***Original Research Article***

**Study of Toxicity, Haematological parameter and Behavioural changes in a fresh water air breathing fish *Anabas testudineus* (Bloch) exposed to Cypermethrin**

**ABSTRACT**: Pesticides are frequently used by farmers to control the pest in the agriculture fields. These pesticides directly or indirectly affecting the aquatic ecosystem including fishes. The present work was carried out to study and evaluate the toxicity of Cypermethrin (25% EC) a common synthetic pyrethroid pesticide frequently used in agriculture fields, in a fresh water air breathing fish *Anabas testudineus* in laboratory condition. 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 concentration to determine some haematological parameters (RBC, WBC, Hb, PCV) in selected fishes. The impact was evaluated using comparative data of control group with experimental groups of fishes subjected to sub lethal concentration of Cypermethrin. The exposure duration was 15, 30 and 45 days respectively. The number of RBC, Haemoglobin percentage and Haematocrit value of treated fishes was found significantly reduced (p< 0.001) comparison 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. Increase in WBC may be due to immune response. During exposure of Cypermethrin, some behavioural alterations (restlessness, erratic movement, hyperactivity) were also recorded, may be due to the effect of stress caused due to sudden habitat change or due to inactivation of specific enzyme acetylcholinesterase activity.

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Key words: 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 the 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 natural ecosystem is increasing day by day. Chemical analysis can identify existence of harmful substances in the environment, but it cannot reveal how those substances will effect 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 the 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 in the gradual degradation of the aquaculture and ecosystem cannot be ignored (Kadam and Patil, 2016)

 Fishes are very rich source of protein and easily digestible food for human beings. It plays a vital role to fulfil our nutritional demand and increase our total production (Pawar and Sonawane, 2014). However, nowadays due to different natural and anthropogenic activities, the number of fishes is declining. (Siddiqua, 2016). Among the different causes of fish decrease, use of pesticides is one of the important issues. Many authors studied the impact of different pesticides on fresh water 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 by different ways such as rain fall, 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 effect to 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). Cypermethrin is extremely poisonous for fishes (Bradbury and Coats 1989). Between 0.7 and 350 µg L-1 are 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 are sprayed in the fields ultimately reaches into water bodies, causing serious threats to aquatic life particularly to fishes (Akhtar *et al*., 2021). Fishes due to its aquatic habitat is directly exposed to environmental noxiousness including harmful insecticides which effects its profitable worth and rearing ability (Firat *et al*., 2011; Georgieva *et al*., 2014; Akhtar *et al*., 2021).

 Cypermethrin has drastic effect on both invertebrates and vertebrate’s species (Das and Mukharjee., 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 health status of the fish (Lerman *et al*., 2004). The evaluation of blood parameters is very effective in detecting the effect of pesticide in fishes. 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. Fresh water fish *Anabas testudineus* were collected from local fish market of Nathnagar & Bhagalpur (Bihar) and bought 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 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 required amount of distill 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 distill 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, 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 fishes were removed from the tank.

 In the present study LC50 value of for fish *Anabas testudineus* were calculated for 96 hours of exposure time by Probit Analysis. Probit analysis is specialized regression model of binomial response variable. Regression is a technique for analyzing data by fitting line to it in order to compare variable, 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 aquarium. To guarantee outcome, the current study was carried out three times. Ten fishes were used per replicates 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 by high pesticide concentration. The 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 concentration of pesticide in ppm (parts per million) taken for the determination of LC50 and fishes (in number and in percentage) died at different interval of time at different concentration. LC50 is calculated as 3.2ppm.

Graph 1; showing normal probability between the numbers of fish died and sample percentile.

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

**BEHAVIORAL RESPOSNSE DURING TOXICITY ASSESEMENT**

 Over the course of 96 hours, fishes (*Anabas testudineus*) showed some alterations in their behaviour. Over the course of the trial, the control group shows 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 coloration.

**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 concentration 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**: showing the increase or decrease in the level of behavioural parameters is shown by numbers of (+) sign. –, 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 fishes *Anabas testudineus* showed very little alteration in behaviour. Normal behaviour was observed in the fishes of 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 day), some changes began to appear in the behaviour of the experimental fishes like hyperactivity, increase in grasping and settling at the bottom of the experimental tank. Gradual color change was also noticed in the fishes treated with Cypermethrin sub lethal doses (0.32 & 0.064ppm). No mortality was recorded during the course of experiment.

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

Fish treated with Cypermethrin (25% EC) showed a significant decline in the number of RBC. It was observed that as the duration of treatment increased, the number of RBC showed a decline trend. At the 15th, 30th and 40th day of treatment with the dose 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 0.32ppm, at the 15th, 30th, 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 of number of RBC was observed in the fishes treated with cypermethrin for the duration of 45 day of treatment.

**(Red Blood Cell)**

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

**Figure 1**- Showing the impact of Cypermethrin on RBC of Anabas testudineus after 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 parameter of *Anabas testudineus* after 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 count. At the beginning of the experiment, it was observed that as the dosing days of experimental group fishes were increasing, the number of WBC was also increasing in compare to control group. But after 15th day it was notice that, as the dosing day increased, the WBC count started decreasing with respect to control group. At 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 0.32ppm, at the 15th, 30th, 40th day of treatment, the mean was observed 320.66±0.819, 260±0.5773, 240±0.5773 respectively (p<0.05) in compare to 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 WBC of *Anabas testudineus* after 15th, 30th and 45th days of exposure. Data is expressed as mean ± SEM (N=10), p < 0.05.

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

Cypermethrin treatment showed some significant effect on the gram percentage hemoglobin of *Anabas testudineus* fish blood. In the experiment, it was found that as the number of days of Cypermethrin dosage was increased, the hemoglobin level decreased in the experimental fishes in compare to control group. At 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, at the 15th, 30th, 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 Hb level of *Anabas testudineus* after 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 compare to control group. At 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, at the 15th, 30th, 40th day of treatment, the mean were observed 27.71±0.0572, 24.22±0.2309, 20.84±0.1154 respectively (p<0.05) in compare to control group. The percentage 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 day 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 in concentration significantly decreased in 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 higher behavioural alteration during toxicity assessment test. The reduction of red blood cells primarily resulted from the development of hypoxic condition during treatment which intern lead to increase destruction of RBCs or decrease 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 decrease synthesis of haemoglobin and RBC numbers in hemopoietic organs (Masud & Singh, 2013) and increase in the rate at which haemoglobin is destroyed or a decrease in the HB synthesis could possibly due to the cows of notable drop in HB levels (Reddy & Bashamohideen, 1989).

According to Kumari and Banerjee,1986 factors such as the animals age stress levels sex and accessibility of food in a given medium main effect the RBC count and haemoglobin concentration decline. Lower HB level and decrease in PCB in fishes exposed to cypermethrin in may be due to decrease RBC count (Paul, 2004). Fishes treated with cypermethrin (25% EC) showed the significant decline in the number of WBC count. 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 decline trend in comparison to the fish of control group. Increase in WBC count during early period of treatment may be due to some pathological response because WBC play a great role during infestation by stimulating the hematopoietic tissue and the immune system by producing antibodies working as defence against any infection (Lebelo et.al., 200; Hassen, 2002; Masud & Singh, 2013). During toxic exposure period of cypermethrin in the WBC count were 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 with an increase in antibody production which helps in Survival and recovery of fish exposed to cypermethrin whereas reduced number of leukocytes in the exposed fishes after 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 the 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, may be due to the stress caused by 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 causing changes in the number and distribution of chromatophore (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 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), the restlessness and hyperactivity in fishes occurs due to stress or inactivation of acetylcholineasterase enzyme leading to accumulation of acetylcoline at synaptic junction. Finally, fishes 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 maybe due to the above mention reason.

**Conclusion:**

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.

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