# Observation of blood Infected by Cypermethrin and Fenvalerate through Immunological Test in Clarias Batrachus (Linnaeus, 1758)

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***Abstract*—The observational relevance of two different immunological tests conducted on Clarias batrachus (Linnaeus, 1758) using two pesticides, Fenvalerate and Cypermethrin, reveals significant changes in the blood smear slides prepared during the tests. The fish were acclimatized to the pesticides for 96 hours (4 days) in two separate tanks. The concentrations of the toxic agents, Cypermethrin and Fenvalerate, were set at 0.25 µg/L (LC50 ¼) from a 20EC stock solution and 25 µg/L (LC50 1/10), respectively. These concentrations were maintained throughout the study, with observations made at 24-hour intervals to assess acute toxicity at lethal concentrations.**

***Index Terms*—Blood Smear Slide preparation, Clarias batrachus (Linnaeus, 1758), Cypermethrin, Fenvalerate, Immunological test, Immunotoxicities, Toxicology.**

## I. INTRODUCTION

Fenvalerate and Cypermethrin are types of pyrethroidal group of insecticides which give support for the rise of toxicity in the blood and other body system of either in insects or in other animals, when these are conveyed via the different fruits and vegetables taken as food.

As per USEPA cypermethrin is a type of cyanophenoxybenzyl pyrethroid and is categorized as restricted because of its high toxicity to fish (ETN [Extension Toxicology Network], 1996 and 2007).

**Table 1:** *Biochemical Results*

| Test Level | Range | Normal | Fenvalerate | Cypermethrin |
|---|---|---|---|---|
| Bilirubin (Total Serum) | 0.08-1.02 mg/l | 0.11 (+) | 0.05 (-) | 0.06 (-) |
| ESR | 20- 34 mm | 22 (+) | 10 (-) | 22 (+) |
| SGOT (AST) | 155.0-162.72 u/L | 161.5 (+) | 72.4 (-) | 261.0 (-) |
| SGPT (ALT) | 40.17-54.01 u/L | 45.3 (+) | 31.3 (-) | 48.7 (+) |
| Post Prandial Plasma Glucose (After Meal) | 109.8-184.5 mg/dl | 151.6 (+) | 71.4 (-) | 271.9 (-) |

**Legends:** (+): Significant towards Non-toxicity
(-): Significant towards toxicity

The concentration of Cypermethrin was taken as LC50 1/4 for 24h, was found to be 0.25 µg/l and for Fenvalerate as LC50 1/10 for 24 h, was 25 µg/l, which was continued for 96 h of every 24 h of time interval as lethal concentration for acute toxicity studies. A great biochemical variation found between the results of Normal, Fenvalerate and Cypermethrin during the blood biochemical examination in Clarias batrachus shown in Table-1 (Kumar and Dhakad 2017).

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Immunology has its origins in the study of how the body protects itself against infectious diseases caused by microorganisms such as bacteria, viruses, protozoa, and fungi, and also, parasitic organisms such as helminth worms.

Immunotoxicities are varied and include pathology of immune organs, decreased humoral or cell mediated immunity, decreased nonspecific immunity, decreased host resistance, hypersensitivity and autoimmunity (Galloway and Handy, 2003).

The first publication about Skin Prick test (SPT) by Helmtraud Ebruster in 1959, it has been used as a primary diagnostic tool to detect type I hypersensitivity reactions under the Immunological test. Skin prick testing or SPT demonstrates an allergic response to a specific allergen and is a simple, safe and quick test, providing results within 10-20 minutes.

Bryan's Leukocytotoxic test was originally developed in 1956 by Black, and further elucidated by Bryan in 1960. The basis of the test is that if the patient's white blood cells are mixed with the offending allergen, they swell. The test then measures any swelling of the leukocytes or blood indicates a problem with that food.

According to Heidel and Smith (2007), a variety of changes in cell morphology and function in response to injury are adaptive and are compatible with cell survival. These changes include acute cellular swelling, hydropic change, and fatty change. Reversible injuries result in structural and functional changes, but adaptation by the affected cells can maintain cell viability. Without adaptation, the changes caused by the injury can progress and may lead to cell death (necrosis).

Abnormalities in the shape or number of white blood cells may be signs of a Platelet disorder which affect duration of blood's ability to clot and changes in the appearance, movement or size of blood cells can be studied by the help of a Microscope slide.

## II. METHODOLOGY

### A. Experimental Design, Units and Stocking of Fish

Catfish Clarias batrachus of the same stock and mean weight of of 470 ±70 grams and the length were ranging from 38 to 53 cm were purchased from a nearby hatchery dealer acclimated to the laboratory conditions for two days that is 48 hours in tank in natural environmental condition with water pH 7. Fishes were fed daily with commercial pedigree pellets (Discus Tokyu) and wheat flour mixed meat mincer pellets according to 3% of body weight of fish. Total weight of fishes combinedly was approximately ± 10 kg. The amount of food was given two times daily 10 kg× 3% = 10000× 3/100 = 300 grams with mixed fed of both wheat flour and commercial pellets in the fish tank.

The complete experiment proceeded in winter season in the month of December to January within normal room temperature (± 22 – 27°C). The design of the experiment was a Completely Randomized Design (CRD) and Acute toxicity.

After acclimatization period of 48 hours (2 days) in Normal temperature, fishes were randomly divided in two groups in 2 separate water tanks. Fishes were acclimatized with pesticides Fenvalerate and Cypermethrin mixed toxic agent in two different tanks for 96 hours (4 days).

*Catfish Clarias batrachus (Linnaeus, 1758)* exposed under the Acute toxicity at the lethal concentration dose of LC50 1⁄4 of 1 µg/ litre for 24 hours; that is 0.25 µg/litre for Cypermethrin. On the other hand, Fenvalerate lethal concentration for 24 hours was LC 50 (250µ g/litre) selected by its 1/10 portion that is LC50 (25 µg/ litre).

Then Immunological test done in two forms-

- Skin Prick Test
- Leucocytotoxic Test

### B. Blood Collection (For the Leucocytotoxic Test)

2-3 drops of clove oil were given as anesthesia before blood sampling of randomly selected fish. Fishes were caught individually with the help of conical hand net. Blood samples were collected from the caudal vessels with the help of 5ml disposable heparinized plastic syringe fitted with a 21-gauge hypodermic needle, and immediately transferred to EDTA tubes and for analyzing blood parameters. Blood samples collected in three levels of Normal, Catfish infected by Fenvalerate and Catfish infected by Cypermethrin.

### C. Immunological test done during Experiment

Following two types of Immunological test done during the Research-

1 Skin Prick Test:

Initially, one catfish was placed in the freshwater glass aquarium and 3 drops of clove oil given as anesthesia. After 3-5 minutes the fish became slow in movement and settled down in bottom of aquarium and became unconscious. It was then taken out from aquarium by fish conical net and kept placed on a tray. Fish was marked with digit number 1 and 2 at two places for Skin prick test, one for Cypermethrin and another for Fenvalerate, respectively on the two distances of body. Fish was weighed 430 gm and length 38 cm. A droplet of toxic agent Cypermethrin was dropped where the digit 1 was marked and the other toxic agent Fenvalerate was dropped on the marked digit 2 on the skin surface. The skin was pricked then by soft hand with help of pin on the surface side where the toxic agent was dropped and marked as 1 and 2 to facilitate better dissolving of toxic agent in the skin and was kept stay for 15 minutes. After the completion of 15 minutes the areas of skin exposed to toxic agent were examined and total area and length of allergic reaction produced due to toxicity of both the agents Cypermethrin and Fenvalerate is noted.

2. Leucocytotoxic Test:

To performance this test, one fish was initially selected from both toxic tank of Cypermethrin and Fenvalerate was taken outside with the help of conical fish net. Fishes were anesthetized by three drops of clove oil in the glass aquarium. After unconsciousness of fish, the blood samples were withdrawn from the caudal vessel and proceeded for the next step for blood analysis through the help of Smear Slide preparation.

Smear slide was prepared by following two different methods:

Method 1: Blood withdrawal during the different doses evenly in normal and toxic phase, then proceeded for the next step of smear slide preparation.

Method 2: Blood withdrawn from normal fish and then transferred in two test tubes. One drop of each toxic agent Fenvalerate (25 µg/litre) and Cypermethrin (0.25 µg/litre) were mixed with normal fish blood tubes separately and then the smear slide was prepared from these three blood samples (normal blood, blood mixed with Fenvalerate, and blood mixed with Cypermethrin).

*Types of Smear Slide preparation:*

a. Thick Smear Spreading technique for Fenvalerate as per Method 2 only

b. Thin Smear Spreading technique for Normal and Cypermethrin infected blood with Method 1 and Method 2.

D. Procedure to make Smear Slide

Blood samples were withdrawn from normal Catfish and transferred into three glass test tubes. A smear was prepared using the blood from the first test tube containing normal fish blood. In the other two test tubes, each of the two toxic agents was added separately to the blood samples.

In the case of Fenvalerate, blood clotted in few seconds after mixing with the toxic agent. Therefore, all the slides of Fenvalerate were prepared in clotting condition using Thick Smear Spreading technique.

In the case of Cypermethrin and normal blood, smear slides were prepared using Thin Smear Spreading technique.

To prepare the smear slide, initially a drop of blood sample spread on slide to create a blood film. The slide was then dipped in Methanol which is used as fixative. The slide was dried in a few seconds and then transferred in FS-B for 15 seconds. It was washed in distilled water and again transferred in FS-B for 30 seconds. Subsequently the slide was washed in normal water and cover slip was placed on every processed film, and slide was preserved as a smear slide. Images of all the prepared smear slides of Normal, Fenvalerate and Cypermethrin were captured and viewed using Photographic microscope.

## III. RESULT AND DISCUSSION

Following observation were made during the Immunological test:

*Leucocytotoxic Test (LCT):*

Initially two types of methods of smear slide were prepared: -

Thin and Thick Smear slide. Results of each slide were observed and are described as below:

1. Thick Smear slide (Blood mixed with Fenvalerate): The blood cell ruptured after interaction of normal blood with Fenvalerate and clumped in seconds before preparation of smear slide film, which indicated that severe impact of given concentration on the fish's blood due to the toxicity of experimental pesticides.

2. Thin Smear slide (Normal and Cypermethrin mixed blood)

Different blood cells were seen in microscopic slide plates as results are shown in below images: -

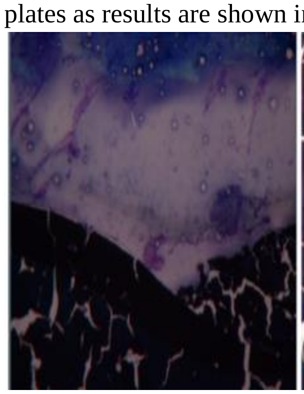

PLATE A-1

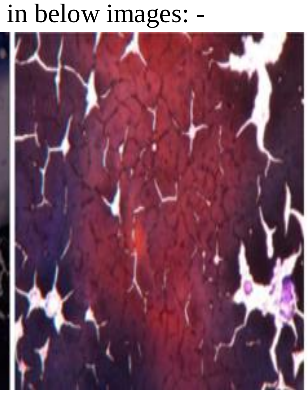

PLATE A-2

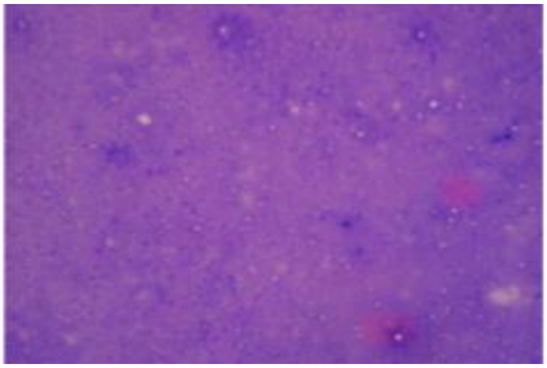

PLATE B

PLATE A-1 & A-2: Leucocytotoxic test of Pesticide (Blood clumping and rupturing).

PLATE B: Thin Smear Film (Cyp).

*Skin Prick Test (SPT):*

Following physical observations were found as results of Skin Prick Test (SPT) which was performed on normal water Clarias batrachus with each pesticide Fenvalerate and Cypermethrin:

A. Fenvalerate:

- Tail upside twisted abnormally
- The pricked area became red covering larger area.
- Big cut and scars appeared
- Effective length was measured as 8mm (at the tail side).

B. Cypermethrin:

- Skin became red and blurred
- Scars appeared on skin
- Heart and swim bladder portion swollen
- Body portion twist like curve and stiffy.
- Patches on the skin at the pricked area.
- Infected length of effective area was measured as 4mm (at the swollen heart portion) and 2mm (at patches and scars) that is total 6mm effective length was measured. Plates prepared are shown as below:

FIGURE-A


FIGURE-B

FIGURE-C

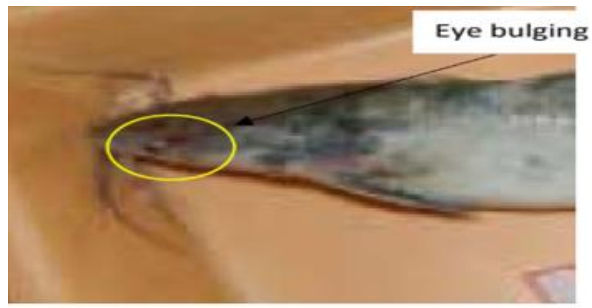


FIGURE-D

FIGURE- A: Appearance of Scars and Patches after Skin Prick Test (SPT)

FIGURE-B: Head portion infected by Melanoma

FIGURE-C: Stiffy, swell and twisted whorl body

FITURE-D: Eye bulging.

## IV. CONCLUSIONS

- Smear slide prepared during the Immunological test shows stages of blood cell growth and degeneration in toxic, protective and normal blood films.
- Demerits of agents are also observed in behavioral and physiological such as aggression, slum secretion, without shoal, active less, stiffy barbels, discoloration of skin color, depressive skin. These symptoms highlighted the extent of suffering experienced by the fish during exposure to toxicity.
- In Skin Prick Test (SPT) it is observed that the entire body severely affected by toxicity of

Fenvalerate and Cypermethrin. The fish's body became stiff and twisted after application of Fenvalerate and Cypermethrin on the body surface of fish.

- The head and tail portion of fish was widely affected after interaction of Fenvalerate and Cypermethrin.
- In Leucocytotoxic test after interaction of Fenvalerate and Cypermethrin, blood film ruptures and clump in seconds before preparation of smear slide film, which indicates that severity of toxicity the given concentration of experimental pesticides on fish's blood.
- Infectious Hematopoietic Necrosis Virus (IHN) disease that results in a severe anaemia found in *Clarias batrachus (Linnaeus ,1758),* during research done**.** Clinical signs are usually exophthalmia, darkened colour, severe mortality, petechial haemorrhages, extremely pale gills and abdominal distention from ascites.

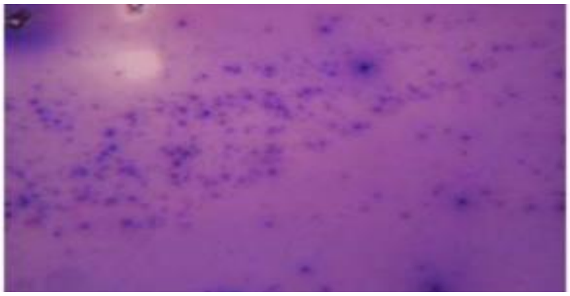

PLATE C

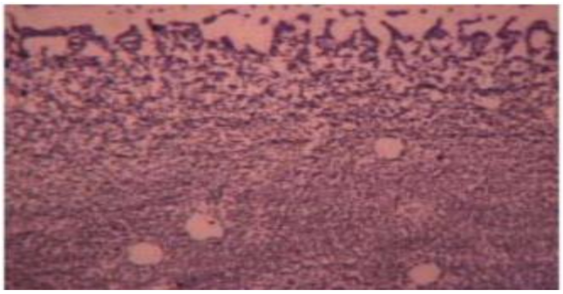

PLATE D

PLATE C: Blood film showing IHN Virus

PLATE D: Disturbances of Circulation: Haemorrhage.

Haemorrhage is the escape of blood from the vascular system. It is caused by an injury to vascular endothelium; this can be due to infection, inflammation, necrosis, neoplasia, or trauma. In the present study the fish also exhibited hemorrhage, and eye bulging was observed as well.

## V. GLOSSARY

SPT- Skin prick testing
LCT - Leucocytotoxic test
CYP- Cypermethrin
FEN-Fenvalerate
IHN Virus- Infectious Hematopoietic Necrosis Virus

## VI. ACKNOWLEDGEMENT

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