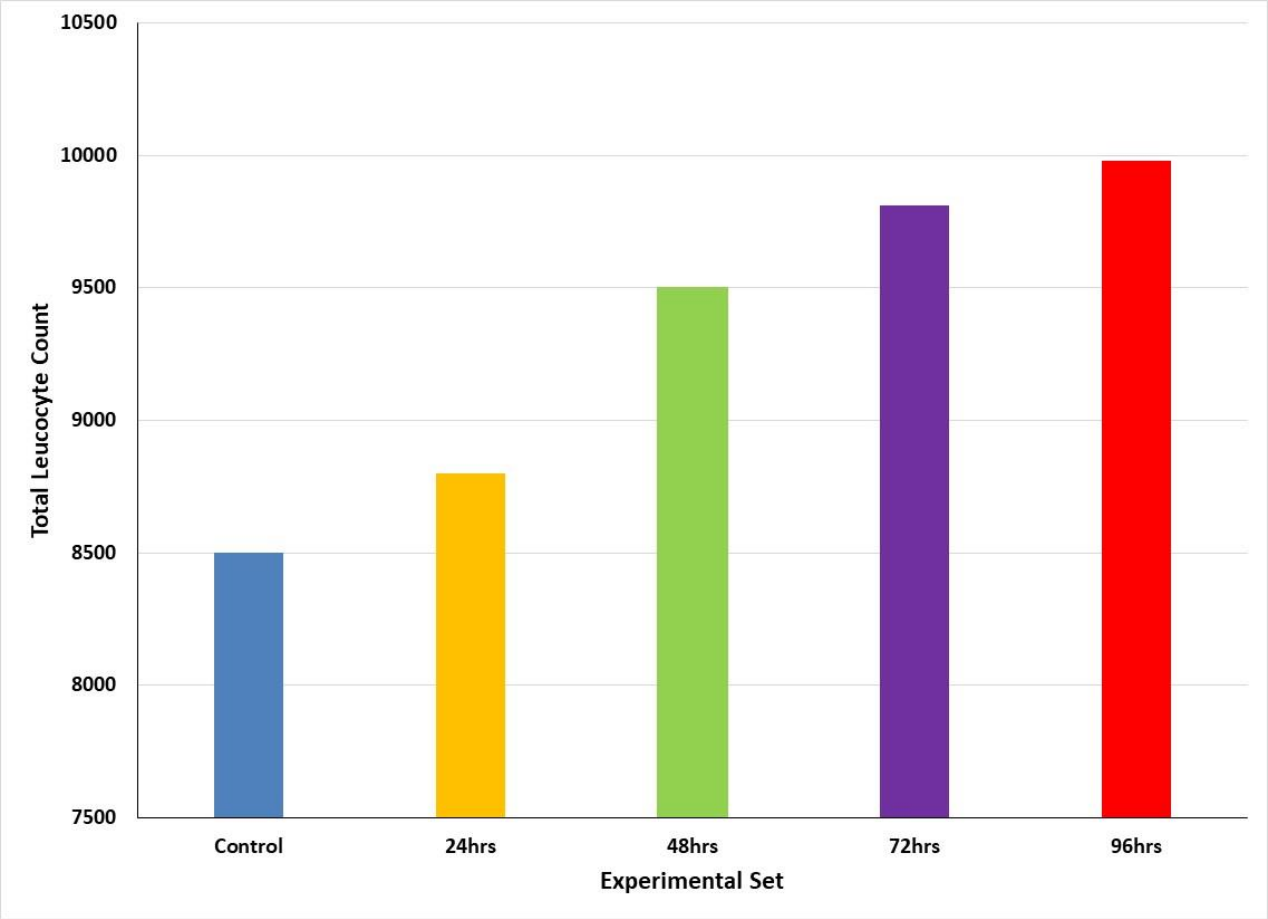


Table 4: Haemoglobin concentration (g/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

Hb Conc.	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	12.8	11.50	10.20	9.35	8.80
±S.Em.	±0.21	±0.32	±0.38	±0.20	±0.28
Level of significance	-	P< 0.05	p< 0.05	p< 0.001	p< 0.001

S.Em. = Standard error of mean

Fig. 4: Haemoglobin concentration (g/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

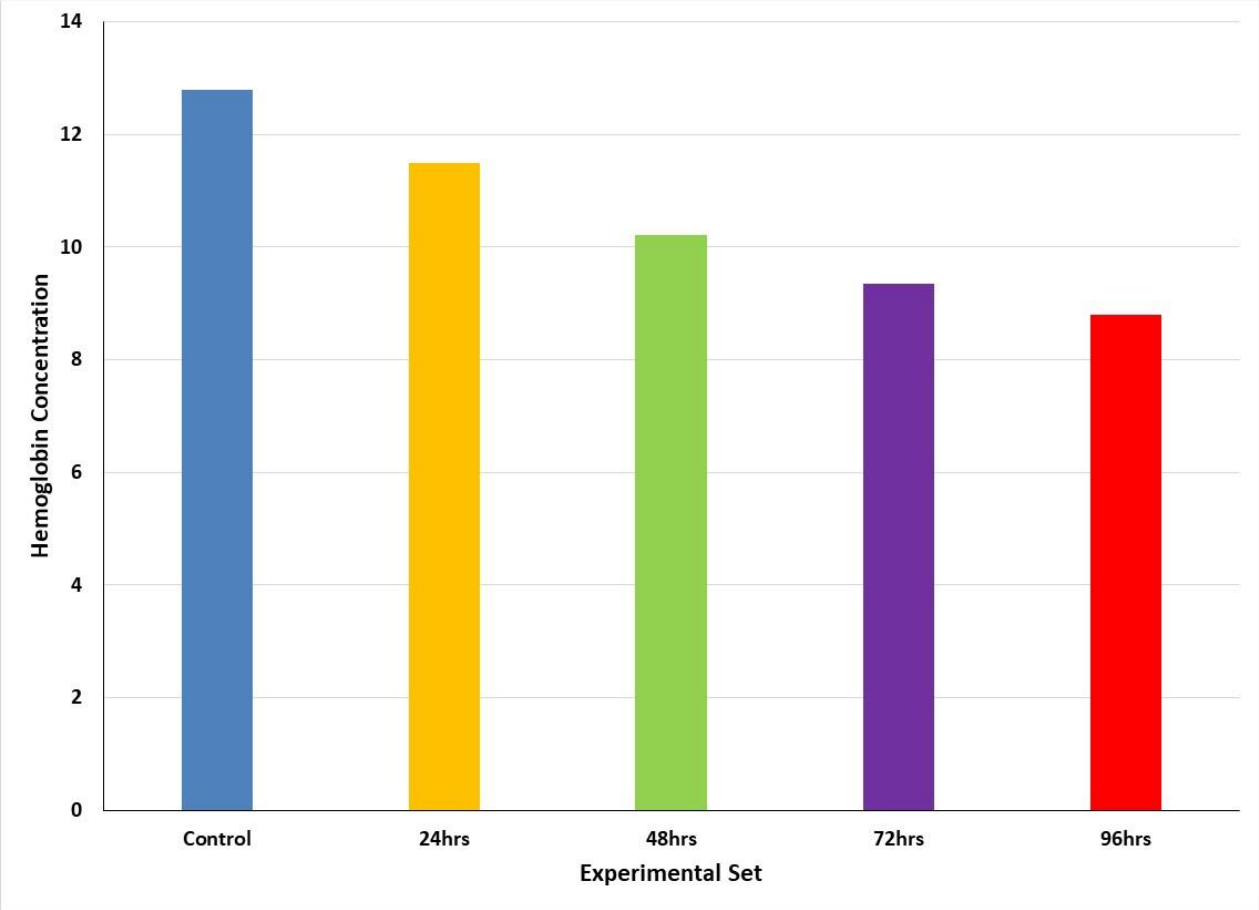


Table 5: Packed cell volume (%) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

PCV	**Control**	**Exposure Hours**			
		24 hours	**48 hours**	**72 hours**	**96 hours**
Mean	45.45	40.50	34.40	30.10	28.50
±S.Em.	±0.25	±0.30	±0.34	±0.66	±0.33
Level of Significance	-	P< 0.05	p< 0.05	p< 0.001	p< 0.001

S.Em. = Standard error of mean

Fig. 5: Packed cell volume (%) in *Channa punctatus* after sub-lethal mancozeb+malathion intoxication

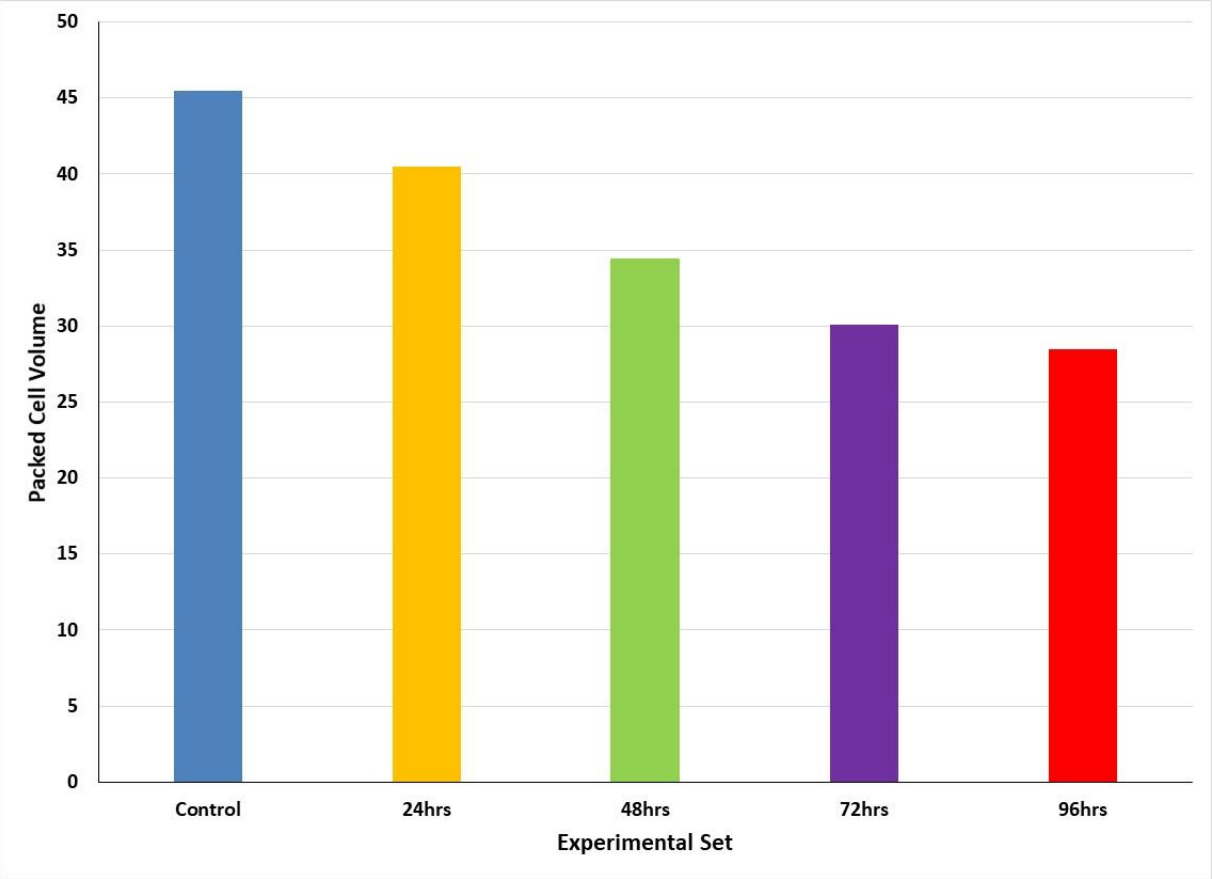


Table 6: Erythrocyte sedimentation rate (mm/hr) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

ESR	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	2.66	2.77	3.35	3.90	4.38
±S.Em.	±0.33	±0.67	±0.33	±0.65	±0.25
Significance level	-	P< 0.05	p< 0.01	p< 0.001	p< 0.001

S.Em. = Standard error of mean

Fig. 6: Erythrocyte sedimentation rate (mm/hr) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

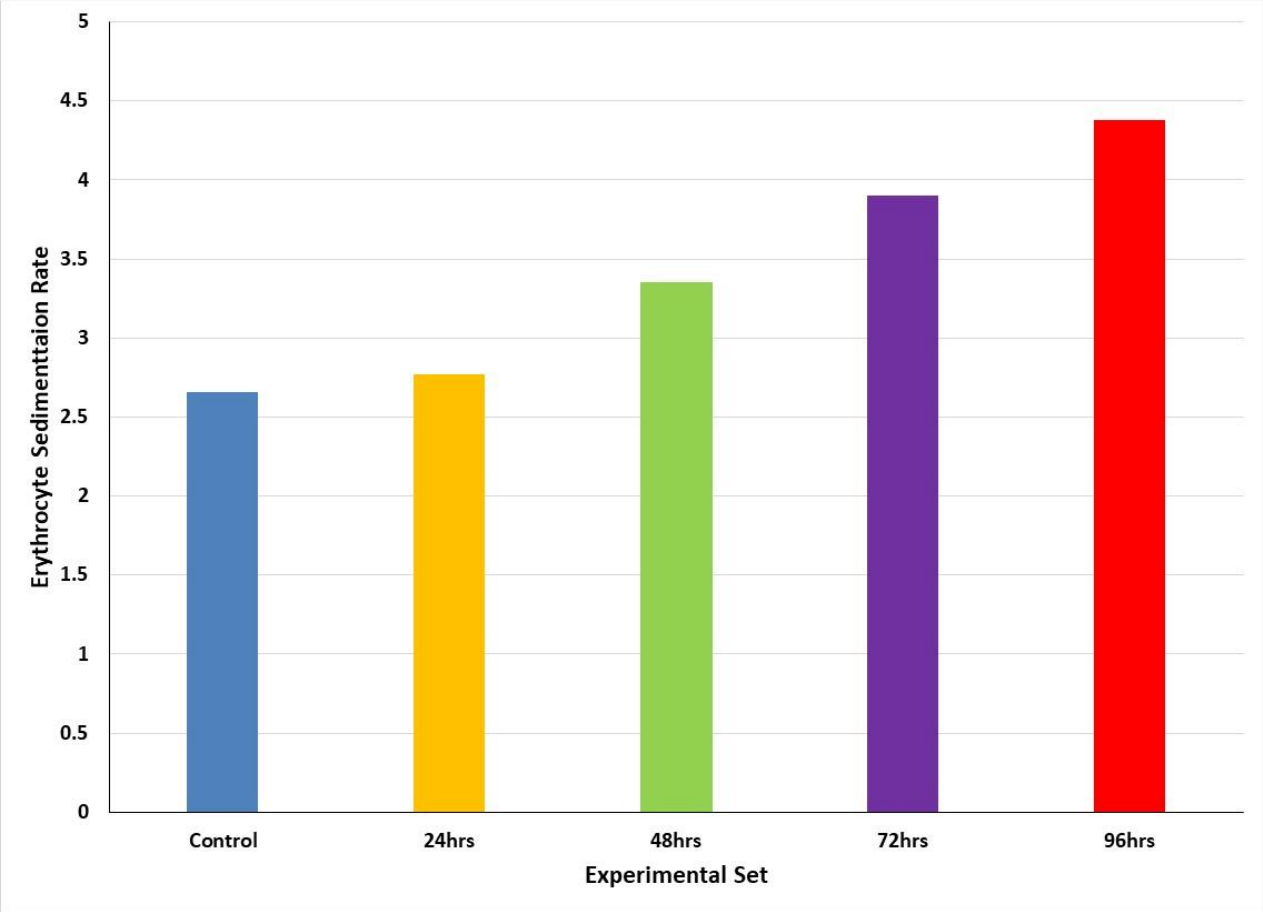


Table 7: Mean corpuscular volume (fl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

MCV	**Control**	**Exposure Hours**			
		24 hours	**48 hours**	**72 hours**	**96 hours**
Mean	30.35	28.35	25.20	21.35	19.28
±S.Em.	±0.18	±0.20	±0.22	±0.15	±0.20
Level of Significance	-	P> 0.05	p< 0.05	p< 0.05	p< 0.01

S.Em. = Standard error of mean

Fig. 7: Mean corpuscular volume (fl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

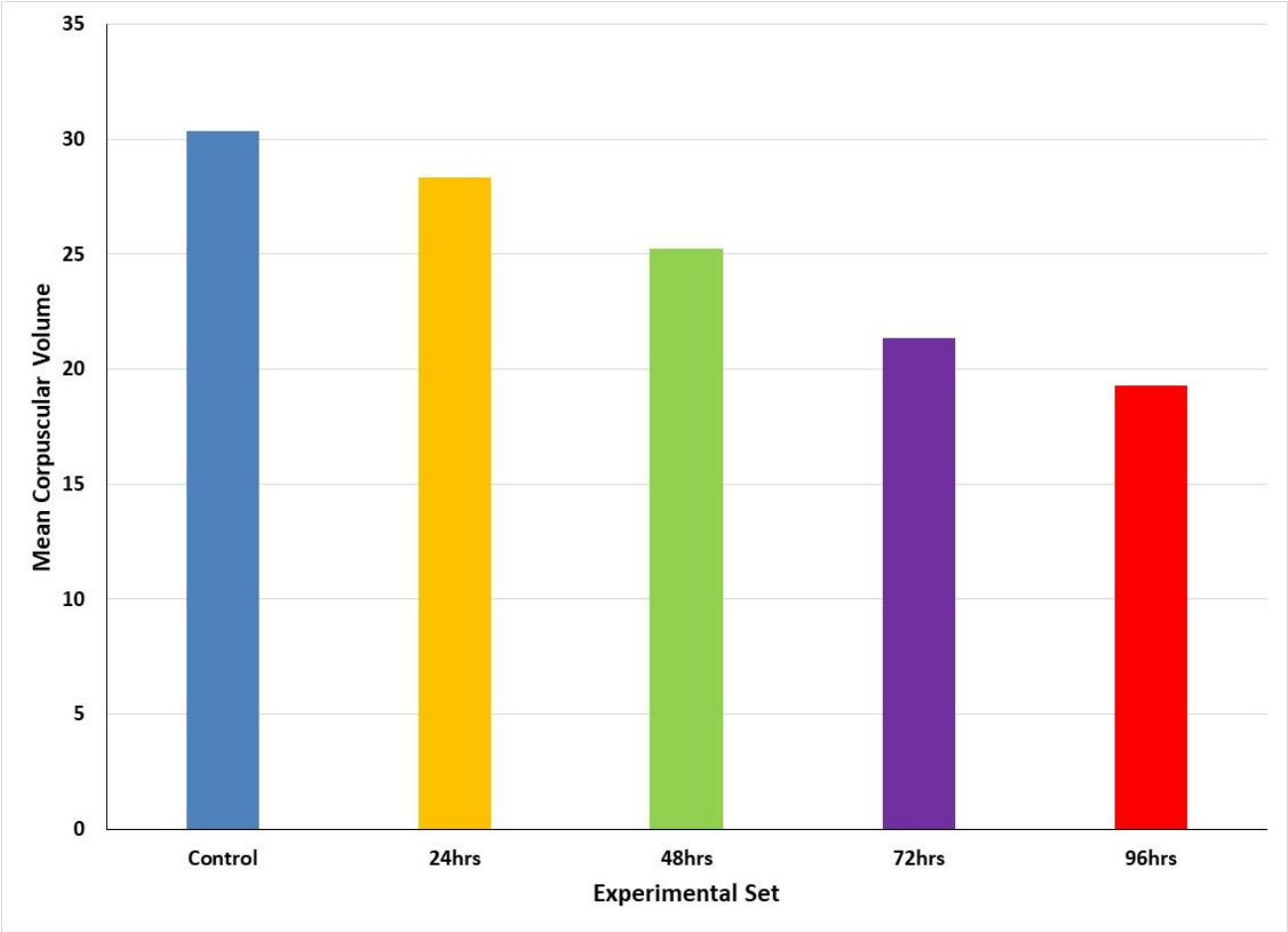


Table 8: Mean corpuscular hemoglobin (pg) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

MCH	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	25.50	22.50	20.67	19.33	17.24
±S.Em.	±0.67	±0.37	±0.25	±0.21	±0.32
level of significance	-	P> 0.05	p< 0.05	p< 0.05	p< 0.01

S.Em. = Standard error of mean

Fig. 8: Mean corpuscular hemoglobin (pg) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

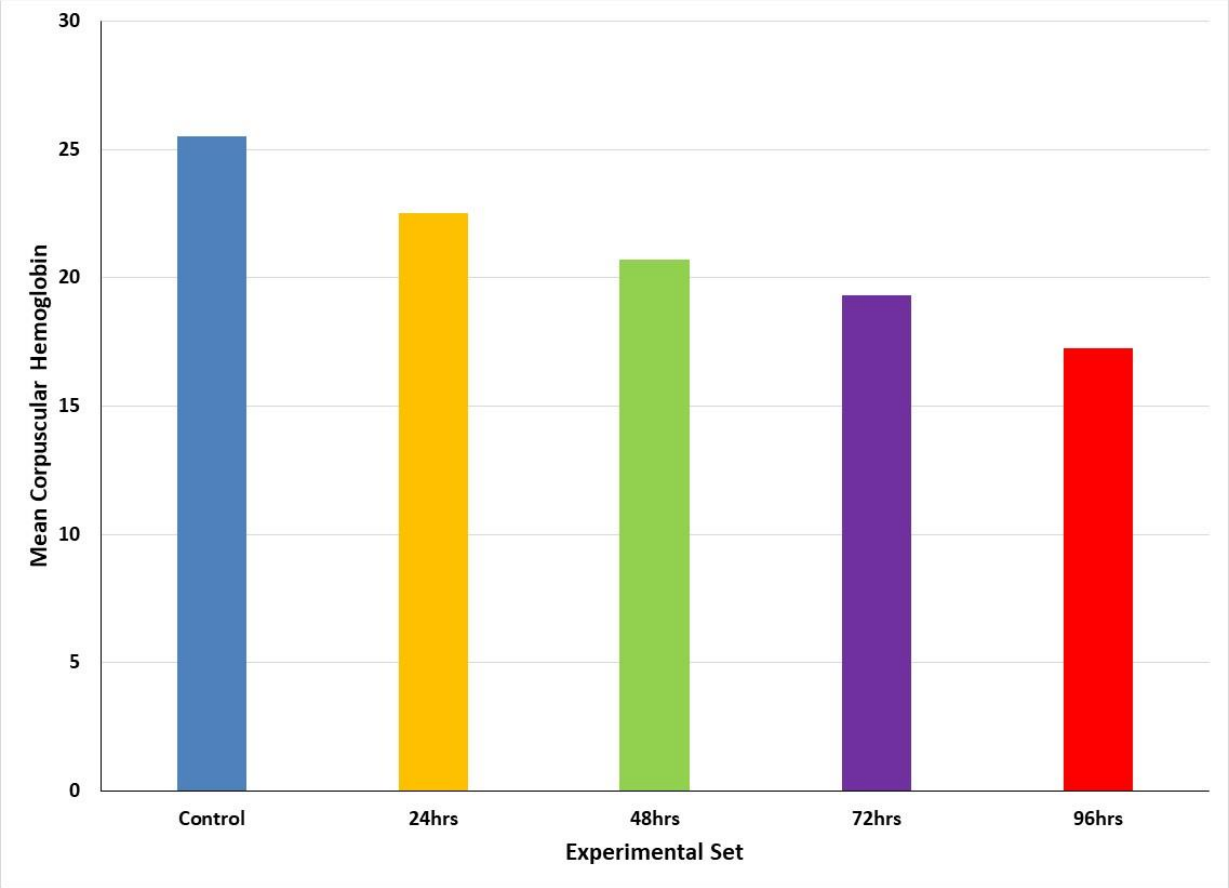


Table 9: After sub-lethal mancozeb+malathionintoxication, mean corpuscular haemoglobin content (mg/dl) in *Channa punctatus*

MCHC	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	20.50	18.33	15.35	12.10	10.18
±S.Em.	±0.23	±0.15	±0.18	±0.27	±0.10
Significance level	-	P> 0.05	p< 0.05	p< 0.001	p< 0.001

S.Em. = Standard error of mean

Fig. 9: After sub-lethal mancozeb+malathionintoxication, mean corpuscular haemoglobin content (mg/dl) in *Channa punctatus*

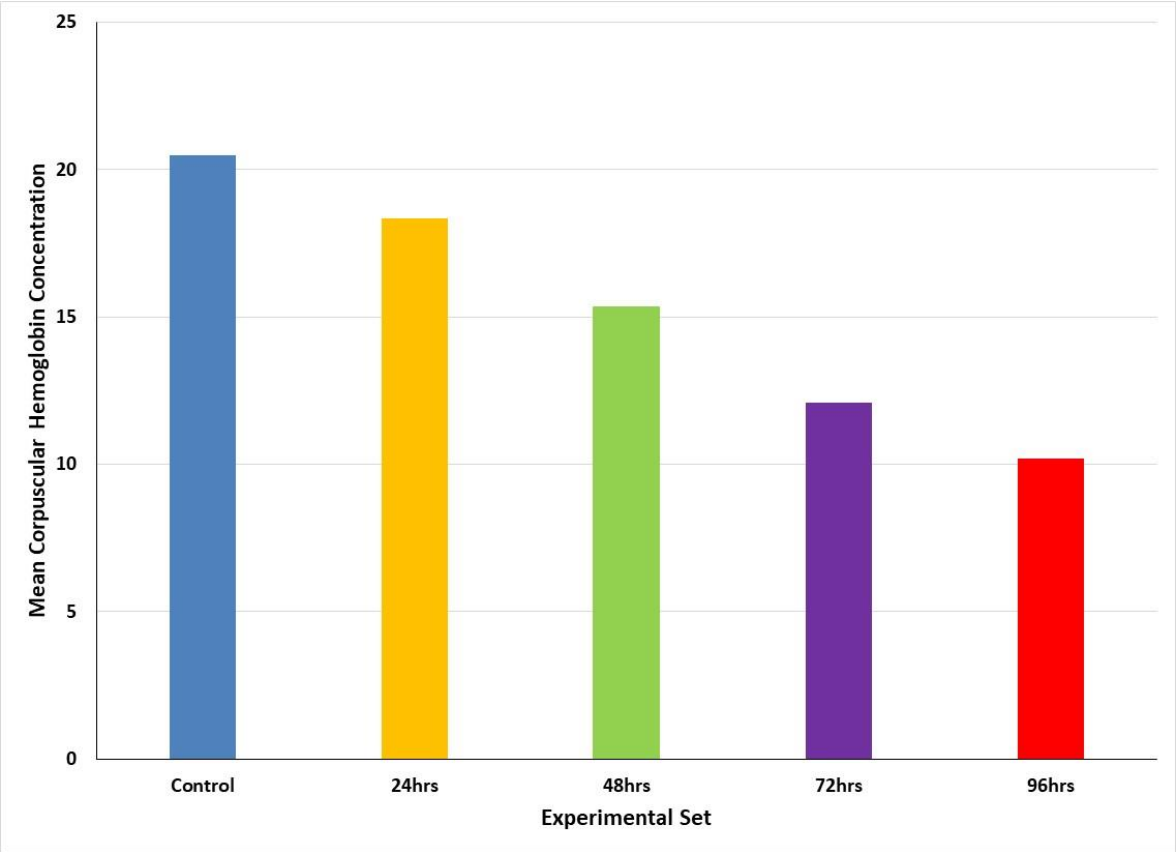


Table 10: Total protein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

Total protein	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	84.50	72.50	65.70	60.10	57.75
±S.Em.	±0.30	±0.62	±0.38	±0.60	±0.28
Level of Significance	-	P< 0.05	p< 0.05	p< 0.01	p< 0.01

S.Em. = Standard error of mean

Fig. 10: Total protein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

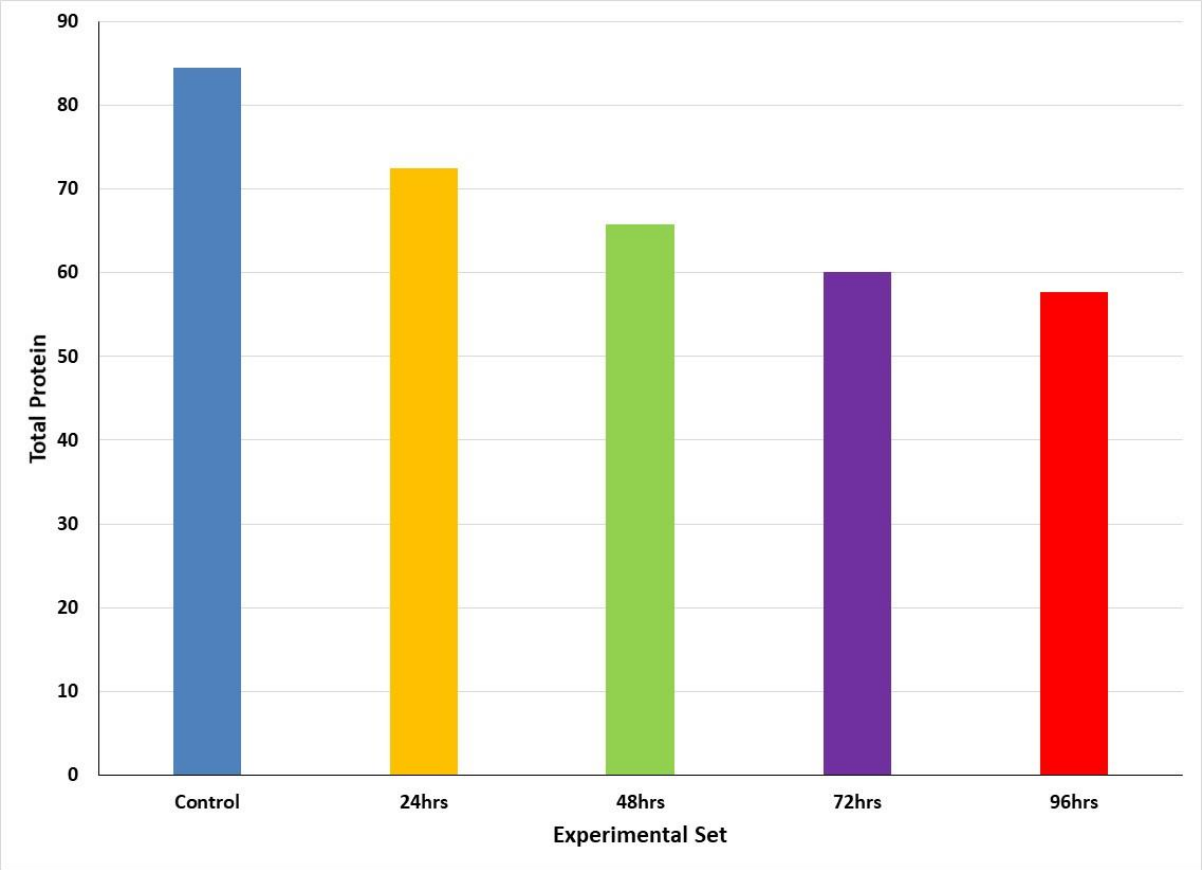


Table 11: Albumin (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

Albumin	**Control**	**Exposure Hours**			
		24 hours	**48 hours**	**72 hours**	**96 hours**
Mean	26.67	20.13	17.15	15.50	13.70
±S.Em.	±0.18	±0.20	±0.13	±0.15	±0.20
Level of Significance	-	P< 0.05	p< 0.01	p< 0.001	p< 0.001

S.Em. = Standard error of mean

Fig. 11: Albumin (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

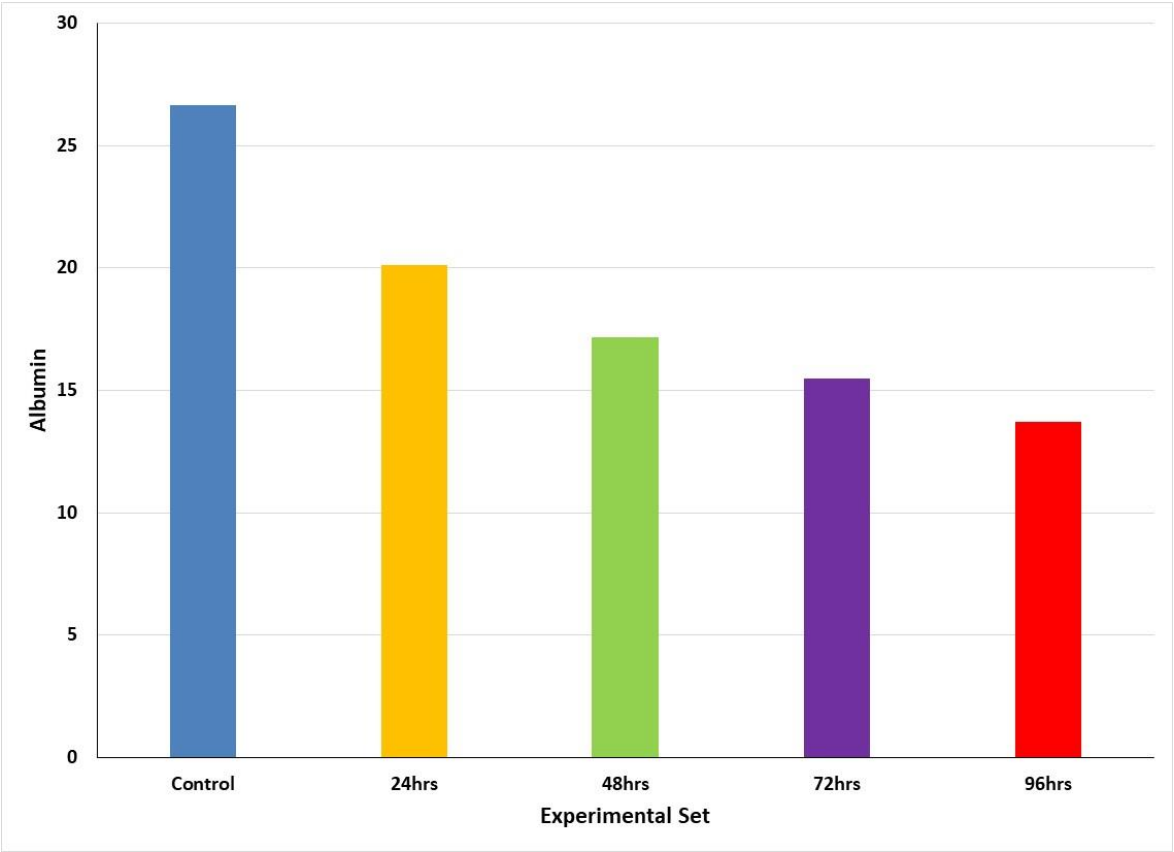


Table 12: Globulin (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

Globulin	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	14.25	12.20	10.10	9.85	8.50
±S.Em.	±0.33	±0.67	±0.33	±0.65	±0.25
Significance level	-	P< 0.05	p< 0.01	p< 0.001	p< 0.001

S.Em. = Standard error of mean

Fig. 12: Globulin (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

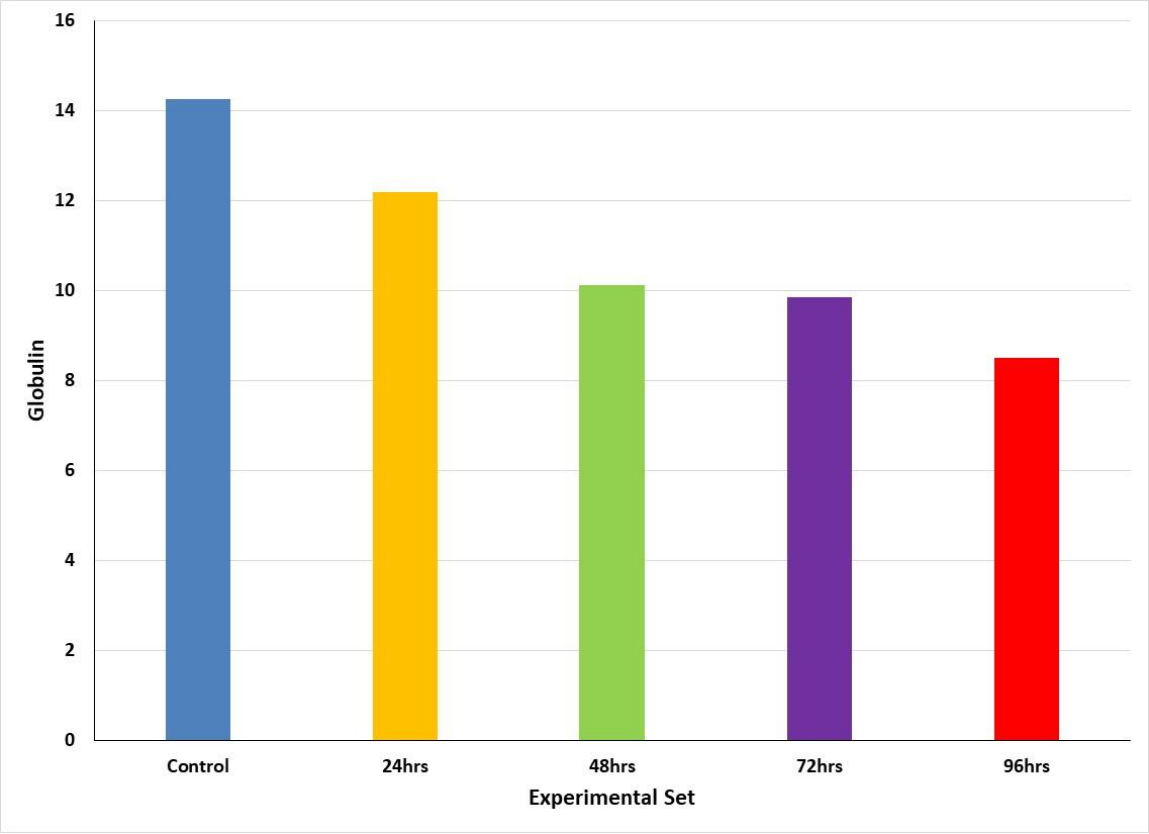


Table 13: Albumin-Globulin Ratio in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

A/G	Control	Exposure Hours			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	1.87	1.64	1.69	1.57	1.61
±S.Em.	±0.10	±0.14	±0.30	±0.33	±0.67
Significance level	-	P< 0.05	p< 0.05	p< 0.01	p< 0.01

S.Em. = Standard error of mean

Fig. 13: Albumin-Globulin Ratio in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

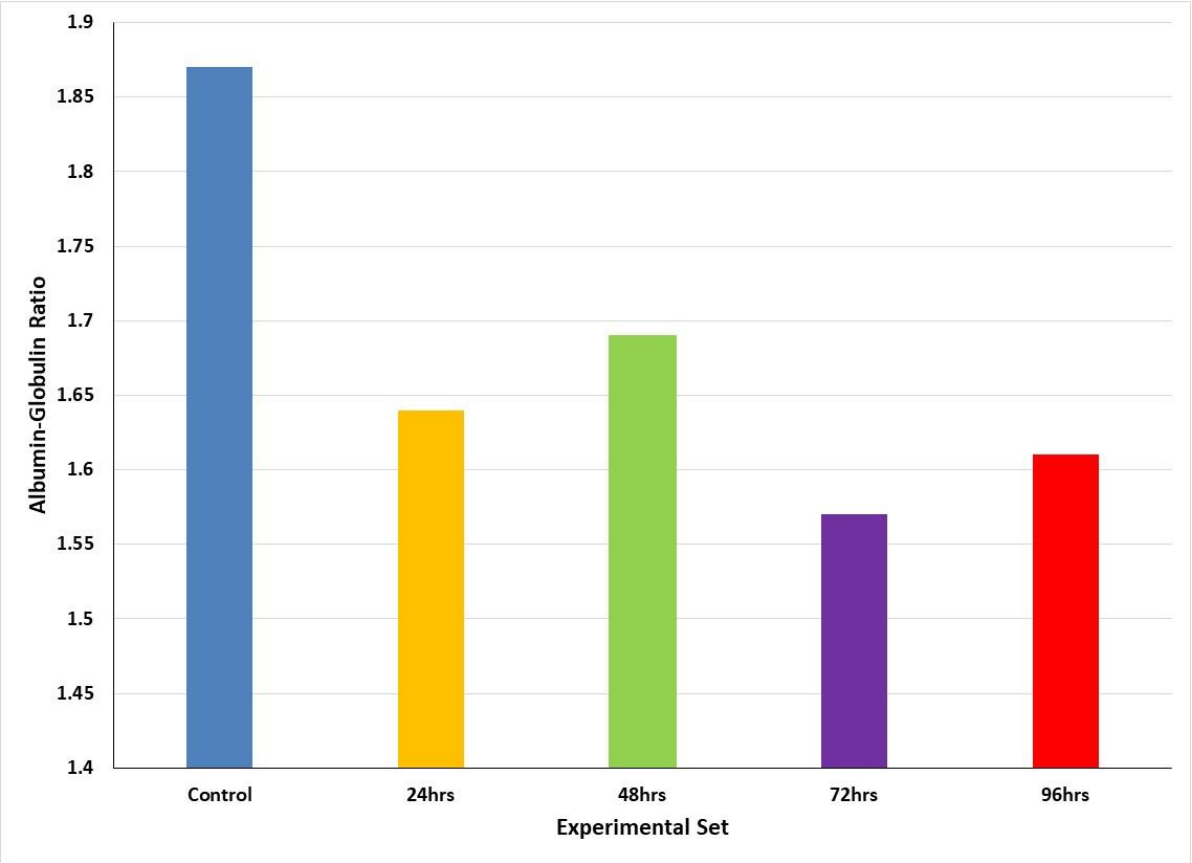


Table 14: Cholesterol (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

Cholesterol	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	155.50	162.40	168.70	175.80	182.50
±S.Em.	±0.55	±0.50	±0.88	±0.95	±0.90
Significance level	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Fig. 14: Cholesterol (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

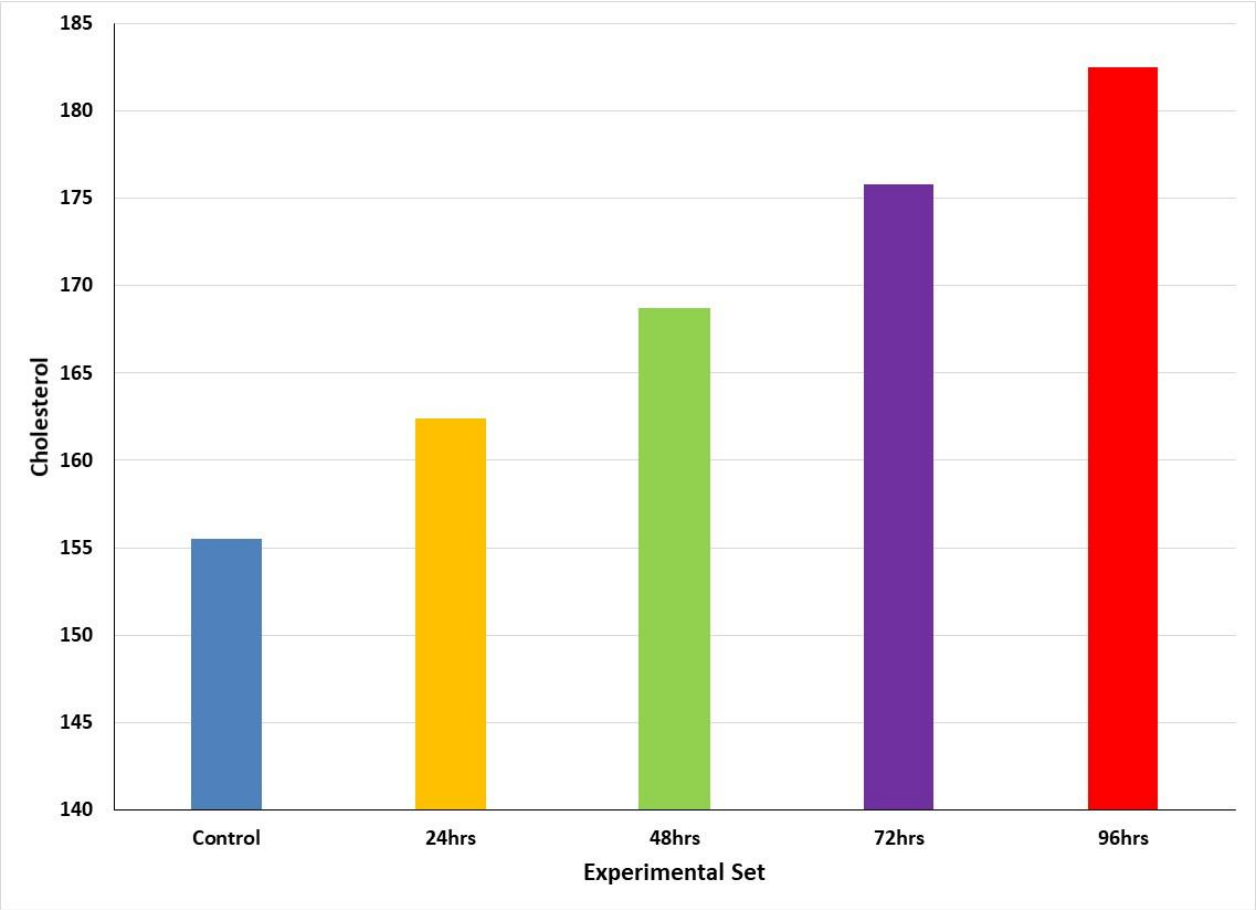


Table 15: Triglyceride (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

TG	Control	Exposure Hours			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	112.50	120.67	128.50	135.45	142.50
±S.Em.	±0.60	±0.50	±0.55	±0.52	±0.48
Level of significance	-	P> 0.05	p< 0.05	p< 0.01	p< 0.01

S.Em. = Standard error of mean

Fig. 15: Triglyceride (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

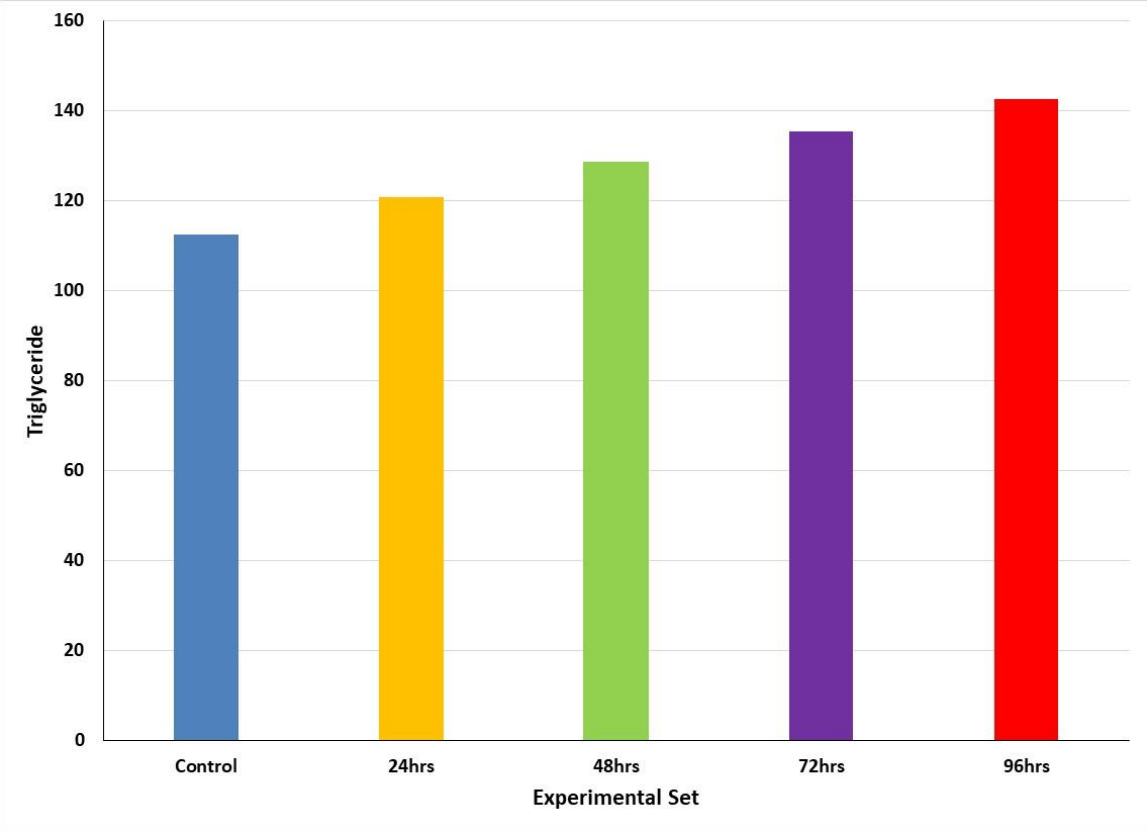


Table 16: High density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

HDL	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	55.67	52.50	46.67	42.30	38.50
±S.Em.	±0.45	±0.37	±0.33	±0.38	±0.28
Significance level	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Fig. 16: High density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

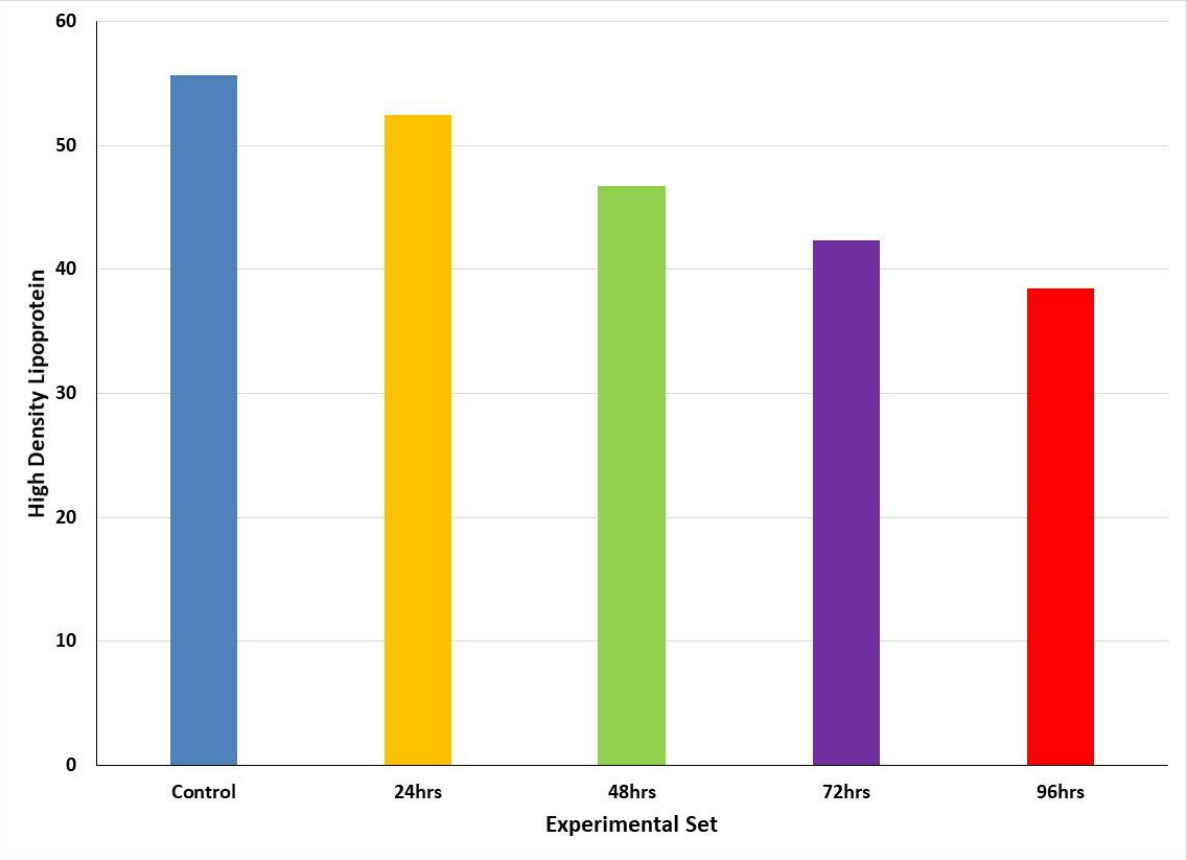
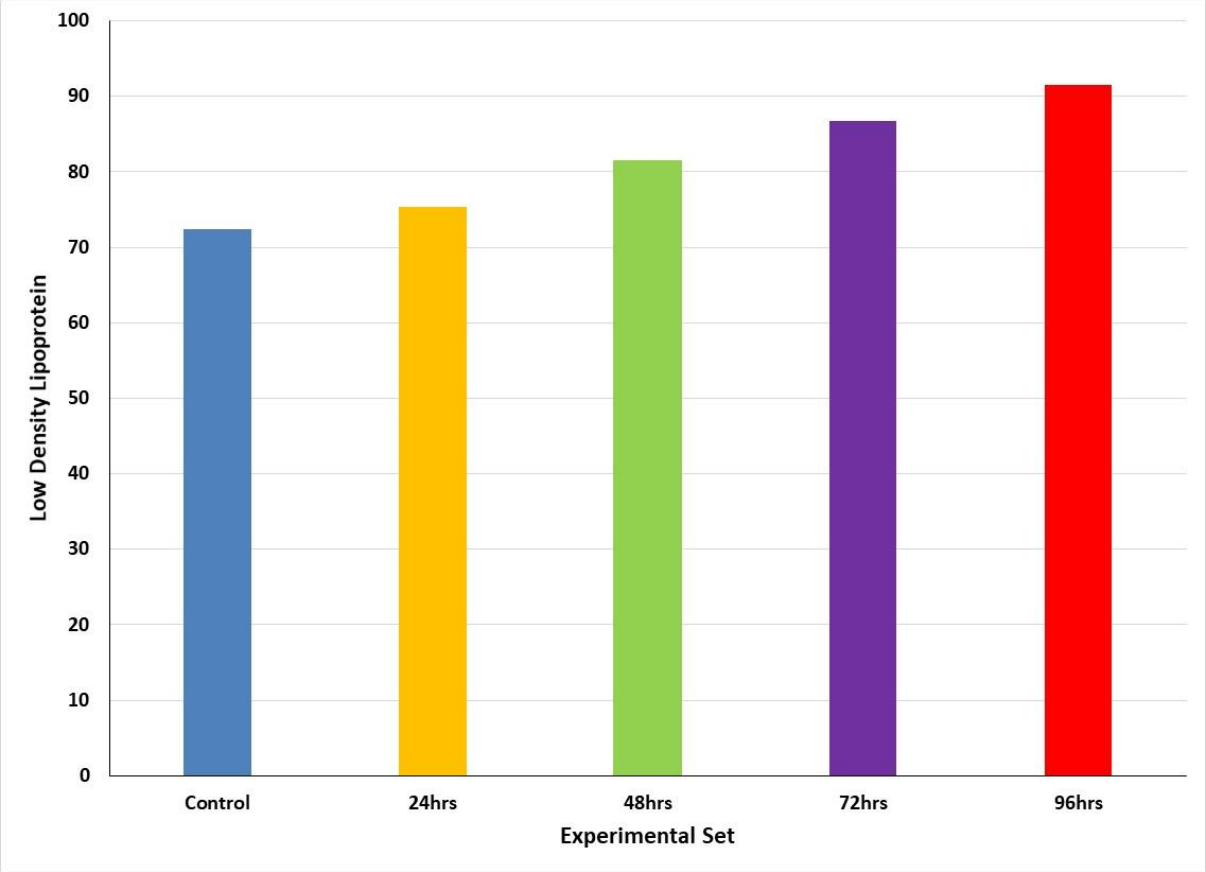


Table 17: Low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

LDL	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	72.40	75.37	81.50	86.70	91.57
±S.Em.	±0.50	±0.45	±0.33	±0.37	±0.62
Level of significance	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Fig. 17: Low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

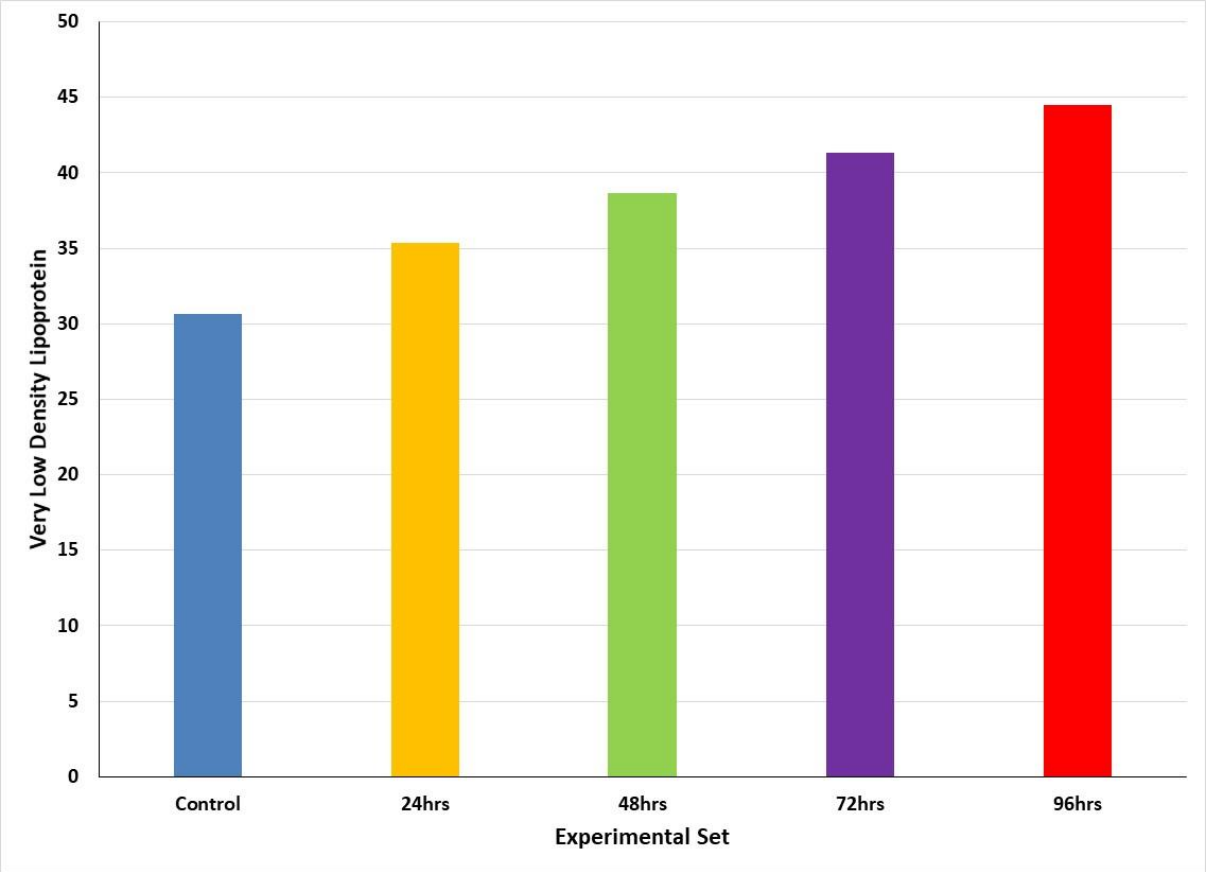


Taable 18: Very low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication

VLDL	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
Mean	30.66	35.33	38.65	41.30	44.50
±S.Em.	±0.18	±0.19	±0.25	±0.33	±0.35
Significance level	-	P> 0.05	p< 0.05	p< 0.05	p< 0.01

S.Em. = Standard error of mean

Fig. 18: Very low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb+malathionintoxication



Result
&
Discussion

RESULT & DISCUSSION

Aquatic pollution has evolved into a global issue that poses a major threat to aquatic organisms' survival. This pollution is because of many reasons of which anthropogenic, agricultural are main causes. Pesticide use if extensive now days in agriculture to enhance production and protect crops from pests. Various categories of pesticides are used in crops. The toxic studies are mainly done on single pesticide while farmers use more than one pesticide in one crop which results in harmful combinations. These combinations go to aquatic system with runoff water and rain. The present study is an effort to observe hematological and biochemical effects of combination of two pesticides viz. mancozeb and malathion.

Among the various aspects of study the bioassay investigation on the fishes were considered of prime importance. Pesticides, such as fungicides and insecticides, are commonly employed in agricultural and in home settings. Their yearly global use is estimated to be between 20,000 and 35,000 tonnes. The organochlorine insecticides, which had been restricted over the world, were replaced by this class of compounds. Organophosphate

pesticides, unlike organochlorine pesticides, do not linger in the environment for lengthy periods of time and do not bioaccumulate to harm us and our environment. However organophosphatesare toxic to non-target wild life the fishes appear to be more sensitive than mammals to the organophosphates (Grue *et al*., 1983).

The natural environment is contaminated with different sorts of anthropogenic pollutants (Jemal *et al*., 2002). Insecticides are considered one among the anthropogenic pollutants for the aquatic environment. They are commonly used in the forestry, veterinary medicine, agriculture and public health in general. Insecticides are valuable in forestry and forestry, but their role in the destruction of the aquatic ecology cannot be overlooked (Basak and Konar, 1977).

Haematological parameters of fishes constitute a crucial biological system for his or her survival against diseases. Insecticidal contamination to aquatic ecosystem can affect haematological parameters of fish (Summarwar, 2012). It was reported that carbofuran caused the reduction in the value of hemoglobin in teleost fishes (Singh and Srivastava, 2010). A significant decrease within the haemoglobin content was observed when the experimental fish (rain-bow trout) was exposed to diazinon (Far *et al*., 2012). A significant decrease in haemoglobin content was considered in *Cyprinus carpio* (Adedeji *et al*., 2009).

In the Nile Tilapia (Orecochromis niloticus) a marked decrease in haemoglobin content was also observed thanks to exposure to dimethoate and malathion within the same species (Sweilum, 2006).

In the present study, decline in total RBCs cout, PCV, Hb concentration, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular Hb concentration has been observed while increase in total leucocyte count and erythrocyte sedimentation rate has been observed after 24hrs, 48hrs, 72hrs and 96hrs treatment of mancozeb+malathion in experimental fish *Channa punctatus*.

Because erythropoiesis is inhibited or in haemopoietic tissue the rate of erythrocyte destruction increases, the total erythrocyte count drops. Hb conc. and PCV value are directly co-related with RBCs count because of the synergistic link among these blood parameters in all vertebrate. Similar to the present findings, Lakshmaiah (2014) observed decrease in total erythrocyte count after intoxication of carbofuran.

Present findings are in agreement with the findings of Svoboda *et al*. (2001) found decline in Hb concentration, packed cell volume and total RBCs count due to decrease in heamopoiesis followed by anaemia induction after exposure to organophosphate pesticide in fish. In support of present findings Chindah *et al*.

(2004) observed the decrease in RBCs count after intoxication of organophosphate in wet land fishes. Similar to the present findings Nithiyanandam *et al*. (2007) showed the reduce in total erythrocyte count, packed cell volume and haemoglobin concentration and this reduction related to haemolysis, haemorrhage and reduced erythropoiesis in fishes after exposure to monocrotophos pesticide (organophosphate). Similarly Banaee *et al*. (2008) observed that decline in (TEC) total erythrocyte count, packed cell volume, Haemoglobin concentration and stated that lower PCV value related to the decrease in number of RBCs which also decrease the amount of space they occupied. Sekhar *et al*. (2011) observed decrease value of total erythrocyte count and haemoglobin content because of the disruptive effect on erythropoietic tissue, which harmed cell viability after intoxication of monocrotophos which is conformity with our present findings. In support of findings, Kaushal (2012) reported the significant decrease in RBC count in *Channa punctatus* (Bloch.) due to haemolysis and blood cell shrinkage caused by the toxic action of malathion, while Lakshmanan *et al*. (2013) observed the reduction in Hb concentration, PCV and total RBCs count,which is similar to present finding. In support of present findings, Ranjeet *et al*. (2013) suggested that decrease in RBC

count due to an appreciable decrease in haemopoiesis leading to different type of anaemia.

Mishra *et al*. (2015) observed the decrease in total erythrocyte count with increase in time of exposure in *Channa punctatus* (Bloch.), while Kulkarni and Bhilave (2015) observed significant decrease in total erythrocyte count and haemoglobin concentration after intoxication of organophosphate which is conformity with our findings. In support of present findings, Shahbazi *et al*. (2015) observed the decrease in Hb concentration, packed cell volume and total RBCs count due to inhibitory impact of pollutants and their metabolites on erythropoiesis and increase in destruction of erythrocytes in haemopoietic organs.

A reduction in TLC was observed in *C. punctatus* after chronic exposure of freshwater teleosts to monotrophos (Singh *et al*., 1992). A significant decline in leucocyte count because of the exposure of *Cyprinus carpio* to toxic environment of diazinon (Banaee *et al*., 2008) was reported. However, a significant rise in leucocyte content was reported in *C. punctatus* due to toxic effects of malathion (Magar and Duve, 2012).

The increase in total leucocytes count is due to protective response of defence mechanism of treated fishes to compensate the pesticidal stress. Similar to the present findings, Lakshmaiah

(2014) observed significant increase in total leucocyte count after intoxication of Carbofuran in *Cyprinus carpio*.

In support of present findings, Nithiyanandam *et al*. (2007) suggested that increase in total leucocyte count in *Cyprinus carpio* due to enhanced release of lymphocytes or lymphopoiesis from lymphomyeloid tissue. Similar to the present findings, Sekhar *et al*. (2010) observed significant increase in white blood cell count indicates a hypersensitivity of leucocyte to monocrotophos and These changes occur as a result of an immune reaction that synthesises antibodies (Abs) in response to pesticide-induced stress, while Kaushal (2012) stated that increase in total leucocyte count due to an adaptive value for the tissue under chemical stress after intoxication of organophosphate in *Channa punctatus* (Bloch.). Lakshmanan *et al*. (2013) observed the increase in total leucocyte count (TLC) which is conformity with present findings, while Kulkarni and Bhilave (2015) reported significant increase in total leucocyte count after intoxication of organophosphate in *Labeo rohita*.

Increase in erythrocyte sedimentation rate is due to decrease in total erythrocyte count. Similar to the present findings, Joshi *et al*. (2002) and Malla *et al*. (2009) recorded the significant raise in erythrocyte sedimentation rate after intoxication of

organophosphate. Ranjeet *et al*. (2013) also observed increase value of erythrocyte sedimentation rate which is conformity with our present findings.

An increase in ESR (mm/hr) has been reported in C. batrachus after exposure to savin (Kumar and Benergee, 1990) and in *Heteropneustes fossilis* when exposed to alachlor and royor (Chaturavedi and Agarwal, 1993). The present study reveals that ESR is correlated negatively with (TEC) total erythrocyte count that is reduce the number of erythrocytes The ESR will be higher. Carbofuran toxicity may disrupt erythropoetic activity in *C. punctatus*. Above all, a sublethal concentration of Carbofuran enhanced the number of leucocytes, lymphocytes, monocytes and neutrophils and dropped the content of haemoglobin in the fresh water fish, *C. punctatus* (Shahi *et al*., 2013).

In the present research, reduction in total no. of proteins, albumin, globulin and albumin-globulin ration has been observed after 24hrs, 48hrs, 72hrs and 96hrs treatment of mancozeb+malathion in experimental fish *Channa punctatus*.

This decline in total content of protein may be due to utilization of protein as it attribute to abnormalities in fat deposit cell of liver following disturbance in the protein metabolism. The high energy demand and cellular damage that occurred in the tissue

of toxicated fish may have contributed to the loss in muscle total protein. Haggag *et al*. (1993) assigned similar reason for the decrease in protein content in toxicated fish. The results are in agreement in Verma and Tonk (1983) observed mercury-exposed fish, *Notopterus notopterus*, have lower muscle protein concentration. The present findings are also in affirmation to the findings of Shukla and Sastry (1988) in *Channa punctatus* after exposure of endosulfan. Singh and Bhati (1994) evaluated the toxic effect of 2,4-D intoxication a herbicide in *Channa punctatus* and noted that the sub lethal concentration changed the nature of *Channa punctatus* and a decline in protein content of liver. Further, Gautam and Gautam (2001) also observed marked decrease in proteins of basic nature in gastro intestinal zone of *Channa punctatus*.

Again, Shrivastava *et al*. (2004) also reported the decrement of liver total protein after mercury toxicity in *Heteropneustes fossilis*. In continuation, a decrement in total protein is also observed by David *et al*. (2003) in malathion toxicity in *Catla catla* and depletion of total protein in *L. rohita* and *C. mrigala* are also observed by Sarkar *et al*. (1993).

In contradictory findings, the restoration of protein damage is done by supplementation of ascorbic acid as it supports growth of

fish reported by Abdel-Tawwab *et al*. (2001) in *Oreochromis niloticus* after supplementation of dietary L-ascorbic acid.

The triglyceride, low density lipoprotein, cholesterol, very low density lipoprotein have been observed to be increased, while a decrease in high density lipoprotein has been observed after 24hrs, 48hrs, 72hrs and 96hrs exposure to mancozeb+malathion in experimental fish *Channa punctatus*. It could be because treated fish use cholesterol and other lipid fractions to combat toxic stress and stabilise toxicant molecules and their secretion in blood increases the serum levels. Further, this could potentially be related to a major obstacle in lipid metabolism which results in accumulation of lipid content in blood. In accordance to the present findings, similar Ghosh (1988), who studied changes in the blood cholesterol in *Channa punctatus* under the influence of Cr (Chromium), reported an elevated lipid profile, Sivaramakrishna and Radhakrishna (1998) in *Cyprinus carpio*, Kahre *et al*. (2000) in *Clarias batrachus* exposed to malathio, Rani *et al*. (2001) in *Tilapia mossambuca*, Radha *et al*. (2005) in *Cyprinus carpio*, Karthikeyan *et al*. (2007) in *Cirrhinus mrigala* and Shankar and Kulkarni (2007) in *Notopterus notopterus*.

These findings are in favour of the explanation of the present work. The changes are due to alteration in enzymes governing lipid, lipoprotein and triglyceride metabolism.

Summary

SUMMARY

The first chapter introduction is about the introductory part and inform about pesticide use and its harmful impact on aquatic system. The second chapter review of literature collects the related recent literature to highlight work of other researchers. In third chapter material and methods of standard laboratory practices has been described. In fourth chapter observations have been summed up in table and graphs with description. In discussion chapter, the findings of present study have been discussed in light of other references and possible reasons for alterations in parameters due to mancozeb and malathion treatment in combination. The summary part is extract of whole thesis with concluding remarks. The bibliography contains all the references used in thesis.

Pesticide use if extensive now days in agriculture to enhance production and protect crops from pests. Various categories of pesticides are used in crops. The toxic studies are mainly done on single pesticide while farmers use more than one pesticide in one crop which results in harmful combinations. These combinations go to aquatic system with run off water and rain. The present study is

an effort to observe hematological and biochemical effects of combination of two pesticides viz. mancozeb and malathion.

Among the various aspects of study the bioassay investigation on the fishes were considered of prime importance. Pesticides, such as fungicides and insecticides, are extensively applied in agricultural and in domestic settings. Their annual consumption is estimated to be between 20,000 and 35,000 tonnes worldwide. This category of chemicals replaced the organochlorine insecticides which have been banned around the world. In contrast to organochlorine pesticides, organophosphates they do not stay in the environment for lengthy periods of time and do not bioaccumulate in such a way that they can affect us and our surroundings. However organophosphates are toxic to non-target wild life.

The natural environment is contaminated with different sorts of anthropogenic pollutants. Insecticides are considered one among the anthropogenic pollutants for the aquatic environment. They are widely employed in agriculture, forestry, public health, and veterinary medicine in general. Insecticides are valuable in forestry and agriculture, but its role in the destruction of the aquatic ecology cannot be unseen.

Haematological parameters of fishes constitute a crucial biological system for his or her survival against diseases.

Insecticidal contamination to aquatic ecosystem can affect haematological parameters of fish.

In the present study, decrease in total erythrocyte cout, PCV, Hb concentration, mean corpuscular hemoglobin concentration, mean corpuscular volume and mean corpuscular Hb has been observed while increase in total leucocyte count and erythrocyte sedimentation rate has been observed after 24hrs, 48hrs, 72hrs and 96hrs treatment of mancozeb+malathion in experimental fish *Channa punctatus*.

Because erythropoiesis is inhibited or the rate of RBCs damage in haemopoietic tissue increases, the total erythrocyte count falls. Hb conc. and PCV value are directly co-related with RBCs count because of the synergistic link among these blood parameters in all vertebrate. Increase in erythrocyte sedimentation rate is due to decrease in total erythrocyte count.

In the present study, reduction in total protein, albumin, globulin and albumin-globulin ration has been observed after 24hrs, 48hrs, 72hrs and 96hrs treatment of mancozeb+malathion in experimental fish *Channa punctatus*.

This decline in total protein content may be due to utilization of protein as it attribute to abnormalities in fat deposit cell of liver following disturbance in the protein metabolism. The high energy

demand and cellular destruction that happened in the tissue of toxicated fish may be responsible for the decrease in muscle total protein.

The triglyceride, low density lipoprotein, cholesterol, very low density lipoprotein have been observed to be increased, while a decrease in high density lipoprotein has been observed after 24hrs, 48hrs, 72hrs and 96hrs exposure to mancozeb+malathion in experimental fish *Channa punctatus*. It could be because treated fish use cholesterol and other lipid fractions to counteract toxic stress and stabilise toxicant molecules, and their release in the blood raises serum levels. This could potentially be due to a halt in lipid metabolism, resulting in a buildup of lipids in the blood.

Finally, the main outcome of present study is that the use of pesticide is very harmful to aquatic systems specially when used in combination because they act as synergist and enhance the toxicity manifolds. The farmers should be educated to minimize the use of synthetic pesticides and increase the use of natural and organic pesticides for farming and whenever needed, avoid eth use of multiple pesticides in crops.

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IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA SHORT COMMUNICATION

DOI: .10.15515/iaast.0976-4828.10.2.236238

Combined Effect of Mancozeb and Malathion Hematological Parameters in *Channa Punctatus*

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ABSTRACT
Channa punctatus Bloch (Actinopterygii: Channidae), one of the most common edible fish, if exposed to pesticides, maybe a serious threat to human health. In the present study, an attempt was made to understand the effect of combined efficacy of Mancozeb and Malathion on hematological profiles of Channa punctatus after exposure to 96 hours. In the present study, decrease in total erythrocyte cout, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration has been observed while increase in total leucocyte count and erythrocyte sedimentation rate has been observed after 24hrs, 48hrs, 72hrs and 96hrs treatment of mancozeb+malathion in experimental fish Channa punctatus. Thus present study concludes that the estimation of the hematological profile of fish will certainly detect early signs of stress physiology concerning their habitat.
***Key words**: Channa punctatus, Mancozeb, Malathion, Hematology*

Received 14.04.2019 Revised 27.04.2019 Accepted 25.05.2019

CITATION OF THIS ARTICLE
A Singh and R Babu. Combined Effect of Mancozeb and Malathion Hematological Parameters in *Channa punctatus*. Int. Arch. App. Sci. Technol; Vol 10 [2] June 2019: 236-238

INTRODUCTION

The overall impact of above activities related to pesticides and insecticides is contamination of aquatic bodies adversely. This affect ecosystem at every trophic level the pesticide can accumulate in aquatic organisms or directly kill them and destroy balance of ecosystem. The past work on this phenomenon is done in vast aspects. Water pollution is major issue form the last many decades. it's far more effective in rivers and water bodies almost dense cities [1]..

The contamination of water with a good range of pollutants has become a matter of great concern over the previous couple of decades, not only due to the threat to public water supplies, but also with the damage caused to the aquatic life. Pollution may be a serious matter for the planet. Because many water resources are polluted thanks to different quite pollutants. The power to predict the impact of commercial waste water and municipal sewage discharge during a particular ecosystem would undoubtedly be enormously useful within the area of escalating industrialization [6]. Hence, the present investigation is aimed to study the effect of sublethal concentrations of Mancozeb and Malathion on the hametaological parameters of *Channa punctatus*.

MATERIAL AND METHODS

PROCUREMENT OF TEST FISH.

Healthy specimens of snake-headed fish, Channa punctatus Bloch (Actinoptrygii: Channidae) with bodyweight 45±5 g and body size 12±5 cm, were collected from a local fish

farm Lucknowr (Uttar Pradesh), India, and were transported to the laboratory. The fishes were carefully examined for any injury and then kept in 1 % solution of KMnO4 for few hours to get rid of dermal infection. These were further kept in a large plastic jar containing 50 L of clean tap water and acclimatized for 15 days to the laboratory conditions. During these periods, the fishes were fed on boiled egg yolk and commercial fish food.

ANALYSIS OF LC_{50}:

LC_{50} value of mancozeb+malathion was 27.28mg/25L with variance 0.0003, fiducial limits 1.4416(+) and 1.4352(-) and regression equation Y = 4.56+4.85 (X-1.34) for the fish *Channa punctatus* (Bloch.). The sublethal concentration is $1/10^{th}$ of LC_{50} i.e. 2.728mg/25L [2].

EXPERIMENTATION:

The experiment was conducted in five aquariums one was used for control and rest are used for pollution study. Each aquarium contains 10 fishes, which were exposed to sub lethal concentration of mancozeb and malathion in combination at different time interval (24, 48, 72 and 96 hour). The sub lethal concentration was selected on the basis of LC_{5o} value.

COLLECTION OF BLOOD:

The blood samples were collected from live fishes through a cardiac puncture in both experimental and control groups at 24, 48, 72, and 96 hours exposures. These were allowed to stand for some time and, after that, centrifuged at 3500 rpm for 10 min to obtain serum.

HAEMATOLOGICAL ANALYSIS

Total Erythrocyte Count (TEC): The total erythrocyte count was estimated with the help of improved Standard Neubaur haemocytometer described by Kit Method.

Total erythrocyte count (million/mm^3) = Total number of RBCs counted in five squares ×10,000

HAEMOGLOBIN CONCENTRATION:

The haemoglobin concentration was estimated by the standard Sahli's method. The value of haemoglobin concentration of blood is expressed in g/dl.

TOTAL LEUCOCYTE COUNT (TLC):

The total leucocytes counted with help of improved standard Neubaur chamber haemocytometer.

Total Leucocyte Count (cells/mm^3) = Total number of WBC counted in four square X 100.

STATISTICAL ANALYSIS

The statistical analysis was performed using advanced numerical tools and the data presented in the manuscript as mean ± standard error (S.E.) unless otherwise stated. Student's t-test calculated the statistical significance of the difference between the control and experimental group.

RESULTS AND DISCUSSION

In the present study, decrease in total erythrocyte cout, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration has been observed while increase in total leucocyte count and erythrocyte sedimentation rate has been observed after 24hrs, 48hrs, 72hrs and 96hrs treatment of mancozeb+malathion in experimental fish *Channa punctatus* (Table1-3, Fig. 1-3).

Haematological parameters of fishes constitute a crucial biological system for his or her survival against diseases. Insecticidal contamination to aquatic ecosystem can affect haematological parameters of fish [5]. Total erythrocyte count decreases due to inhibition of erythropoiesis or increase in rate of erythrocyte destruction in haemopoietic tissue. Hb conc. and PCV value are directly co-related with RBCs count because of the synergistic link among these blood parameters in all vertebrate. Similar findings observed decrease in total erythrocyte count after intoxication of carbofuran and increase in total leucocytes count is due to protective response of defence mechanism of treated fishes to compensate the pesticidal stress [5]. Adhikari *et al* [1] who observed effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton); Kavitha *et al* [4] examined the toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major

carp, *Catla catla*; Talas and Gulhan [7] who observed effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*).

Table 1: Total erythrocyte count (million/mm^3) in *Channa punctatus* after sub-lethal mancozeb+malathion (2.728mg/25L) intoxication

TEC	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	3.65	3.20	2.55	2.35	2.10
±S.Em.	±0.10	±0.11	±0.12	±0.15	±0.18
Significance level	-	p< 0.05	p< 0.01	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Table 2: Total leucocyte count (TLC) (cells/mm^3) in *Channa punctatus* after sub-lethal mancozeb+malathion (2.728mg/25L) intoxication

TLC	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	8500	8800	9500	9810	9980
±S.Em.	±32.10	±55.50	±50.15	±58.90	±55.20
Significance level	-	p> 0.05	p< 0.05	p< 0.01	p< 0.01

S.Em. = Standard error of mean

Table 3: Haemoglobin concentration (g/dl) in *Channa punctatus* after sub-lethal mancozeb+malathion (2.728mg/25L) intoxication

Hb Conc.	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	12.8	11.50	10.20	9.35	8.80
±S.Em.	±0.21	±0.32	±0.38	±0.20	±0.28
Significance level	-	P< 0.05	p< 0.05	p< 0.001	p< 0.001

S.Em. = Standard error of mean

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Journal of the Maharaja Sayajirao University of Baroda
ISSN: 0025-0422

EFFECT OF SUBLETHAL TOXICITY OF MANCOZEB AND MALATHION ON HAEMATOLOGICAL PROFILE OF *Channa punctatus (Bloch.)*

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Abstract:
Although, present observations conducted only on the toxic effect of single pesticide combination (mancozeb and malathion), while farmers use more than combinations pesticide in even single crop which turn out to be more harmful to our natural ecosystem or environment. These combinations go to aquatic system with runoff water and rain and affect the life of water bodies. The hematological effects against the combination of two pesticides viz. mancozeb and malathion were observed on edible fish *Channa punctatus* at the interval of 24 hours, 46 hours, 72 hours and 96 hours of treatment and also with untreated. The effect on total erythrocyte count (TEC) significant decrease with increase of exposure to mancozeb+malathion (at 24 hours 3.20, at 48 hours 2.55, at 72 hours 2.35 and at 96 hours 2.10 million/mm^3). However, total leucocyte count (TLC) showed opposite pattern and significantly decreased with increase exposure (at 24 hours 8800, at 48 hours 9500, at 72 hours 9810 and at 96 hours 9980 cells/mm^3). Similarly, erythrocyte sedimentation rate (ESR) also showed decreased pattern with increase exposure (at 24 hours 2.77, at 48 hours 3.35, at 72 hours 3.90 and at 96 hours 4.38 mm/hr). The overall study revealed a significant toxic effect on the hematological observations in *C. punctatus*.
Keywords: Edible fishes, erythrocyte count, leucocyte count, erythrocyte sedimentation rate, toxic.

Introduction:
Pollution is major issue form the last few decades in India. Water pollution affects the rivers, lakes, ponds and water bodies almost in the dense cities. The waste of houses and industries contain various pollutants like detergents, sewage, heavy metals, insecticides etc. Pesticides are a diverse group of compounds with widely varying modes of action which affects metabolism of the body (Cappello *et al.*, 2016). The dangers of poisoning are proportional to the dose, duration of exposure and sensitivity and toxicity. Around the mid-twentieth century, insecticidal use in agriculture exploded. Fungicides are also used in agriculture to keep seed corn free of mycosis. These compounds are then released into neighbouring water bodies, where they are devoured by fish and other aquatic life. Frequent and miss used of pesticide disrupt the natural equilibrium of balanced ecosystem by affecting food chain, community and also to the fishes in water body (Cappello *et al.*, 2016).
Among the various aspects of study the bioassay investigation on the fishes has considered of prime importance. Pesticides, such as fungicides and insecticides, are commonly employed in agricultural and in home settings. Their yearly global use is estimated to be between 20,000 and 35,000 tones. The organochlorine insecticides had been restricted all over the world. Organophosphate pesticides, unlike organochlorine pesticides, do not linger in the environment for lengthy periods of time and do not bioaccumulate to harm us and our environment. However organophosphatesare observed as toxic to non-target wildlife (Chindah *et al.*, 2004).
Fishes are the most sensitive to pollution and also known as indicator of water body (Farombi *et al.*, 2007; Ahmad, 2012). Although, effluents are one of the most important components in organic phenomena, their accumulation becomes hazardous to aquatic organisms. As fish are the simplest source of protein and minerals but they are facing the environmental contamination. The pollutants accumulate in body of fishes and affect their physiology. The injurious effect of certain fungicide on various vitals and their accumulation within the muscles of inhabitants has attracted the eye of variety of workers. Therefore, present experiment designed to study the sublethal toxicity of mancozeb and malathion pesticides on the haematological aspect of fresh water edible fish *i.e.*, *Channa punctatus*.

Materials and methods
To start experiment small sized (16 to 18 cm) freshwater edible *Channa punctatus* (Bloch*)* collected from local source in the month of September and October. They were thoroughly and treated with 0.2 % KMnO4 solution to clear skin infections. Finally, they were kept in a huge glass aquarium for 15 days in a laboratory setting. Thereafter, they were treated with two pesticides mancozeb and malathion separately. After 96 hours of exposure, the median lethal concentration (LC_{50}) was calculated for the population of *C. punctatus* under specific set of testing conditions. The experiment was carried out in different aquariums under laboratory conditions. Among them one utilize as control or untreated and others as treated with sub-lethal doses of mancozeb and malathion in combination at various time interval (24, 48, 72 and 96 hr).
After exposure of pesticides, five fish were collected from each group of control and treated for the investigations. The blood was collected after severing the caudal peduncle of the living fish using a scissor. The collected blood centrifuged for 30 minutes at 2500rpm and to stand in a slanting position for serum collection. Haematological examination was performed on blood samples

that had been treated with the anticoagulant EDTA. Total Erythrocyte Count (TEC) examined by Neubaur Chamber Haemocytometer and Total Leukocyte Count (TLC) by Neubaur Chamber Haemocytometer.

Statistical calculations

For each biochemical parameters a minimum of 50 replicates were done and the data was statistically examined using the student's t test.

Mean (x)

The following formula was used to compute the mean:-

$$\overline{X} = \frac{\Sigma x}{N}$$

Where,

$\sum x$ = Addition of individual observation

N = Number of observations.

Standard Deviation (S.D.)

The S.D. was computed using the given formula-

$$S.D. = \sqrt{\frac{\Sigma(x - \overline{x})^2}{N - 1}}$$

Where,

$\Sigma(x-\overline{x})^2$ = The sum of all deviations' squares.

Standard Error of Mean (S.Em)

The following formula was used to compute the S.E. (standard error) of the mean:-

$$S.Em. = \frac{S.D.}{\sqrt{N}}$$

Where,

N = No. of observations.

S.D. = Standard deviation

Analysis of Variance (ANOVA)

ANOVA was calculated by the following sequential steps.

(i) Sum of Squares (S.S.) -

Total S.S. = ? $(X - X)^2$

If zero in taken as the arbitrary mean, the deviations of the variates from zero will be the variates themselves.

Total S.S. = $(X_1^2 + X_2^2 + ...X_n^2) - \frac{(\Sigma \overline{X})^2}{n}$

Where,

ΣX = Grand total of Variates

n = Number of variates

Between groups sum of squares -

S.S. = $\Sigma(T_a^2 + T_b^2 + ...T_n^2) - \frac{(\Sigma \overline{X})^2}{n}$

Where = $T_a T_b$ ---------- T_n = Group total of Variates.

Within group S.S. = Total S.S. - B/W group S.S.

(ii) Degree of freedom:

Total Degree of freedom = N - 1

N = No. of observation

Degree of freedom b/w groups = K-1

K = No. of groups

Degree of freedom within groups = N - K

Result and discussion

Haematological parameters of fishes constitute a crucial biological system for its survival against diseases. Insecticidal contamination to aquatic ecosystem can affect haematological parameters of fish (Summarwar, 2012). In the present study, the haematotoxic effects of mancozeb and malathion pesticide have been observed after 24, 48, 72 and 96 hours after the intoxication of pesticide as well as in control edible fish *Channa punctatus*.

The observations showed that total erythrocyte count (TEC) was recorded as average of 3.65 million/mm^3 in control set of fishes, and also in treated fishes, a total erythrocyte count after intoxication of mancozeb+malathion pesticide at 24 hours was observed an average of 3.20 million/mm^3, while after 48 hours 2.55 million/mm^3, after 72 hours 2.35 million/mm^3 and after 96 hours 2.10 million/mm^3. The observation clearly showed that a significant decrease pattern in total erythrocyte count recorded with increase of exposure to mancozeb+malathion (Table 1). Due insecticidal activity, erythropoiesis inhibited or in haemopoietic tissue the rate of erythrocyte destruction increases, therefore, total erythrocyte count dropped. Similar to the present findings, Banaee *et al*. (2008) and Lakshmaiah (2014) also observed decrease in total erythrocyte count after intoxication of carbofuran. Moreover, Sekhar (2011) recorded decrease value of total erythrocyte count because of the disruptive effect on erythropoietic tissue, which harmed cell viability after intoxication of monocrotophos which showed conformity with our present findings. The observations of Mishra *et al*. (2015) on decrease of total erythrocyte count with increase in time of exposure in *Channa punctatus* (Bloch.) was also corroborative study. Moreover, Kulkarni and Bhilave (2015) observed significant decrease in total erythrocyte count after intoxication of organophosphate and showed conformity with present findings.

As far as total leucocyte count (TLC) was concern, it was recorded after intoxication of mancozeb+malathion pesticide at 24 hours as an average of 8800 cells/mm^3, after 48 hours 9500 cells/mm^3, after 72 hours 9810 cells/mm^3 and after 96 hours 9980 cells/mm3 with comparison of control which observed as an average of 8500 cells/mm^3 (Table 1). The total leucocyte count (TLC) of *Channa punctatus* increased significantly with increase of exposure to mancozeb+malathion (Table 1). The increase in total leucocytes count may be due to protective response of defense mechanism of treated fishes to compensate the pesticide stress. Similar to the present findings, Lakshmaiah (2014) also observed significant increase in total leucocyte count after intoxication of Carbofuran in *Cyprinus carpio*. In support of present findings, Nithiyanandam *et al*. (2007) also reported increased total leucocyte count in *Cyprinus carpio* due to enhanced release of lymphocytes or lymphopoiesis from lymphomyeloid tissue. Similarly, a significant rise in leucocyte content was reported in *C. punctatus* due to toxic effects of malathion by Magar and Duve (2012) and showed complete agreement to present findings. In contrast, reduction in TLC was observed in *C. punctatus* after chronic exposure of freshwater teleosts to monotrophos by Singh *et al*., (1992). A significant decline in leucocyte count because of the exposure of *Cyprinus carpio* to toxic environment of diazinon was recorded by Banaee *et al*., (2008). Moreover, Shahi *et al*., (2013) reported enhanced number of leucocytes, lymphocytes, monocytes and neutrophils in the freshwater fish, *C. punctatus*.

During the experimentation, erythrocyte sedimentation rate (ESR) was recorded an average of 2.66 mm/hr for control *C. punctatus*. For treated fishes, ESR observed after intoxication of mancozeb+malathion pesticide at 24 hours an average of 2.77 mm/hr, after 48 hours 3.35 mm/hr, after 72 hours 3.90 mm/hr and after 96 hours of 4.38 mm/hr. Similar to TLC, the rate of erythrocyte sedimentation increased significantly with increase of exposure to mancozeb+malathion (Table 1). The present study revealed that erythrocyte sedimentation rate was negatively correlated with total erythrocyte count. Similar to the present findings, Joshi *et al*. (2009), Malla *et al*. (2009) and Ranjeet *et al*. (2013) recorded raise in erythrocyte sedimentation rate after intoxication of organophosphate. An increase in ESR (mm/hr) has also been reported in *C. batrachus* after exposure to savin by Kumar and Benergee (1990), and in *Heteropneustes fossilis* when exposed to alachlor and royor by Chaturavedi and Agarwal (1993) which showed conformity with our present findings.

Table 1: Sublethal toxicity of Mancozeb and malathion on haematological profile of *Channa punctatus* (Bloch.)

Haematological observations	**Control**	**Exposure Hours**			
		24 hrs	**48 hrs**	**72 hrs**	**96 hrs**
TEC (million/mm^3)	3.65±0.10	3.20±0.11	2.55±0.12	2.35±0.15	2.10±0.18
TLC (cells/mm^3)	8500±32.10	8800±55.50	9500±50.15	9810±58.90	9980±55.20
ESR (mm/hr)	2.66±0.33	2.77±0.67	3.35±0.33	3.90±0.65	4.38±0.25
Level of significance	-	$p< 0.05$	$p< 0.01$	$p< 0.01$	$p< 0.001$

TEC = Total erythrocyte count, TLC = Total leucocyte count, ESR = Erythrocyte sedimentation rate, the values given are the means ± standard error of mean

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Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [6] May 2021 : 198-200

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

SHORT COMMUNICATION **OPEN ACCESS**

Assessment of Lipoproteins in *Channa punctatus* under Toxic Stress of Mancozeb and Malathion in Combination

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ABSTRACT
In the present study, an attempt was made to understand the effect of sublethal concentrations of paper mill effluent on lipid profiles of Channa punctatus after exposure to 96 hours. The low density lipoprotein, very low density lipoprotein have been observed to be increased, while a decrease in high density lipoprotein has been observed after 24hrs, 48hrs, 72hrs and 96hrs exposure to mancozeb+malathion in experimental fish Channa punctatus.
Keywords: Channa punctatus, mancozeb+malathion, lipoproteins

Received 12.03.2021 Revised 09.04.2021 Accepted 12.05.2021

INTRODUCTION

Insecticidal use in agriculture gained momentum round the mid twentieth century. Fungicides also are utilized in agriculture for the prevention of mycosis in seed corn. Later these compounds discharge in nearby water bodies and consumed by fishes and other aquatic life. These fat soluble contaminants concentrate within the fat of fishes by bioaccumulation and bio- magnification [1]. The fishes, best indicator of water body pollution, are the foremost sensitive of all the aquatic animals towards the pollutant. The buildup of effluents becomes hazardous to the aquatic organism because they're the foremost important factors of organic phenomenon. The desperate and uncared use of fungicides in agriculture practices has further enhanced the matter to the worldwide importance [5]. Lipids play an important role in the architectural dynamics of the cell and transport mechanism across the cell membrane. Lipids also contribute to energy production as they have high caloric values and play a vital role in the biochemical adaptations of animals to stress conditions [3]. Hence, the present investigation is aimed to study the effect of sublethal concentrations of mancozeb+malathion on the lipoprotein metabolism of Channa punctatus.

MATERIAL AND METHODS

PROCUREMENT OF TEST FISH.

Healthy specimens of snake-headed fish, *Channa punctatus* Bloch (Actinoptrygii: Channidae) with bodyweight 45±5 g and body size 12±5 cm, were collected from a local fish farm Lucknowr (Uttar Pradesh), India, and were transported to the laboratory. The fishes were carefully examined for any injury and then kept in 1 % solution of KMnO4 for few hours to get rid of dermal infection. These were further kept in a large plastic jar containing 50 L of clean tap water and acclimatized for 15 days to the laboratory conditions. During these periods, the fishes were fed on boiled egg yolk and commercial fish food.

ANALYSIS OF LC_{50}:

LC_{50} value of mancozeb+malathion was 27.28mg/25L with variance 0.0003, fiducial limits 1.4416(+) and 1.4352(-) and regression equation Y = 4.56+4.85 (X-1.34) for the fish *Channa punctatus* (Bloch.). The sublethal concentration is $1/10^{th}$ of LC_{50} i.e. 2.728mg/25L [2].

EXPERIMENTATION:

The experiment was conducted in five aquariums one was used for control and rest are used for pollution study. Each aquarium contains 10 fishes, which were exposed to sub lethal concentration of mancozeb

and malathion in combination at different time interval (24, 48, 72 and 96 hour). The sub lethal concentration was selected on the basis of LC_{50} value.

COLLECTION OF BLOOD

The blood samples were collected from live fishes through a cardiac puncture in both experimental and control groups at 24, 48, 72, and 96 hours exposures. These were allowed to stand for some time and, after that, centrifuged at 3500 rpm for 10 min to obtain serum.

ESTIMATION OF HIGH DENSITY LIPOPROTEIN (HDL): High density lipoprotein was estimated by the Warnick *et al* [8].

$$\text{Serum HDL (mg/dl)} = \frac{\text{O.D. of 'Test'}}{\text{O.D. of 'Standard'}} \times 75$$

ESTIMATION OF LOW DENSITY LIPOPROTEIN (LDL): Low density lipoprotein (LDL) was calculated from the values of serum cholesterol, very low density lipoprotein (VLDL) and high density lipoprotein (HDL) by using following formula given by Friedwald *et al*. [2].

LDL = CHOLESTEROL – (VLDL + HDL)

ESTIMATION OF VERY LOW DENSITY LIPOPROTEIN (VLDL): Very low density lipoprotein (VLDL) was calculated by the following formula given by Friedwald *et al*. [2].

$$\text{VLDL} = \frac{\text{Triglyceride (TG)}}{5}$$

RESULTS AND DISCUSSION

The low density lipoprotein, very low density lipoprotein have been observed to be increased, while a decrease in high density lipoprotein has been observed after 24hrs, 48hrs, 72hrs and 96hrs exposure to mancozeb+malathion in experimental fish *Channa punctatus* (Table-1-3).

Table 1: High density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication

HDL	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	55.67	52.50	46.67	42.30	38.50
±S.Em.	±0.45	±0.37	±0.33	±0.38	±0.28
Significance level	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Table 2: Low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication

LDL	Conrol	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	72.40	75.37	81.50	86.70	91.57
±S.Em.	±0.50	±0.45	±0.33	±0.37	±0.62
Significance level	-	P> 0.05	p< 0.05	p< 0.01	p< 0.001

S.Em. = Standard error of mean

Table 3: Very low density lipoprotein (mg/dl) in *Channa punctatus* after sub-lethal mancozeb + malathion intoxication

VLDL	Control	Exposure Hours			
		24 hours	48 hours	72 hours	96 hours
Mean	30.66	35.33	38.65	41.30	44.50
±S.Em.	±0.18	±0.19	±0.25	±0.33	±0.35
Significance level	-	P> 0.05	p< 0.05	p< 0.05	p< 0.01

S.Em. = Standard error of mean

The lipoproteins alterations are significant after treatment. It may be intoxication of pesticides on cholesterol and other lipid metabolism and may increased level of LDL, VLDL, while decreased in HDL levels in blood.. Further, this may also be due to hindrance in lipid metabolism which results in

accumulation of lipid content in blood. In accordance to the present findings, similar increased lipid profile has been reported by Ghosh [1] who observed the alterations of cholesterol in blood of *Channa punctatus* over the influence of Chromium and Radha *et al*. [4] observed blood and hepatic cholesterol HDL, VLDL and LDL inhibited throughout the experimental period under stress in *Cyprinus carpio,* and similar results observer by [6, 7]. These findings are in favour of the explanation of the present work. The changes are due to alteration in enzymes governing lipid, lipoprotein and triglyceride metabolism.

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CITATION OF THIS ARTICLE

A Singh and R Babu. Assessment of Lipoproteins in *Channa punctatus* under Toxic Stress of Mancozeb and Malathion in Combination. Bull. Env. Pharmacol. Life Sci., Vol10 [6] May 2021: 198-200

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Mancozeb and Malathion Induced Alterations in Lipoproteins in *Channa punctatus*

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ABSTRACT

Insecticidal use in agriculture gained momentum round the mid twentieth century. Fungicides also are utilized in agriculture for the prevention of mycosis in seed corn. Later these compounds discharge in nearby water bodies and consumed by fishes and other aquatic life. Pesticides are related chemicals; destroy the fragile balance between species that characterizes a functioning ecosystem. Pesticides are economical way of controlling pests. Pesticides are often wont to stop the spread of pests in imports and exports, preventing weeds in gardens and protecting house and furniture from destruction. Pesticides include a good sort of chemicals with great difference in their mode of action, uptake by the body, metabolism and elimination from the body and toxicity to focus on and non-target organisms. Poisoning risks depend upon dose, toxicity, duration of exposure and sensitivity.

Key words: Mancozeb, Malathion, Channa punctatus, Lipoproteins

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ALTERATIONS IN HEMATOLOGICAL PARAMETERS IN *CHANNA PUNCTATUS* FOLLOWING TOXIC STRESS OF MANCOZEB AND MALATHION IN COMBINATION

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ABSTRACT

The overall impact of above activities related to pesticides and insecticides is contamination of aquatic bodies adversely. This affect ecosystem at every trophic levelthe pesticide can accumulate in aquatic organisms or directly kill them and destroy balance of ecosystem. Problem of pesticidal pollution is very severe in the era of excess use of pesticides in every field like houses, crops, industries etc. in every house there is some kind of mosquito repellent or insecticide for flies, mosquito and other harmful insects, even ratkill is very common for control of rats. In crops, it is very evident fact that the farmers use excess and excess of pesticides to increase crop yield by reducing damage by pests and fertilizers. In pesticide making industry, the waste was run off in water bodies. The power to predict the impact of commercial waste water and municipal sewage discharge during a particular ecosystem would undoubtedly be enormously useful within the area of escalating industrialization.

Key words: *Channa punctatus*, Mancozeb, Malathion, Hematology