**Effect of Delthamethrin on the activities of the alkaline and acid phosphatases in the air breathing fish *Clarias batrachus* (Linn.)**

**ABSTRACT-**

Intensive use of pyrethroids as a new class of agricultural insecticides to control agricultural pesticides crops has concomitantly increased the level of pesticides in aquatic ecosystems. It is against this backdrop that the present study was undertaken, which reflects the changes in the activities of alkaline and acidic phosphatases in the plasma, liver and kidney of the fish *Clarias batrachus* exposed to sub-lethal (0.145ppm) concentration of deltamethrin, a synthetic pyrethroids for a period of 35 days. A significant (P<0.05) increase, amounting to 92 %, in alkaline phosphatase (ALP) activity, was observed in plasma of fish treated with sub-lethal doses of deltamethrin when compared to control group. However, due to exposure to deltamethrin, the activity of acid phosphatase (ACP) decreases significantly (P<0.05) in the liver and kidney. The result of the present study suggest that the tested concentrations of deltamethrin cause significant adverse effects on the enzymological parameters of air-breathing fish *C. batrachus*. The alterations of these parameters can be effectively used to monitor the impact of deltamethrin on aquatic ecosystems.

**Keywords:** Alkaline phosphatase, Acid phosphatase, Deltamethrin, *Clarias batrachus*, Aquatic ecosystem.

1. **INTRODUCTION-**

In the past few decades, there has been a large-scale indiscriminate use of pesticides, including the synthetic pyrethroids to meet the food requirements of the rising population. These are used to control the harmful targeted organisms such as insects, weeds, mollusks, harmful bacteria and viruses (Pathak et. al., 2022; Mohamed et al., 2020). In fact, pesticides affect the whole ecosystem. Of late, synthetic pyrethroids are preferred over organochlorine, organophosphate and carbamates due to their proven low toxicity to mammals and, hence, are widely used in India (Devi and Gupta, 2014). Deltamethrin is a synthetic type II pyrethroid insecticide, classified as a moderately toxic substance (WHO Class II) used widely in agriculture and forestry against a broad spectrum of insect pests due to their short biodegradation period and low tendency to accumulate in organisms (Velısek et al*.,* 2007; Marques et al*.,* 2014). Synthetic pyrethroids in general and deltamethrin in particular have been reported to be highly toxic to fishes (El-Sayed et al., 2007; Jiang et al., 2021; Banaei et al., 2022), due to deficiency of the enzymes for their hydrolysis required and also due to their high rate of absorption through gills makes these insecticides highly susceptible to ecosystem toxicity (Haya, 1989; Sayeed et al*.,* 2003).

Keeping the above facts in view, the present study was undertaken which reports the changes in the activity of the key enzymes, namely alkaline and acid phosphatase, under lethal and sub-lethal exposure of the air breathing fish *Clarias* *batrachus.*

The selection of this fishas anexperimental fish for the present study was in consideration of the fact that this fish can survive for a longer period in laboratory conditions due to the presence of air-breathing organs besides its medical and convalescence value.

1. **MATERIAL AND METHODS-**

**2.1-Procurment, Acclimation and Feeding of fish**

The fish *C. batrachus* (average length: 30-40 cm and average weight: 45-60±2 g) were purchased from a local fish market and were transported to a laboratory in wide-mouthed earthen pots. They were first rinsed in 0.1% KMnO4 to remove any dermal infection. Fishes were acclimatized to laboratory conditions for 15 days in a large 60 L glass tank under natural photoperiod and room temperature. No aeration was provided. They were fed *ad libitum* with standard fish meal (rice bran and groundnut oil cake) every day at 11:30 AM. Water was renewed every day before renewal of the exposure medium to avoid accumulation and contamination of excretory materials.

**2.2-Pesticides-**

Deltamethrin (C22H19Br2NO3) (25% EC), a technical grade broad spectrum insecticide manufactured by Bayer Crop Science Ltd., Gujarat, India was used for evaluation of its toxicity.

 

**2.3-Lethality-**

To determine the LC50 concentration, ten tanks of 20 L capacity, each filled with 10 L of water, were taken. Ten well-acclimated fish in each of the tanks (A to K) were transferred from the acclimatizing tank to the experimental tanks with the help of a hand net. Tank-A was designated as control and Tanks-B to K as experimental ones. Ten different concentrations of deltamethrin were poured into each of the experimental tanks. The mortality rate/ percentage was counted for 24 hr, 48 hr., 72 hr., and 96 hr. respectively, at each of the concentrations. Feeding was stopped during acute toxicity bioassay.

The LC50 value was calculated by probit regression analysis method of Finney (1978). Determination of experimental dose (0.145ppm) for chronic experimentation was done following the method of Hart et al., (1945).

**2.4-Experimental Design**

Two aquaria (A+B) each filled with 10 L of water were taken. A was designated control and B the experimental one. Ten well-acclimated fish were transferred to each of the aquariums. The feeding schedule was strictly maintained each day.

Fish from tank-B were exposed to a sub-lethal dose of deltamethrin (0.145 ppm) for a period of 35 days. The exposure medium was renewed every 96 hr. in order to maintain an effective concentration of the test chemical.

 At the end of 35 days, fish of both control and treated groups were anesthetized with 1:4000 MS 222 (Tricane, methane, sulfonate, sandoz) and blood was collected by gill puncture from the fish for determining alkaline phosphatases and dissected out to test tissues (liver and kidney) for acid phosphatases estimated by the method of King and Jegatheesan (1959).

The blood samples were centrifuged at 12,000 g for 3 min at room temperature to harvest the plasma. The harvested plasma was utilized for ALP studies.

**2.5-Statistical analysis-**

The LC50 for 24 hr. to 96 hr. were calculated by probit analysis method of Finney (1978). The significance of data obtained from sample between control and deltamethrin treated fish was tested using student-t test.

**RESULTS-**

ALP percent change was increased significantly in plasma of fish exposed to deltamethrin for a period of 35 days. The increase was 15.3%, 24.5%, 17.6%, 70.00% and 92.00% over that of the control values on 7, 14, 21, 28 and 35 days respectively (-1). The enzyme activity was found to be decreased in deltamethrin-treated fish throughout the study period when compared with the control groups (P< 0.05) (-1). A significant (P < 0.05) difference in ACP and ALP activities was found among the treatments.

A significant decrease in ACP activity of 25.5, 40.9, 43.18, 50 and 60.86 percentages was found in both livers of *C. batrachus*respectively over a time period of 7-35 days (-2) when compared to control groups. In sub-lethal treatment, in the exposure period from 7 to 35 days, the percentage decrease is 43.2 to 77.14 in the experimental group as compared to the control group. In the liver as well as kidney, a maximum percent inhibition in ACP was 60.86 and 77.14 of ACP activity was observed after 35 days, respectively (-2).

1. **DISCUSSION-**

A fundamental goal to testing ecotoxicology is commonly used to evaluate the toxic effect of chemical pesticides on aquatic ecosystems and to monitor the water quality (Brugs et al., 1977). Limited information reported regarding sub-lethal toxicity of chemical pesticides against air-breeding fish. The findings of the present study to an increase in alkaline phosphatase (ALP) activity and decreased acid phosphatase (ACP) activity in lethal and sub-lethal concentrations were supported by previous work (Bradbnury and Coats, 1989; Datta and Kaviraj, 2003; Svobodava et al., 2003).

“Pyrethroids act mainly on the voltage-dependent Na+ channels of the nerve cell membrane and induce toxicity” (Oliveira et al*.,* 2012). “In addition, the oxidative stress caused by deltamethrin also induces toxic effects in the physiological system of fish” (Yonar and Sakin, 2011). “Any environmental disturbance can be considered to be a potential source of stress and can be detected by changes of hormone concentrations in plasma” (Donaldson, 1981). “In general, the LC50 value varies with respect to species and size of fish. Deltamethrin induces toxic effects in fish through its high rate of gill absorption, lipophilicity and deficiency in fish, enzyme system to hydrolyze pyrethroids” (Viran et al*.,* 2003). These insecticides act mainly on the voltage-dependent Na+ channels of the nerve cell membrane and induce toxicity.

“The enzyme acid phosphatase is a lysosomal enzyme that hydrolyzes the phosphoesters in acidic medium” (Agrahari and Gopal, 2009) and “possesses different properties in different biological materials” (Sarsiek et al*.,* 2005). “Similarly, alkaline phosphatase catalyzes dephosphorylation of many molecules at alkaline pH. In this study, a significant decrease in the levels of ACP in both the liver and kidney of *C. batrachus* exposed to lethal and sub-lethal treatments of deltamethrin might have resulted from the increase of glycogenolysis or damage in the kidney and liver” (Saha and Kaviraj, 2009; Adeyemi et al*.,* 2010). The present work supported by Guardiola et al*.,* (2014) found “deleterious morphological changes in the liver of gilthead sea bream (*Sparus* *aurata* L.) upon exposure to deltamethrin. Increased level of ALP activity in blood plasma is an indicator of hepatic tissue damage, kidney dysfunction and bone disease” (Barse et al*.,* 2006). “In the present investigation, a significant higher level of plasma ALP activity in Indian *C. batrachus* may be due to damage in the tissues of liver and kidney and an increase in the lysosomal mobilization by deltamethrin toxicity” (Rao, 2006). “In general, synthetic pyrethroids are neuropoisons and can act on the axons in the peripheral and central nervous systems by interacting with sodium channels” (Narahashi, 1982).

The observations made in the present study will be a baseline to initiate in-depth studies and would help to guide insecticide-based strategies. In addition, broader monitoring of synthetic pesticides is required as a continuous process to determine the behavioral, physiological and biochemical effect on aquatic ecosystem organisms.

1. **Conclusion:**

From the above findings, it is concluded that stressful environmental conditions, especially hypoxia, affect the normal metabolic activities in *C. batrachus*. The results of the present study conclude that the exposure of Indian air-breathing fish to acute and sub-lethal concentrations of deltamethrin has significantly altered the enzymological (ALP and ACP) responses. The present study reports that deltamethrin is a highly toxic pesticide to Indian air-breathing fish and the presence of deltamethrin even at very low concentrations in the aquatic environments may cause harmful effects on aquatic organisms.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that no generative AI technologies such as large language, models (Chat GPT,COPILOT etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**Table 1: Changes in the plasma ALP activity in an Indian air breathing fish (*Clarias batrachus*) treated with a sub-lethal concentration of deltamethrin (0.145 ppm) for the period of 35 days**

|  |  |
| --- | --- |
| **Exposure period****In days** | **ALP activity in plasma (U/L)** |
| **Control**  | **Experiment**  | **Percent change**  |
| 7 | 95.80 ± 0.50 | 113.9 ±3.0 | + 15.3 |
| 14 | 94.42 ± 0.50 | 120.0 ± 2.5 | + 24.5 |
| 21 | 92.25 ± 0.40 | 148.5 ± 3.6 | + 17.6 |
| 28 | 93.17 ± 0.01 | 160.45 ± 4.5 | + 70 |
| 35 | 94.12 ± 0.05 | 183.2 ± 0.6 | + 92 |

Values are mean ± S.E of five individual observations. (+) denotes increase percentage over control. Means in a column bearing same letter do not differ significantly according to DMRT (p < 0.05)

**Table 2: Changes in ACP activity in liver and kidney of an Indian air breathing fish (*Clarias batrachus*) treated with a sub-lethal concentration of deltamethrin (0.145 ppm) for the period of 35 days**

|  |  |  |
| --- | --- | --- |
| **Exposure period****In days** | **ACP activity in Liver (U/L)** | **ACP activity in Kidney (U/L)** |
| **Control**  | **Experiment**  | **Percent change (%)** | **Control**  | **Experiment**  | **Percent change (%)** |
| 7 | 2.0 ± 0.01 | 1.49 ± 3.0 | - 25.5 | 3.7 ± 0.01 | 2.1 ± 0.01 | - 43.20 |
| 14 | 2.2 ± 0.01 | 1.30 ± 2.5 | - 40.9 | 3.6 ± 0.01 | 1.9 ± 0.01 | - 47.21 |
| 21 | 2.2 ± 0.01 | 1.25 ± 3.6 | - 43.18 | 3.6 ± 0.01 | 1.5 ± 0.01 | - 58.33 |
| 28 | 2.3 ± 0.01 | 1.15 ± 4.5 | - 50 | 3.6 ± 0.01 | 1.1 ± 0.01 | - 69.44 |
| 35 | 2.3 ± 0.01 | 0.9 ± 0.6 | - 60.86 | 3.5 ± 0.01 | 0.8 ± 0.01 | - 77.14 |

Values are mean ± S.E of five individual observations. (-) denotes decrease percentage over control

Means in a column bearing same letter do not differ significantly according to DMRT (p < 0.05)

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