

## Comparative enzymological study of Liver induced by Boss organic (Biopesticide) & Agroban (Chemical pesticide) in Catfish *Heteropneustes fossilis* (Bloch)

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**Abstract:** In the present study authors studied the Comparative enzymological study of liver induced by Boss organic (Biopesticide) & Agroban (Chemical pesticide) in Catfish *Heteropneustes fossilis* (Bloch). The biochemical parameters studied were Ribose Nucleic Acid (RNA), Total Protein (TP), Alkaline Phosphates (ALP), Glutamate Pyruvate Transaminase (GPT), Glutamate Oxaloacetate Transaminase (GOT). These parameters in the experimental animals were found a decline. The order of toxicity of Boss organic (Biopesticide) & Agroban (Chemical pesticide) to biochemical parameters were decreased level RNA (77.5%) (Biopesticide) and (80%) (Chemical pesticide), Total Protein (13.77%) (Biopesticide) and (17.34%) (Chemical pesticide), Alkaline Phosphates (94.13%) (Biopesticide) and (84.0%) (Chemical pesticide), (GPT) (55.56%) (Biopesticide) and (50.01%) (Chemical pesticide), (GOT). (66.65%) (Biopesticide) and (62.48%) (Chemical pesticide). Thus, on the basis of obtained results of the presents investigation it can be concluded that the 96 hours exposure of 32ppm biopesticide and 10ppm chemical pesticide aqueous solution has a toxic effect and alter the biochemistry of liver. Therefore it is recommended to the user of this biopesticide and chemical pesticide that they should be careful about the dose they are using.

**Key Words:** Biopesticide, Chemical pesticide, biochemistry, toxicity, *Heteropneustes fossilis* (Bloch).

### 1. INTRODUCTION:

Nowadays, pesticides are being used extensively in the control of crop pests. Increased used of pesticide results in the excess inflow of toxic chemicals, mainly into the aquatic ecosystem (Baskaran et al., (1989), Kalavanthy et al., 2001). The aquatic environment is currently under threat by the indiscriminate use of synthetic pesticides by the human activities and causing high risk to non-target organisms (Kumar et. al., (2010). The excess use of this pesticide may enter into natural waters through agricultural run-off and ultimately cause damage to non-target organisms such as fish. (Stephenson, 1982; Prashanth and Neelagund, 2008; Singh et al., 2010). The freshwater catfish *Heteropneustes fossilis* is widely cultivated in rice fields, swamps and derelict water bodies (Chondar, 1999) and is thus frequently exposed to agricultural runoff. Synthetic insecticides are used widely for the control of various insect pests because they can be applied whenever and wherever needed, economical and most important thing is the reliability of control method. Hence, the production and consumption of pesticides have greatly increased in recent years. The contribution of pesticides to increase agricultural production cannot be denied, but synthetic pesticides have also caused unprecedented ecological damage, also induced serious health hazard among workers during manufacture, formulation and field applications. To overcome the problems of synthetic chemical hazards, one of the best control measures is the use of biopesticide products. The popularity of the plant products increasing day by day because of their biodegradability, least persistence, and least toxic to non-target organisms, economic and easy availability. Today about 200 plants with insecticidal activities are known. Among the natural product is one of the most promising natural compounds is Boss organic (biopesticide), and its biological base is Cashew nut. A biopesticide is active in nearly 550 insect species, mostly in earthworms, rats, rabbits, daphnia, mice, fishes, etc.

### 2. MATERIALS AND METHODS:

Healthy *Heteropneustes fossilis* fishes were purchased from the local fish market of Indore and acclimatized to the laboratory conditions for one week, during which they were regularly fed with Prawn powder and Soya meal.

**Experimental Design:**

In the present study experimental fishes were divided into three major groups viz.

**1. Control group :** - The control group was run with tap water as vehicle. Water used in the experiment was dechlorinated and was having pH 8 and temperature 25-30°.

**2. Experimental group:-**

**Set A: Control group:** In this group, 10 fishes were kept and exposed to normal tap water.

**Set B:** In this group, 10 fishes were used. The test fishes were exposed to sub-lethal dose of biopesticide was 32ppm.

**Set C:** In this group, 10 fishes were used, and test fishes were treated with chemical pesticide was 10ppm.

**Experimental duration:**

In both control and experimental group, fishes were exposed to maximum 96 hrs.

**AUTOPSY:** Fishes of control and experimental groups were sacrificed at 24 hours, 48 hours, and 72 hours 96 hours respectively. The liver lobes were removed, blotted and weighed and then processed various biochemical tests.

**3. METHODS:**

Following standard biochemical methods were used, which are described in the laboratory manual in Biochemistry.

- (1) Extraction and Estimation of RNA.
- (2) Estimation of total protein by Biuret method.
- (3) Determine the Alkaline phosphates by King and King Method.
- (4) Estimation of Activity of Glutamate Pyruvate Transaminase enzyme.
- (5) Estimation of Activity of Glutamate Oxaloacetate Transaminase enzyme.

**4. RESULTS:**

**RNA content:** The total content of RNA in the liver of *Heteropneustes fossilis* was found to be decreased gradually after the exposure of 32ppm of Boss organic (biopesticide) and 10ppm of Agroban (chemical pesticide). The normal value of RNA content in the animal was observed 16 mg/gm wt. of liver which after Boss organic (biopesticide) exposure of 24 hours, 48 hours, 72 hours, and 96 hours reduced to 8.4, 5.2, 4.0, and 3.6 mg/gm of tissue wt. respectively, and Agroban (chemical pesticide) exposure of 24 hours, 48 hours, 72 hours, and 96 hours reduced to 14, 6.2, 6.0, and 3.2 mg/gm of tissue wt. respectively. In the experiment total, 77.5 percent of biopesticide and 80 percent of chemical pesticide inhibition in RNA activity was observed after 96 hours. (Table No.1 Biopesticide fig.1 & Table No.1 Chemical pesticide fig.1).

**Total protein:** The quantity of total protein in the liver of experimental fish was found decreased order. The value of total protein after 24 hours, 48 hours, 72 hours and 96 hours exposure of Boss organic (biopesticide) and Agroban (chemical pesticide) were found decreased in the order of 9.69%, 12.75%, 12.75%, 13.77% biopesticide and 7.65%, 11.22%, 13.77%, 17.34% of chemical pesticide respectively. The important observation in this experimental was that the protein value decreases up to 13.77% biopesticide and 17.34% chemical pesticide just within 96 hours.

This shows that initially, toxicant exposure induces the utilization of stored protein and further stimulates protein synthesis to cope additional demand of protein to fight against biopesticide chemical pesticide toxicant. (Table No.2 Biopesticide fig. 2 & Table No.2 Chemical pesticide fig.2).

**Alkaline Phosphates Protein:** The effect of biopesticide and chemical pesticide on alkaline phosphates activity of *Heteropneustes fossilis* was found to decrease gradually as the duration of exposure of biopesticide and chemical pesticide. The total inhibition in alkaline phosphates activity after 24 hours, 48 hours, 72 hours, 96 hours, exposure of biopesticide were 56.71%, 83.13%, 84.72%, 94.13% and 1.84%, 58.6%, 81.9%, 84.0% chemical pesticide respectively. (Table No.3 Biopesticide fig. 3 & Table No.3 Chemical pesticide fig.3).

**GPT:** The Glutamate Pyruvate Transaminase activity was found affected in the exposure of Boss organic (biopesticide) and Agroban (chemical pesticide) in *Heteropneustes fossilis*. The decrease in GPT after 24 h, 48 h, 72 h, 96 h, were found in 0%, 11.13%, 50.01%, 55.56% biopesticide and 20.01%, 33.36%, 42.90%, 50.01% chemical pesticide respectively. The inhibition of GPT activity of liver was very high and reaches up to 55.56% biopesticide and 50.01% chemical pesticide. (Table No.4 Biopesticide, fig.4 & Table No.4 Chemical pesticide fig.4).

**Glutamate Oxaloacetate Transaminase (GOT):** The Glutamate Oxaloacetate Transaminase activity was found affected in the exposure of Boss organic (biopesticide) and Agroban (chemical pesticide) in *Heteropneustes fossilis*. After 24 h., 48 h., 72 h., 96 h., of test of chemicals the reduction in enzymatic activity (GOT) were found 24.98%, 33.31%, 57.13%, 66.65% biopesticide and 14.27%, 24.98%, 39.98%, 62.48% chemical pesticide respectively. The inhibition of GOT activity of liver was very high and reaches up to 66.65% biopesticide and 62.48% chemical pesticide. (Table No.5 Biopesticide fig.5 & Table No.5 Chemical pesticide fig.5).

**RNA change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide). Table No.1.**

S. No.	Duration (hours)	RNA (µg/mg)		Difference	% Alter
		Control	Experimental		
1	24	16	8.4	7.6	47.5
2	48	16	5.2	10.8	67.5
3	72	16	4.0	12	75
4	96	16	3.6	12.4	77.5

**RNA change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemicalpesticide). Table No.1.**

S. No.	Duration (hours)	Total protein (µg/mg)		Difference	% Alter
		Control	Experimental		
1	24	16	14	2	12.5
2	48	16	6.2	9.8	61.2
3	72	16	6.0	10	62.5
4	96	16	3.2	12.8	80

**Total protein change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide). Table No.2**

S. No.	Duration (hours)	Total protein (µg/mg)		Difference	% Alter
		Control	Experimental		
1	24	0.0196	0.0177	0.0019	9.69
2	48	0.0196	0.0171	0.0025	12.75
3	72	0.0196	0.0171	0.0025	12.75
4	96	0.0196	0.0169	0.0027	13.77

**Total protein change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemicalpesticide). Table No.2**

S. No.	Duration (hours)	Total protein ( $\mu\text{g}/\text{mg}$ )		Difference	% Alter
		Control	Experimental		
1	24	0.0196	0.0181	0.0015	7.65
2	48	0.0196	0.0174	0.0022	11.22
3	72	0.0196	0.0169	0.0027	13.77
4	96	0.0196	0.0162	0.0034	17.34

**Alkaline phosphatase activity in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (bipoesticide). Table No.3**

S. No.	Duration (hours)	Alkaline phosphatase activity in KA units/100/ml		Difference	% Alter
		Control	Experimental		
1	24	18.92	8.181	10.73	56.71
2	48	18.92	3.181	15.73	83.13
3	72	18.92	2.888	16.03	84.72
4	96	18.92	1.11	17.81	94.13

**Alkaline phosphatase activity in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemical pesticide). Table No.3**

S. No.	Duration (hours)	Alkaline phosphatase activity in KA units/100ml		Difference	% Alter
		Control	Experimental		
1	24	18.92	18.57	0.35	1.84
2	48	18.92	7.804	11.1	58.6
3	72	18.92	3.333	15.5	81.9
4	96	18.92	3.015	15.9	84.0

**GPT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (bipoesticide). Table No.4**

S. No.	Duration (hours)	GPT activity in $\mu\text{g}$ mole/liter/min		Difference	% Alter
		Control	Experimental		
1	24	33.33	33.33	0	0
2	48	33.33	29.62	3.71	11.13
3	72	33.33	16.66	16.67	50.01
4	96	33.33	14.81	18.52	55.56

**GPT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemical pesticide). Table No.4**

S. No.	Duration (hours)	GPT activity in $\mu\text{g}$ mole/liter/min		Difference	% Alter
		Control	Experimental		
1	24	33.33	26.62	6.67	20.01
2	48	33.33	22.21	11.12	33.36
3	72	33.33	19.03	14.03	42.90
4	96	33.33	16.66	16.67	50.01

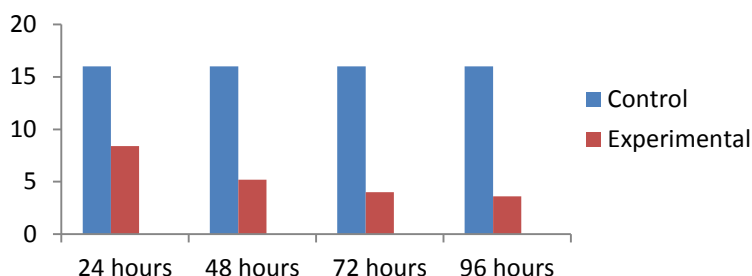
**GOT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (bipoesticide). Table No.5**

S. No.	Duration (hours)	GOT activity in $\mu\text{g mole/liter/min}$		Difference	% Alter
		Control	Experimental		
1	24	22.21	16.66	5.55	24.98
2	48	22.21	14.81	7.4	33.31
3	72	22.21	9.519	12.69	57.13
4	96	22.21	7.405	14.80	66.65

**GOT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agrobano (chemical pesticide). Table No.5**

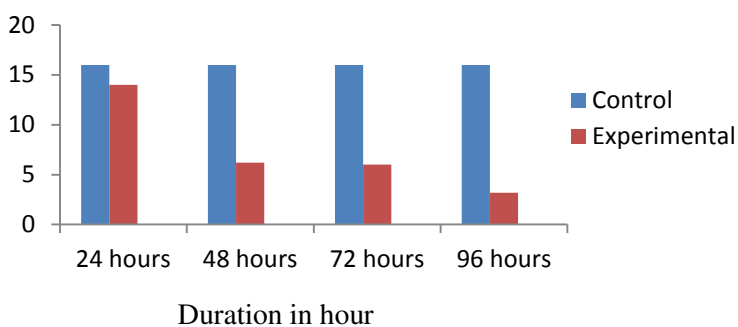
S. No.	Duration (hours)	GPT activity in $\mu\text{g mole/liter/min}$		Difference	% Alter
		Control	Experimental		
1	24	22.21	19.04	3.17	14.27
2	48	22.21	16.66	5.55	24.98
3	72	22.21	13.33	8.88	39.98
4	96	22.21	8.332	13.8	62.48

**RNA change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide).**

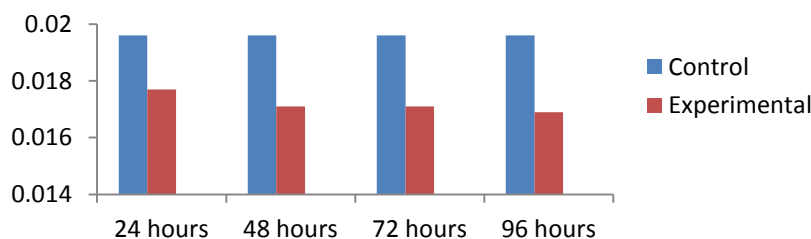


Duration in hour

**RNA change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agrobano (chemical pesticide).**

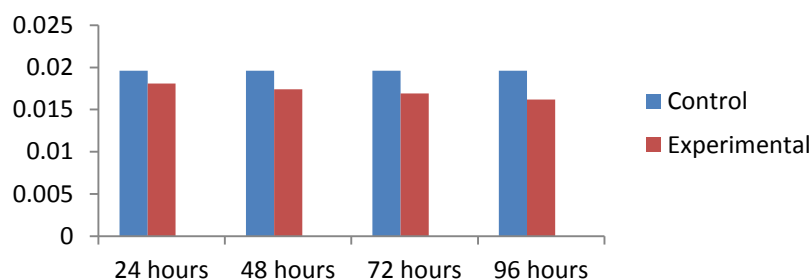


**Total protein change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide).**



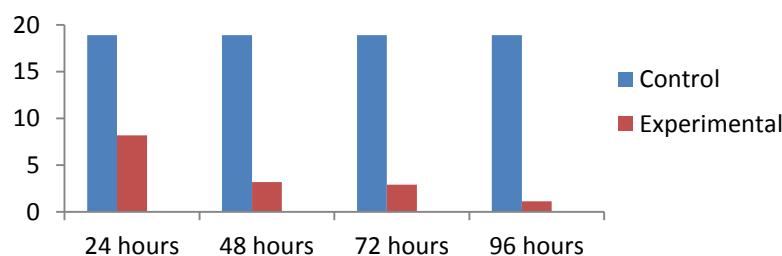
Duration in hour

**Total protein change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemical pesticide).**



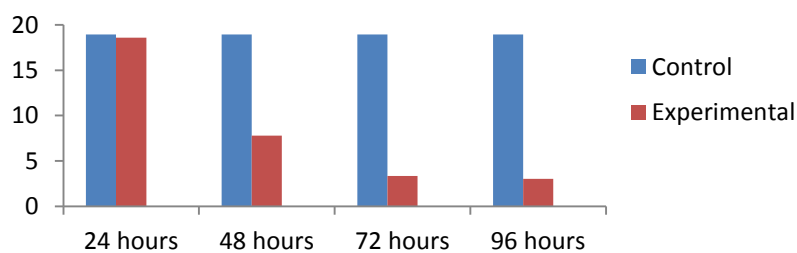
Duration in hour

**Alkaline phosphatase activity in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide).**



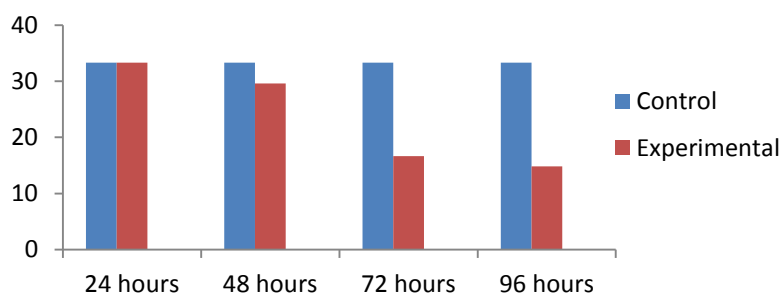
Duration in hour

**Alkaline phosphatase activity in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemical pesticide).**



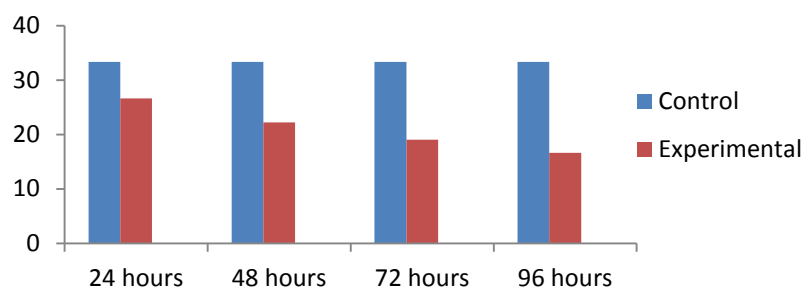
Duration in hour

**GPT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide).**



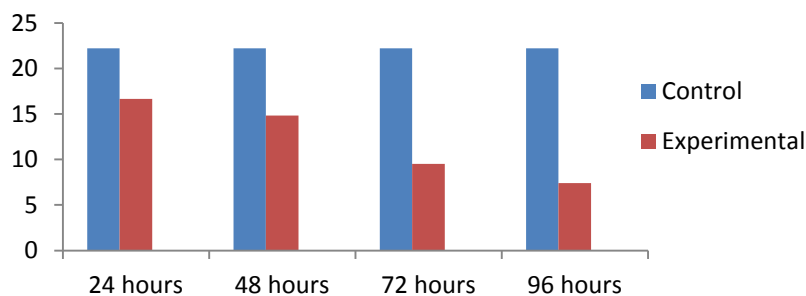
Duration in hour

**GPT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemicalpesticide).**



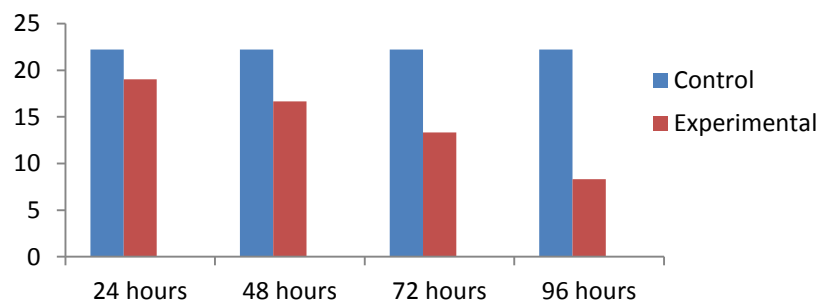
Duration in hour

**GOT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 32ppm concentration of Boss organic (biopesticide).**



Duration in hour

**GOT change in the liver of *Heteropneustes fossilis* (Bloch) Exposed to 10ppm concentration of Agroban (chemical pesticide).**



Duration in hour

**5. DISCUSSION:**

In the present study, the pesticide containing Boss organic (biopesticide) is less toxic to fish compared to Agroban (chemical pesticide). The 96 h LC50 of Chemical pesticide is 10ppm. Whereas Biopesticide is much higher 32ppm indicating the less toxic nature of the plant based pesticide. Das et al., 2002 have studied the acute toxicity of biopesticide in the fingerlings of Indian major carps, i.e., Labeo rohita, Catla catla and Cirrhinus mrigala and the 96h LC50 values were found to be 32ppm respectively. Hassanein et al., 2007 reported the 96h LC50 value of a biopesticide (Trilogy) on the grass carp fish, Ctenopharyngodon idella and was found to be 112ppm. Cagauan et al. (2004) showed that the lethal concentration of biopesticide to Nile tilapia Oreochromis niloticus L. was 12.4 ml/L and mosquito fish Gambusia affinis Baird and Girard was 8.31 ml/L and the corresponding 96 h LC50 values were 2.57 and 3.0 ml/L. In the present investigation biopesticide 32ppm and

chemical pesticide 10ppm exposure for 96 hours to *Heteropneustes fossilis* (Bloch.) was found toxic as it is altered rather decreased all the studied biochemical parameters (RNA, TP, ALP GPT and GOT.) of liver and thus support the observation of previous authors. Therefore it is recommended to the user of this biopesticide and chemical pesticide that they should be careful about the dose they are using.

## 6. SUMMARY:

In the present investigation biopesticide 32ppm and chemical pesticide 10ppm exposure to *Heteropneustes fossilis* for 96 hours alone decrease the activity of Total protein, Alkaline phosphates, Glutamate Oxaloacetate Transaminase, Glutamate Pyruvate Transaminase. The whole experimental study showed that biopesticide and chemical toxicity causes comparatively more and severe damaged to protein, and Alkaline phosphates. However RNA activities not much affected. The order of toxicity can put in the following decreasing order.

Protein > GPT > GPT > Alkaline Phosphates > RNA.

This showed that biopesticide and chemical pesticide also able to change the biochemistry of liver. Present work deals with the effect of biopesticide and chemical pesticide in biochemistry of liver of *Heteropneustes fossilis*. Remarkable finding of the present investigation are summarized below.

1. Test fishes were exposed for 24 hours, 48 hours 72 hours, 96 hours with Biopesticide (32ppm) and Chemical pesticide (10ppm).
2. Fishes were treated with pesticide feed with artificial food on alternate days. Fishes were sacrificed for different examination along with control group fishes.
3. Physiochemical properties (Temperature PH, DO, BOD, COD, Hardness, Alkalinity, Chloride) of test medium were analysed during experiment.
4. LC<sub>50</sub> values of pesticides were determined exposing the fish for 24 hours, 48 hours, 72 hours, 96 hours respectively for biopesticide and chemical pesticide.
5. Ribose Nucleic Acid (RNA) was slightly changed in experimental fishes.
6. Total protein was found reduced in the experimental fish.
7. Alkaline phosphates activity was also found decreased in the experimental fishes.
8. The Glutamate Pyruvate Transaminase activity was found affected in the experimental fishes.
9. The Glutamate Oxaloacetate Transaminase activity was also found affected in the experimental fishes.

## 7. CONCLUSION:

The present experiment study reveals that biopesticide and A chemical pesticide affects the biochemical parameter Viz. Prorein, Phosphate, GPT, GOT, by effecting the survivility of the fishes. Biopesticide can be used in the less of chemical pesticide as it is environment friendly, which does not toxify the air we breathe, food we eat, soil we cultivate nor water we use for irrigation. In short, these biopesticides are equally effective, for cheaper and fully eco-friendly. Cashew nut obtain from cashew nut seed was subjected to toxicological testing to document is safe for use as pesticide and it become necessary to use as it is ecologically safe and biologically active botanical substance that are metabolized and are not passed on the next trophic level. These pesticides should be further studied so as to establish the clear mechanism of action in fishes.

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