***Original Research Article***

**Assessment of Cypermethrin-Induced Toxicity on Blood Parameters and Behavior in the Air-Breathing Fish Anabas testudineus (Bloch)**

**ABSTRACT**

**Aim**: The present study aims to evaluate the toxicity of Cypermethrin (25% EC) a common synthetic pyrethroid pesticide frequently used in agriculture fields, in a freshwater air-breathing fish Anabas testudineus in laboratory condition. **Background:** Pesticides are frequently used by farmers to control the pests in the agriculture fields. These pesticides directly or indirectly affect the aquatic ecosystem including fishes. **Method**: The experiment was carried out in the University Department of Zoology, T.M.B.U. Bhagalpur, Bihar, India. Freshwater fish Anabas testudineus were collected from the local fish market of Nathnagar & Bhagalpur (Bihar) and brought to the Departmental laboratory without any physical injury. The LC50 value was found 3.2 ppm and 1/10 & 1/50th of LC 50 i.e. 0.32ppm & 0.064ppm respectively were selected as sub-lethal concentrations to determine some haematological parameters (RBC, WBC, Hb, PCV) in selected fishes. The impact was evaluated using comparative data of the control group with experimental groups of fishes subjected to sub-lethal concentrations of Cypermethrin. The exposure duration was 15, 30 and 45 days respectively. **Results:** The number of RBC, Haemoglobin percentage and Haematocrit value of treated fishes was found significantly reduced (p< 0.001) compared to control fishes, whereas the number of WBC was showed increased at the beginning of the experiment but after 15th days of exposure it showed a decreasing trend till the end of the treatment. An increase in WBC may be due to immune response. The result demonstrated that the fish exposed to high cypermethrin concentration significantly decreased the RBC count, haemoglobin and PCV levels. **Conclusion**: During exposure to Cypermethrin, some behavioural alterations (restlessness, erratic movement, hyperactivity) were also recorded, which may be due to the effect of stress caused due to sudden habitat change or due to the inactivation of specific enzyme acetylcholinesterase activity. To promote sustainable development, using insecticides at the lowest effective concentration is recommended to control insects while minimizing harm to aquatic and terrestrial environments.

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

Keywords: Cypermethrin, *Anabas testudineus*, Toxicity, Haematological parameters, behavioural changes

**INTRODUCTION**

 India is an aquacultural country. It is totally dependent on agriculture and agricultural products to feed people. So, a large amount of Agro-chemicals and insecticides are used to increase agricultural production (Kumar,2019). In the last few decades pollutants have entered the environment due to various human activities (Awoyinka *et al.,* 2019) and now a day’s contamination of the natural ecosystem is increasing day by day. Chemical analysis can identify the existence of harmful substances in the environment, but it cannot reveal how those substances will affect the aquatic ecosystem. Therefore, bioassay experiments are necessary to evaluate the effects of harmful compounds on the environment (F. Bagheri *et al*., 2007).

 Pesticides at higher concentrations are known to reduce the survival, growth and reproduction of fishes (Mekim *et al*., 1975) and produce many visible effects on fish fauna (Johenson, 1968 & Kumar, 2019). These are useful tools in agriculture and forestry, on the other hand, their contribution to the gradual degradation of the aquaculture and ecosystem cannot be ignored (Kadam and Patil, 2016). It is confirmed from evidences that direct or indirect prolonged exposure of pesticides inside water is harmful for fishes and other aquatic life, leading to acute and chronic health problems (Bhardwaj et al., 2022). Aquatic toxicity of pesticides is generally difficult to assess but their monitoring is very important, as it makes ecological imbalance leading to loss of economic aquatic organisms (Bhardwaj et al., 2020).

 Fishes are a very rich source of protein and easily digestible food for human beings. It plays a vital role in fulfilling our nutritional demand and increasing our total production (Pawar and Sonawane, 2014). Fishes are important component of food chain so, any effect of toxicant may have adverse impact on the nutritive values of fish and on the human being due to their consumption (Gupta & Srivastava, 2006). However, nowadays due to different natural and anthropogenic activities, the number of fishes is declining. (Siddiqua, 2016). Among the different causes of fish decrease, the use of pesticides is one of the important issues. Many authors studied the impact of different pesticides on freshwater fish species, such as melathion on *Labeo rohita* (Thenmozhi *et al*., 2011*), Barbus gonionetes* (Hoque *et al*., 2000), *Cyprinus carpio* (Sharmin *et al*., 2014), and chlorpyrifos on *Channa gachua* (Kadam & patil, 2016).

 Application of organophosphorus insecticides in crop fields has a great impact on aquatic systems especially on the fish population (Kadam & Patil, 2016). Thus, pesticides are mixed with aquatic systems in different ways such as rainfall, overflow of water bodies, drainage systems etc. (Siddiqua, 2016). These pesticides enter the food chain and thus subsequent bioaccumulation and biotransformation at different trophic levels have disastrous effects on the ecosystem (Grende *et al*., 1994; Zahran *et al*., 2019).

 Cypermethrin is a type of synthetic pyrethroid pesticide. Its low toxicity to birds and animals has led to a rapid global increase in the usage of cypermethrin (US EPA 1989). Pyrethroid pesticides are very commonly used for pest control in agriculture fields and may reach the aquatic system through irrigation and rain. As a result many non-target organisms including fishes of the freshwater ecosystems are adversely affected (ANUPAMA and AMIT, 2020). Cypermethrin is extremely poisonous to fishes (Bradbury and Coats 1989). Between 0.7 and 350 µg L-1 is the range of the 96-hour LC50 values (Sarikaya, 2009). For *Anabas testudineus*, the LC50 (96-hour) of cypermethrin was reported by Velmurugan *et al*., (2014) to be 0.3 µg L-1. Cypermethrin (CYP) is one of the most effective insecticides used in forestry, agriculture, buildings and farmyards (Casida *et al*., 1983; Khan *et al*., 2006; Ullah *et al*., 2015). Commercially CYP is being used against cotton and soybean pests (Carriquiriborde *et al*., 2007). The insecticide which is sprayed in the fields ultimately reaches water bodies, causing serious threats to aquatic life particularly to fish (Akhtar *et al*., 2021). Fishes due to their aquatic habitat is directly exposed to environmental noxiousness including harmful insecticides which affects their profitable worth and rearing ability (Firat *et al*., 2011; Georgieva *et al*., 2014; Akhtar *et al*., 2021).

 Cypermethrin has a drastic effect on both invertebrates and vertebrate species (Das and Mukherjee., 2003). It is a synthetic parathyroid used as an insecticide in large-scale commercial agriculture applications as well as in consumer products for domestic purposes. Cypermethrin is highly toxic to fish (Casida *et al*., 1983; Khan *et al*., 2006; Ullah *et al*., 2015; Kumar, 2019).

 Blood parameters are intensively used as biological indicators for the health status of the fish (Lerman *et al*., 2004). The evaluation of blood parameters is very effective in detecting the effect of pesticides on fish. The present investigation was conducted to evaluate the toxicity of cypermethrin pyrethroid (25%EC) on a freshwater an air-breathing fish *Anabas testudineus*.

**MATERIALS AND METHOD:**

 The experiment was carried out in the University Department of Zoology, T.M.B.U. Bhagalpur, Bihar, India. Freshwater fish *Anabas testudineus* were collected from the local fish market of Nathnagar & Bhagalpur (Bihar) and brought to the Departmental laboratory without any physical injury. The average length of fish was 11.51 ± 1.298. Fishes were acclimatized for about three weeks prior to experiments and screened for any pathogenic infections. The fishes were maintained in glass aquaria of volume (60×30×30) cm3. For the purpose of preventing fungal contamination, glass aquaria were washed with 1% KMNO4 solution (Anupama & Amit, 2023) to remove dermal infections if any. Healthy fishes were transferred to glass aquaria containing a sufficient volume of water (20L). They were regularly fed with commercial fish food.

**EXPERIMENTAL CHEMICAL:**

The commercial-grade pesticide Cypermethrin (25% EC) manufactured by INDIA Pesticide Ltd was selected for this investigation to observe its effect on selected fishes. This chemical was taken into an experimental flask and the required amount of distilled water was added in order to prepare the desired concentration.

**TOXICITY TEST-(96 hours of LC50):**

 The toxicity range-finding experiment was conducted to determine the Cypermethrin concentration that may kill 50% population of *Anabas testudineus* fish in 96 hours. The commercial grade Cypermethrin (25% EC, manufactured by India Pesticide Ltd.) was diluted 1000 times with the distilled water to prepare the stock solution, which is equivalent to 1000ppm. After that, the stock solution was used accordingly to complete the experiment.

 The experiment was performed in the glass aquaria, containing 20L of tap water with dual simultaneous replicates of 8 treatments containing 1, 1.5, 2, 2.5, 3, 3.5, 4.5, and 5 ppm of Cypermethrin. 10 healthy were fishes randomly removed from the glass aquarium and added to the experimental tank. Mortality was recorded after 24, 48, 72, 96 hours of exposure. Dead fish were removed from the tank.

 In the present study LC50 value of fish *Anabas testudineus* was calculated for 96 hours of exposure time by Probit Analysis. Probit analysis is a specialized regression model of the binomial response variable. Regression is a technique for analyzing data by fitting a line to it in order to compare variables, also known as the dependent variable (Y) and the independent variable (X).

 Y = a + bx + e

 Where,

 a = y- intercept

 b = the slope of the line

 e = error term

**ANIMAL & EXPERIMENTAL DESIGN:**

The fishes were separated into three groups Ⅰ, Ⅱ and Ⅲ, and was kept in different glass aquariums. To guarantee an outcome, the current study was carried out three times. Ten fish were used per replicate for each group. To act as a control Group Ⅰ was kept in the water without pesticides. Group Ⅱ & group Ⅲ were exposed to the sub lethal doses of Cypermethrin 0.32 and 0.064 ppm respectively (1/10th and 1/50th of LC50 value).

**RESULTS:**

**TOXICITY STUDIES**

 Acute toxicity is typically used to examine how sensitive various species are to various chemical potencies using LC50 values. The mortality rate of fish increased because of high pesticide concentration. Table 1 displayed lethal concentrations determined by Probit Analysis. Lethal concentration (LC50) was calculated as 3.2 ppm.

**TABLE -1:** Probit analysis on the effect of Cypermethrin to *Anabas testudineus* at 96 hours of exposure.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No. of fishes** | **Conc. In ppm** | **Log****Conc.**  | **Fish died (24 h)** | **Fish died****(48h)** | **Fish died****(72h)** | **Fish dead****(96h)** | **Total fish dead**  | **% kill** | **Probit value** | **LC50** |
| 10 | 1 | 0.176091259 | 0 | 0 | 0 | 0 | 0 | 0% | 0 | 3.2ppm |
| 10 | 1.5 | 0.301029996 | 0 | 0 | 0 | 0 | 0 | 0% | 0 |
| 10 | 2 | 0.397940009 | 0 | 0 | 1 | 0 | 1 | 10% | 3.72 |
| 10 | 2.5 | 0.477121255 | 0 | 0 | 1 | 1 | 2 | 20% | 4.16 |
| 10 | 3 | 0.544068044 | 2 | 0 | 2 | 0 | 4 | 40% | 4.75 |
| 10 | 3.5 | 0.602059991 | 3 | 0 | 0 | 3 | 6 | 60% | 5.25 |
| 10 | 4 | 0.653212514 | 2 | 3 | 1 | 2 | 8 | 80% | 5.84 |
| 10 | 5 | 0.698970004 | 3 | 2 | 4 | 1 | 10 | 100% | 7.33 |

Table showing the concentration of pesticide in ppm (parts per million) taken for the determination of LC50 and fishes (in number and in percentage) died at different intervals of time at different concentrations. LC50 is calculated as 3.2 ppm.

Graph 1; shows the normal probability between the number of fish that died and the sample percentile.

Graph 2: showing Y- Value between pesticide (Cypermethrin) concentration and % mortality of fishes.

**BEHAVIORAL RESPONSE DURING TOXICITY ASSESSMENT**

 Over the course of 96 hours, fishes (*Anabas testudineus*) showed some alterations in their behaviour. Over the course of the trial, the control group showed normal behaviour, and at low concentrations (1 ppm), normal reactions were noted. A considerable increase in hyperactivity was noticed in terms of surfacing, scraping, and schooling moments following a 24-hour exposure to Cypermethrin as compared to the control group. After 72 hours of exposure, there was a decrease in surfacing and jerky movements, as well as an increase in grasping and settling at the bottom of the test chamber. After 96 hours of exposure, fishes in the control group exhibited normal loss of pigmentation, but higher dosages resulted in pale greyish-black colouration.

**TABLE 2:** Impact of Cypermethrin on the behavioural pattern of *Anabas testudineus* (average length 18 ± 2 cm and weight 48 ± 2 g, n ¼ 10) at different concentrations of pesticide after 96 hrs. of exposure.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | Control | 0.32ppm | 0.064ppm |
|  |  |  |  |
| Skin color | \_\_\_\_ | ++ | ++ |
| Loss of Balance  | \_\_\_ | ++ | ++ |
| Surface activity | \_\_\_ | ++ | + |
| Rate of swimming | \_\_\_ | +++ | +++ |
| Hyperactivity  | \_\_\_ | ++ | + |
| Convulsion  | \_\_\_ | ++ | ++ |
| Opercula activity rate | \_\_\_  |  + |  ++ |
|  |  |  |  |

**Table 2**: the increase or decrease in the level of behavioural parameters is shown by the number of (+) signs. –, none (0%); +, mild (<10%); ++, moderate (10 to 50%); +++, severe (> 50%). The (-) sign indicates normal behavioural conditions.

**BEHAVIOURAL RESPONSES DURING SUB-LETHAL DOSING**

 In the course of 45 days of sublethal dosing of 1/10 and 1/50 of LC50, the fish *Anabas testudineus* showed very little alteration in behaviour. Normal behaviour was observed in the fish of the control group. At the beginning of the experiment (15th day), no alteration in the behaviour was observed in the experimental group fishes. But by the end of the experiment (30 to 45th days), some changes began to appear in the behaviour of the experimental fish like hyperactivity, an increase in grasping and settling at the bottom of the experimental tank. Gradual colour change was also noticed in the fishes treated with Cypermethrin sub-lethal doses (0.32 & 0.064 ppm). No mortality was recorded during the course of the experiment.

1. **IMPACT ON RBC (Red blood cell)**

Fish treated with Cypermethrin (25% EC) showed a significant decline in the number of RBCs. It was observed that as the duration of treatment increased, the number of RBCs showed a declining trend. On the 15th, 30th and 40th day of treatment with the dose of 0.064ppm, the mean of the RBC count was 1.02±0.069, 0.97±0.062, 0.90±0.144 (106µL-1)respectively (p<0.05) in compare to control of mean around 2.92±0.3002 at 45th day. Similarly with the dose of 0.32ppm, on the 15th, 30th, and 40th day of treatment, the mean was observed 0.92±0.1677, 0.9±0.064, 0.84±0.049 (106µL-1)respectively (p<0.05) in compare to control group. A significant decrease in the number of RBCs was observed in the fishes treated with cypermethrin for the duration of 45 days of treatment.

**(Red Blood Cell)**

It has been observed that RBC count decreases with an increase in the concentration of Cypermethrin on the 15th, 30th, and 45th days.

**Figure 1**- Showing the impact of Cypermethrin on the RBC of Anabas testudineus after the 15th, 30th and 45th days of exposure. Data is expressed as mean ± SEM (N=10), p < 0.05.

**Table 3:** Haematological values of *Anabas testudineus* exposed to sub-lethal concentrations of cypermethrin.

|  |
| --- |
| **Hematological Cypermethrin 15th day 30th day 45th day****Parameter concentration** (in ppm) |
| **RBC****(106µL-1)** | **Control** | 2.69±0.3505 | 2.89±0.222 | 2.92±0.3002 |
| 0.064ppm | 1.02±0.069 | 0.97±0.062 | 0.90±0.144 |
| 0.32ppm | 0.92±0.1677 | 0.9±0.064 | 0.84±0.049 |
| **WBC****(103µL-1)** | **Control** | 278±1.527 | 284.33±0.769 | 279.66±6.5773 |
| 0.064ppm | 300.33±0.666 | 272.33±0.8819 | 250.66±1.2018 |
| 0.32ppm | 320.66±0.819 | 260±0.5773 | 240±0.5773 |
| **HB****(g dl-1)** | **Control** | 10.76±0.023 | 11.56±0.323 | 11.68±0.969 |
| 0.064ppm | 4.113±0.1241 | 4.11±0.1241 | 3.8±0.1732 |
| 0.32ppm | 3.79±0.1674 | 3.6±0.11547 | 3.32±0.184 |
| **PCV****(%)** | **Control** | 44.81±0.1732 | 46.83±0.115 | 47.74±0.0577 |
| 0.064ppm | 38.89±0.2309 | 30.81±0.0577 | 28.83±0.2309 |
| 0.32ppm | 27.71±0.0572 | 24.22±0.2309 | 20.84±0.1154 |

**Table 3-** showing the impact of Cypermethrin (sub-lethal doses and control group) on different haematological parameters of *Anabas testudineus* after the 15th, 30th and 45th days of exposure. Data is expressed as mean ± SEM (N=10), p < 0.05.

1. **IMPACT ON WBC (White blood cell)-**

Fish treated with Cypermethrin (25% EC) showed a significant decline in the number of WBC counts. At the beginning of the experiment, it was observed that as the dosing days of the experimental group fishes were increasing, the number of WBC was also increasing in comparison to the control group. But after the 15th day it was noticed that, as the dosing day increased, the WBC count started decreasing with respect to the control group. On the 15th, 30th and 40th day of treatment with the dose 0.064ppm, the mean of the WBC count was 300.33±0.666, 272.33±0.8819, 250.66±1.2018 respectively (p<0.05) in compare to control of mean around 279.66±6.5773 at 45th day. Similarly with the dose of 0.32ppm, on the 15th, 30th, and 40th day of treatment, the mean was observed 320.66±0.819, 260±0.5773, 240±0.5773 respectively (p<0.05) in comparison to the control group. The fish treated with cypermethrin showed a notable reduction in WBC over the course of the 45-day treatment period.

 **(White Blood Cell)-**

**Figure 2-** Showing the impact of Cypermethrin on the WBC of *Anabas testudineus* after the 15th, 30th and 45th days of exposure. Data is expressed as mean ± SEM (N=10), p < 0.05.

1. **IMPACT ON HAEMOGLOBIN (HB%)**

Cypermethrin treatment showed some significant effect on the gram percentage haemoglobin of *Anabas testudineus* fish blood. In the experiment, it was found that as the number of days of Cypermethrin dosage was increased, the haemoglobin level decreased in the experimental fishes in comparison to the control group. On the 15th, 30th and 40th day of treatment with the dose 0.064ppm, the mean of the hb% was 4.113±0.1241, 4.11±0.1241, 3.8±0.1732(g dl-1) respectively (p<0.05) in compare to control of mean around 11.68±0.969 at 45th day. Similarly with the dose 0.32ppm, on the 15th, 30th, and 40th day of treatment, the mean was observed 3.79±0.1674, 3.6±0.11547, 3.32±0.184 (g dl-1) respectively (p<0.05) in compare to control group. The hb% of the fish treated with cypermethrin decreased significantly over the 45-day treatment period.

**(Haemoglobin) -**

**Figure 3**- Showing the impact of Cypermethrin on the Hb level of *Anabas testudineus* after the 15th, 30th and 45th days of exposure Data is expressed as mean ± SEM (N=10) p < 0.05.

1. **IMPACT ON PACT CELL VOLUME (PCV)**:

The effect of cypermethrin was also observed on the pact cell volume of fish *Anabas testudineus.* There was a very significant change in fish pact cell volume due to the dosage of cypermethrin. It was observed that as the dosage days of cypermethrin increased, the percentage of packed cell volume decreased in the experimental group fishes when compared to the control group. On the 15th, 30th and 40th day of treatment with the dose 0.064ppm, the mean of the PCT percentage was 38.89±0.2309, 30.81±0.0577, 28.83±0.2309 respectively (p<0.05) in compare to control of mean around 47.74±0.0577 at 45th day. Similarly with the dose 0.32ppm, on the 15th, 30th, and 40th day of treatment, the mean was observed 27.71±0.0572, 24.22±0.2309, 20.84±0.1154 respectively (p<0.05) in compare to the control group. The percentage of PCV of the fish treated with cypermethrin decreased significantly over the 45-day treatment period.

**(Pact cell volume)**

**Figure 4** - Showing the impact of Cypermethrin on PCV% of *Anabas testudineus* after 15th, 30th and 45th days of exposure Data is expressed as mean ± SEM (N=10) p < 0.05.

**DISCUSSION-**

The haematological parameter of Anabas Testudines subjected to sub-little qualitative of cypermethrin on days 15, 30th and 45th reveal a substantial difference in the blood parameters when compared to the fishes of the control group. Any organism’s health status is generally influenced by haematological parameters (Baker et.al.,2001). They are used in the clinical diagnostic of fish physiology which is best on the interaction between the internal and external physical environment (Adeyemo et.al.,2005). The result demonstrated that the fish exposed to high cypermethrin concentration significantly decreased the RBC count, haemoglobin and PCV levels.

 Some sort of behavioural alteration was also noticed among the fishes treated with sub-little doses. Fishes should have higher behavioural alteration during toxicity assessment tests. The reduction of red blood cells primarily resulted from the development of hypoxic conditions during treatment which in turn led to increased destruction of RBCs or decreased rate of RBC formation due to lack of HB in the cellular medium (Chen et.al., 2004). Changes in the haematological parameters might have been brought about by cypermethrin as an anaemic condition due to decreased synthesis of haemoglobin and RBC numbers in hemopoietic organs (Masud & Singh, 2013) and an increase in the rate at which haemoglobin is destroyed or a decrease in the HB synthesis could possibly due to the cows of a notable drop in HB levels (Reddy & Bashamohideen, 1989).

According to Kumari and Banerjee,1986 factors such as the animal's age stress levels sex and accessibility of food in a given medium mainly affect the RBC count and haemoglobin concentration decline. Lower HB levels and decrease in PCB in fishes exposed to cypermethrin may be due to decreased RBC count (Paul, 2004). Fishes treated with cypermethrin (25% EC) showed a significant decline in the number of WBC counts. At the beginning of the exposure, it was noticed that the WBC count was slightly increased but after 15 days the WBC count suddenly showed a declining trend in comparison to the fish of the control group. An increase in WBC count during the early period of treatment may be due to some pathological response because WBC plays a great role during infestation by stimulating the hematopoietic tissue and the immune system by producing antibodies working as a defence against any infection (Lebelo et.al., 200; Hassen, 2002; Masud & Singh, 2013). During the toxic exposure period of cypermethrin the WBC count was enhanced indicating that fish can develop a defensive mechanism to overcome the toxic stress (Masud & Singh, 2013).

The sudden increase in WBC count can be related to an increase in antibody production which helps in the Survival and recovery of fish exposed to cypermethrin whereas a reduced number of leukocytes in the exposed fishes after the 15th day of treatment can result in reduced disease reduction (Kaattari & Piganelli, 1996; Valmurugan et.al., 2016)

**Behavioural change during sublethal dozing:**

The physiological reactions that can reveal stress and connected to behavioural alteration (Little & finger,1990). Fishes exposed to various pesticides exhibit some behavioural alterations in eating, swimming, predation, species aggressiveness change in colour etc. (Cong et.al., 2008 & 2009)

Most of the experimental fishes during the toxicity test were found less active and expressed erratic swimming movement, which may be due to the stress caused by a sudden change in their habitat condition (Shrivastava et.al., 2010; Chaudhary & Azad, 2024). Change in colour or loss of pigmentation was also recorded among the fishes may be due to dysfunction of the pituitary gland. Under stress causes changes in the number and distribution of chromatophores (Yadav et.al.,2007; Ramesh & Saravanan, 2008). Behavioural anomalies as a result of stress are further analysed at the most sensitive induction of adverse effects of the aquatic environment (Nawani et.al., 2010; Chaudhary & Azad, 2024). Faster opercular activity and loss of equilibrium were noticed among the treated fishes. According to Fulton & Kay (2001), restlessness and hyperactivity in fish occur due to stress or inactivation of the acetylcholinesterase enzyme leading to the accumulation of acetylcholine at the synaptic junction. Finally, fish may be paralyzed or settled on the bottom of the tank (Chaudhary & Azad, 2024). Few alterations like increased opercular activity and hyperactivity were also recorded among the fishes treated with cypermethrin lethal doses may be due to the above-mentioned reason.

**Conclusion:**

Based on findings, the study concluded that Higher pesticide concentrations resulted in significant fatality rates and toxicity levels. Cypermethrin was found to induce behavioural and morphological changes in fish, potentially leading to serious physiological issues and death. Despite utilizing lower dosages than recommended for pest control in the field, all fish species in the tests experienced significant fatality rates. To promote sustainable development, it is recommended to use insecticides at the lowest possible concentration to control insects while also protecting the aquatic and terrestrial environments.

**Ethical approval-**

Animal ethic committee approval has been collected and preserved by author(s).

**Disclaimer (Artificial Intelligence)-**

Author(s) here by declare that NO generative AI technologies such as large language models (ChatGPT, COPILOT etc) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

1. Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S (2004). Effect of cypermethrin and carbofuran on certain hematological parameters and prediction of recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Saf. 58:220-226.
2. Agarwal K, Chaturvedi LD (1995). Anomalies in blood corpuscles of *Heteropneustes fossilis* induced by alachlor and rogor. Adv. Bios. 14: 73-80.
3. Akhtar, N., Srivastava, M.K., Raizada, R.B., 2009. Assessment of chlorpyrifos toxicity on certain organs in rat, *Rattus norvegicus*. J Environ Biol 30, 1047-1053.
4. Anupama and Amit, Haematological alterations in a fresh water fish Anabas testudineus under cypermethrin exposure, International Journal of Biology Sciences 2023; 5(1): 32-35.
5. Awoyinka OA, Omodara TR, Oladele FC, Ajayi DD, In-vitro Protein Digestibility of selected Under Utilized Local Wild Bean and Bio-availability in Rats Model. Academia journal Biotechnology 2019; 7(4): 063-067.
6. B. K. Das and S. C. Mukherjee. “Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences,” Comparative Biochemistry and Physiology, vol. 134C, 109–121, 2003.
7. B. Velmurugan, M. Selvanayagam, E. I. Cengiz, S. Bilici and A. Satar A. “Surface structures of gill, scale and erythrocyte of *Anabas testudineus* exposed to sublethal concentration of cypermethrin,” Environmental Toxicology and Pharmacology, vol. 37, no. 3, 1109-1115, 2014.
8. Bagheri F. Study of pessticial Residue (Diazinon, Azinphosmethyl) in River of Golestan Province (Gorganround and Gharehsou). Open Journal of Marine. 2007; 7(2): 1-125.
9. Bunn KE, Thompson HM, Tarrant KA (1996). Effect of agrochemical on the immune system. J. Bull. Environ. Contam. Toxicol. 57(4): 632 639.
10. Carriquiriborde, P., Díaz, J., Mugni, H., Bonetto, C. and Ronco, A.E., 2007. Impact of cypermethrin on stream fish populations under field-use in biotechsoybean production. Chemosphere, 68: 613-621.
11. Casida, J.E., Gammon, D.W., Glickman, A.H. and Lawrence, L.J., 1983. Mechanisms of selective action of pyrethroid insecticides. Annu. Rev. Pharmacol. Toxicol., 23: 413‒438.
12. Chaudhary D.N. and Azad P.K. (2024). Optimizing Acclimatization for Channa punctatus in Different Laboratory Environments. Asian Journal of Fisheries and Aquatic Research. Vol.26 (12): 70-76.
13. Chen X., Yin D, Hu S, Hou Y (2004). Immunotoxicity of penta chlorophenol on macrophage immunity and IgM secretion of the crucian carp (*Carassius auratus*). J. Bull. Environ. Contam. Toxicol. 73: 153-160.
14. Cong NV, Phuong NT, Bayley M. Effects of repeated exposure of diazinon on cholinesterase activity and growth in snaked head fish (*Channa striata*). Ecotoxicology and Environmental Safety, 2009; 72b: 699-703.
15. Cong, N.V., Phuong, N.T. and Bayley, M. 2008. Brain cholinesterase response in the snakehead fish (*Channa striata*) after field exposure to diazinon. Ecotoxicol. Env. Safety. 71(2): 314-318. https://doi.org/10.1016/j.ecoenv.2008.04.005.
16. F. J. Baker, R. E. Silverton and C. J. Pallister. “Introduction to Medical Laboratory Technology,” 7th edition, Bounty Press Limited, Ibadan, Nigeria, 2001, pp.448.
17. Fırat, O., Cogun, H.Y., Yüzereroğlu, T.A., Gok, G., Fırat, O., Kargin, F. and Kotemen, Y., 2011. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia *Oreochromis niloticus*. Fish Physiol. Biochem., 37: 657-666. https://doi.org/10.1007/ s10695-011-9466-3.
18. Fulton, M.H., Key, P.B., 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ. Toxicol. Chem. 20 (1), 37–45.
19. Georgieva, E., Stoyanova, S., Velcheva, I. and Ancheva, V., 2014. Histopathological alterations in common carp (*Cyprinus carpio* L.) gills caused by Thiamethoxam. Braz. Arch. Biol. Tech., 57: 991- 996. https://doi.org/10.1590/S1516-8913201402582.
20. Grande, M., Anderson, S. and Berge, S. 1994. Effects of pesticides on fish. Norwegian J. Agril. Sci. (Suppl.) 13: 195-209.
21. Hassen FEZM (2002). Studies on diseases of fish caused by Henneguya infestation, Ph.D Thesis, Faculty of Veterinary Medicine, Suez Canal University. Egypt
22. Hoque, M.M., Nahar, Z. and Hossain, M.A. 2000. Toxicity of malathion to silver barb (*Barbodes gonionotus* Bleeker) fingerlings. Bangladesh J. Fish. Res. 4(1): 101-104.
23. I. Orun, Z. Selamoglu Talas, M. F. Gulhan and K. Erdogan. “Role of propolis on biochemical and hematological parameters of *Oncorhynchus mykiss* exposed to cypermethrin,” Journal of Survey in Fisheries Sciences, vol. 1, no. 1, 21-35, 2014.
24. Johnson, M.S., Aubee, C., Salice, C.J., Leigh, K.B., Liu, E., Pott, U. and Pillard, D. 2017 A review of ecological risk assessment methods for amphibians: comparative assessment of testing methodologies and available data. Integr. Environ. Assess. Manage., 18: 91-114.
25. Kadam, P. and Patil, R. 2016. Effect of chlorpyrifos on some biochemical constituents in liver and kidney of fresh water fish, *Channa gachua* (F. Hamilton). Int. J. Sci. Res. 5(4): 1975-1979.
26. Khan, B.A., Farid, A., Khan, N., Rasul, K. and Perveen, K., 2006. b Survey of pesticide use on fruits and vegetables in district Peshawar. Sarhad J. Agric., 22: 497.
27. Kumar, A., et al., 2009. k-cyhalothrin and cypermethrin induced in vivo alterations in the activity of acetylcholinesterase in a freshwater fish, *Channa punctatus* (Bloch). Pesticide Biochemistry and Physiology 93, 96–99.
28. Kumari K, Banergi V (1986). Effect of sublethal toxicity to Zinc, Hg and Cd on peripheral haemogram in *A. testudineus*. Uttar Predesh. J. Zool. 6(2): 241-250.
29. Lebelo SL Saunders DK, Crawford TG (2001). Observations on blood viscosity in striped bass, Morone saxtilis (Walbaum) Associated with fish Hatchery conditions. Kansa Acad. Sci. 104: 183-194.
30. Lermen, C.L., Lappe, R., Crestani, M., Vieira, V.P., Gioda, C.R., Schetinger, M.R.C., Baldisserotto, B., Moraes, G. and Morsch, V.M., 2004. Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*. Aquaculture, 239: 497-507. https://doi. org/10.1016/j.aquaculture.2004.06.021.
31. Little, E.E. and Finger, S.E. 1990. Swimming behaviour as an indicator of sub-lethal toxicity in fish. Env. Toxicol. Chem. 9(1): 13- 16. <https://doi.org/10.1002/etc.5620090103>.
32. M. J. Parma, A. Loteste, M. Campana and C. Bacchetta “Changes of hematological parameters in *Prochilodus lineatus* (Pisces, Prochilodontidae) exposed to sublethal concentration of cypermethrin,” Journal of Biology, vol. 28, no. 1, 147-149, 2007.
33. M. N. Cakmak and A. Girgin. “Toxic effect of a synthetic pyrethroid insecticide (cypermethrin) on blood cells of rainbow trout (*Oncorhynchus mykiss*, Walbaum),” Journal of Biological Science, vol. 8, 694-698, 2003.
34. Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwaha, B., Kumar, P., & Lakra, W. S. (2010). Lethal concentration and toxicity stress of carbosulfan, glyphosate, and atrazine to freshwater air-breathing fish *Channa punctatus*. International Aquatic Research, 2, 105–111.
35. O. K. Adeyemo. “Haematological and histological effects of cassava mill effluent in *Clarias gariepinus*,” African Journal of Biomedical Research, vol. 8, 175-183, 2005.
36. Pascual J.A. and Peris S.J. (1992). "Effects of forest spraying with two application rates of cypermethrin on food supply and on breeding success of the blue tit (*Parus caeruleus*)". Environmental Toxicology and Chemistry. 11 (9): PP- 1271–1280.
37. Paul EA (2004). Toxicity of a synergized formulation of sumithrin to *Daphnia magna*. J. Bull. Environ. Contaim. Toxicol. 72(6): 1285-1289.
38. Pawar, S.M. and Sonawane, S.R. 2014. Seasonal variation in muscle glycogen and moisture content of *Garra mullya* and *Rasbora daniconius*. Int. J. Fauna Biol. Stud. 1(5): 91-94.
39. R. Sarikaya. ‘’ Investigation of acute toxicity of alpha-cypermetrhin on adult Nile tilapia (*Oreochromis niloticus* L.),” Turkish Journal of Fisheries and Aquatic Sciences, vol. 9, 85–89, 2009.
40. Ramesh, M., and Saravanan, M., 2008. Haematological and biochemical responses in a freshwater fish Cyprinus carpio exposed to chlorpyrifos. International Journal of Integrative Biology, 3, 80–83.
41. Reddy MP, Bashamohideen M (1989). Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio.* Acta Hydrochim. Hydrobiol. 17:101-107.
42. S. L. Kaattari and J. D. Piganelli. “The specific immune system: humoral defense,” in: G. Iwama, G. and T. Nakanishi (Eds.), The Fish Immune System: Organism, Pathogen and Environment, Academic Press, San Diego, USA, 1996, pp. 207–254.
43. S. Masud and I. J. Singh. “Effect of cypermethrin on some hematological parameters and prediction of their recovery in a freshwater teleost, *Cyprinus carpio*,” African Journal of Environmental Science Technology, vol. 7, no. 9, 852-856, 2013
44. S. P. Bradbury and J. R. Coats. “Comparative toxicology of the pyrethroid insecticides,” Research Reviews, 108: 133-178, 1989.
45. Saxena KK, Seth N (2002). Toxic effects of cypermethrin on certain hematological aspects of fresh water fish *Channa punctatus*. Bull. Environ. Contam. Toxicol. 69:364-369.
46. Sharmin, S., Salam, M.A., Haque, M.A. and Shahjahan, M. 2014. Toxicity bioassay of organophosphorous pesticide malathion in common carp, *Cyprinus carpio*. pp. 99-100. In: Proc. 5th International Conference on Environmental Aspects of Bangladesh.
47. Siddiqa, A., Islam, M.J., Rahman, M.S., Uddin, M.N. and Fancy, R. 2016. Assessing toxicity of organophosphorus insecticide on local fish species of Bangladesh. Int. J. Fish. Aqu. Stud. 4(3): 670-676.
48. Srivastava, G.; Singh, K.; Tiwari, M.N.; Singh, M.P. Proteomics in Parkinson’s disease: current trends, translational snags and future possibilities. Expert Rev. Proteom., 2010, 7, 127-139.
49. Thenmozhi, C., Vignesh, V., Thirumurugan, R. and Arun, S. 2011. Impacts of malathion on mortality and biochemical changes of freshwater fish *Labeo rohita*. Iranian J. Env. Health Sci. Eng. 8(4): 325-332.
50. Ullah, R., Zuberi, A., Naeem, M. and Ullah, S., 2015. Toxicity to hematology and morphology of liver, brain and gills during acute exposure of Mahseer (*Tor putitora*) to cypermethrin. Int. J. Agric. Biol., 17: 199‒204.
51. US EPA (United States Environment Protection Agency). “Pesticide fact sheet 199: Cypermethrin.” Office of pesticides and toxic substances, Washington, 1989.
52. Verma SR, Sarita R, Dalela RC (1982). Indicators of stress induced by pesticides in *Mystus vittatus* Haematological parameters. Indian J. Environ. Health 24: 58-64.
53. Yadav, A., Neralia, S., & Gopesh, A. (2007). Acute toxicity levels and ethological responses of *Channa punctatus* to fertilizer industrial wastewater. Journal of Environmental Biology, 28, 159–162.
54. Zahran, E., Risha, E., Awadin, W. and Palic, D., 2018. Acute exposure to chlorpyrifos induces reversible changes in health parameters of Nile tilapia (*Oreochromis niloticus*). Aquat. Toxicol., 197: 47– 59. <https://doi.org/10.1016/j.aquatox.2018.02.001>.
55. Bhardwaj, J. K., Kamboj, H., & Tyor, A. K. (2022). The Toxicity of Imidacloprid on early embryonic stages and growth rate of hatchlings of common carp, Cyprinus carpio. Toxicology International, 29(1), 105-115.
56. Bhardwaj, J.K., Harkrishan., Tyor, A.K., (2020). Sublethal effects of imidacloprid on haematological and biochemical profile of freshwater fish, cyprinus carpio. J. Adv. Zool. 41 (1&2), 75-88.
57. ANUPAMA, ., & JHA, A. K. (2020). EFFECTS OF CYPERMETHRIN ON HISTOPATHOLOGY OF GONADS OF A FRESH WATER FISH Anabas testudineus (Bloch.). UTTAR PRADESH JOURNAL OF ZOOLOGY, 41(10), 46–50. Retrieved from <https://mbimph.com/index.php/UPJOZ/article/view/1610>
58. Gupta, P., & Srivastava, N. (2006). Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish Channa punctatus(Bloch). Journal of Environmental Biology, 27(2), 211-215.