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# Intoxication of Alphamethrin on the Haematology of *Channa punctatus*

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## ABSTRACT

A bioassay experiment was conducted to examine the effect of alphamethrin on some blood parameters of *Channa punctatus*. Four concentrations viz. 0.0015625, 0.003125, 0.0125 and 0.025 ppm of alphamethrin was prepared. Lethal value was obtained at 0.025 ppm and all the experimental fish died within 24 hrs. At sublethal concentrations, alphamethrin induced remarkable changes in protein concentration, blood density, bleeding time and clotting time of the fish. Protein concentration was decreased after exposure to 0.0125 ppm alphamethrin for 30 days, while little change was observed in the blood density of fish due to the exposure to alphamethrin, but bleeding time and clotting time were enhanced. The changes become more significant with the increase in concentration of alphamethrin and exposure period. The results suggest that alphamethrin is capable of inducing alterations in the normal haematological values of *Channa punctatus*. Possible mode of action of alphamethrin in *Channa punctatus* is discussed.

## 1) INTRODUCTION

Alphamethrin [3-(2,2-dichlorophenyl)-2,2-Dimethyl, cyno (3-Phenoxyphenyl) methyl ester] is an important member of the family of synthetic pyrethroids which are being used very commonly in agriculture and forestry to control several pests of various crops and other plants. But at the same time these compounds when reach in water bodies with runoffs cause toxicity in aquatic non target organisms including fish [1, 2, 3, 4, 5, 6, 7, 8, 9, 10] and their toxicity level increases as we move up in the food chain [11]. The persistence of toxic pyrethroids in aquatic environment is dangerous and toxicity to fish is harmful to human being through fresh water edible fishes.

The study of toxicity to organisms is an essential first step for the evaluation of the effect of pesticides for achieving the objective viz. to determine the safer concentration and to formulate the safe application of pesticides [12, 13, 14]. Due to the highly susceptibility of blood towards external and internal environment, it is known as pathophysiological reflector and physicomorphological changes in blood indicate the alterations in the quality of environment and play an important role in the diagnosis of the functional status of the exposed fish. Therefore, the present work is done to evaluate the effect of alphamethrin on fresh water fish *Channa punctatus* by studying the changes in the haematology of this fish.

## 2) MATERIALS AND METHODS

*Channa punctatus* was obtained from local water bodies and acclimatized in the laboratory in aquarium for 10 days and then divided in four alphamethrin treated groups and one control group in different aquaria containing 0.0015625, 0.001325 and 0.0125ppm of alphamethrin above these concentrations the doe become lethal for the exposed fish. The fish were reared till their death or a maximum period of 30 days whichever was earlier. During this period fish were sacrificed at an interval of 5days to examine the effect of alphamethrin on their hematology.

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Blood was collected by cutting the caudal peduncle, using heparin as anticoagulant. Protein concentration of the blood was estimated by the method of Lowry et al [15] and the density of blood was measured by chloroform benzene method. Bleeding Time and Clotting Time were recorded by capillary tube method.

**Table 1:- Effect of alphamethrin on protein concentration ( in μg/ml ) of *Channa punctatus***

| Conc. of Alphamethrin in ppm | 0 DAY | 5 DAYS | 10 DAYS | 15 DAYS | 20 DAYS | 25 DAYS | 30 DAYS |
|---|---|---|---|---|---|---|---|
| Control | 107.50 ± 5.99[a] | 105.00 ± 5.92<br>2.32[b] | 104.17 ± 5.89<br>3.10 | 102.50 ± 5.84<br>4.65 | 105.00 ± 5.92<br>2.32 | 101.66 ± 5.82<br>5.43 | 106.67 ± 5.96<br>0.77 |
| 0.0015625 ppm | - | 95.83 ± 5.65<br>8.73 | 92.50 ± 5.55<br>11.20 | 86.67 ± 5.37<br>15.44 | 82.50 ± 5.24<br>21.43 | 79.17 ± 5.14<br>22.12 | 73.33 ± 4.94<br>31.25 |
| 0.003125 ppm | - | 92.50 ± 5.55<br>11.90 | 86.67 ± 5.37<br>16.80 | 82.50 ± 5.24<br>19.51 | 77.50 ± 5.08<br>26.19 | 74.17 ± 4.97<br>27.04 | 71.67 ± 4.89<br>32.81 |
| 0.0125 ppm | - | 88.33 ± 5.43<br>15.88 | 85.00 ± 5.32<br>18.40 | 80.00 ± 5.16<br>21.95 | 77.50 ± 5.08<br>26.19 | 74.17 ± 4.97<br>27.04 | 70.00 ± 4.83<br>34.38 |

a = mean of 6 individuals ± S.E.
b = percent decrease in protein concentration

**Table 2:- Effect of alphamethrin on blood density ( in gm/ml ) of *Channa punctatus***

| Conc. ofAlphamethrin in ppm | 0 DAY | 5 DAYS | 10 DAYS | 15 DAYS | 20 DAYS | 25 DAYS | 30 DAYS |
|---|---|---|---|---|---|---|---|
| Control | 1.08 ± 0.6[a] | 1.08 ± 0.60<br>0.00[b] | 1.08 ± 0.60<br>0.00 | 1.08 ± 0.60<br>0.00 | 1.08 ± 0.60<br>0.00 | 1.08 ± 0.60<br>0.00 | 1.08 ± 0.60<br>0.00 |
| 0.0015625 ppm | - | 1.07 ± 0.60<br>0.93 | 1.06 ± 0.60<br>1.85 | 1.05 ± 0.60<br>2.78 | 1.04 ± 0.16<br>3.41 | 1.01 ± 0.23<br>6.11 | 0.99 ± 0.15<br>8.30 |
| 0.003125 ppm | - | 1.04 ± 0.58<br>3.70 | 0.98 ± 0.58<br>9.63 | 0.98 ± 0.58<br>9.63 | 0.98 ± 0.58<br>9.63 | 0.91 ± 0.58<br>15.55 | 0.91 ± 0.58<br>15.55 |
| 0.0125 ppm | - | 0.98 ± 0.58<br>9.63 | 0.91 ± 0.58<br>15.55 | 0.91 ± 0.58<br>15.55 | 0.91 ± 0.58<br>15.55 | 0.91 ± 0.58<br>15.55 | 0.91 ± 0.58<br>15.55 |

a = mean of 6 individuals ± S.E.
b = percent decrease in blood density

**Table 3:- Effect of alphamethrin on bleeding time ( in Seconds ) of *Channa punctatus***

| Conc. of Alphamethrin in ppm | 0 DAY | 5 DAYS | 10 DAYS | 15 DAYS | 20 DAYS | 25 DAYS | 30 DAYS |
|---|---|---|---|---|---|---|---|
| Control | 18.50 ± 2.48[a] | 15.33 ± 2.26<br>17.13[b] | 16.33 ± 2.33<br>11.73 | 16.17 ± 2.32<br>12.59 | 18.50 ± 2.48<br>0.00 | 19.67 ± 2.56<br>6.32 | 24.67 ± 2.87<br>33.35 |
| 0.0015625 ppm | - | 17.33 ± 2.40<br>13.05 | 18.50 ± 2.48<br>13.29 | 20.00 ± 2.58<br>23.68 | 21.50 ± 2.68<br>16.22 | 22.67 ± 2.75<br>15.25 | 24.33 ± 2.85<br>1.38 |
| 0.003125 ppm | - | 18.67 ± 2.49<br>21.79 | 20.33 ± 2.60<br>24.49 | 20.83 ± 2.63<br>28.82 | 22.00 ± 2.71<br>18.92 | 18.33 ± 2.47<br>6.81 | 23.50 ± 2.80<br>4.74 |
| 0.0125 ppm | - | 20.83 ± 2.63<br>35.88 | 22.00 ± 2.71<br>34.72 | 23.17 ± 2.78<br>43.29 | 25.00 ± 2.89<br>35.13 | 26.33 ± 2.96<br>33.86 | 29.17 ± 3.12<br>18.24 |

a = mean of 6 individuals ± S.E.
b = percent increase in bleeding time

**Table 4:- Effect of alphamethrin on clotting time ( in Seconds ) of *Channa punctatus***

| Conc. of Alphamethrin in ppm | 0 DAY | 5 DAYS | 10 DAYS | 15 DAYS | 20 DAYS | 25 DAYS | 30 DAYS |
|---|---|---|---|---|---|---|---|
| Control | 12.50 ± 2.04[a] | 12.33 ± 2.03<br>1.36[b] | 13.00 ± 2.08<br>4.00 | 12.83 ± 2.07<br>2.64 | 15.83 ± 2.30<br>26.64 | 16.00 ± 2.31<br>28.00 | 18.17 ± 2.46<br>45.36 |
| 0.0015625 ppm | - | 13.50 ± 2.12<br>9.49 | 15.33 ± 2.26<br>17.92 | 17.00 ± 2.38<br>32.50 | 18.50 ± 2.48<br>16.87 | 19.67 ± 2.56<br>22.94 | 20.67 ± 2.62<br>13.76 |
| 0.003125 ppm | - | 15.17 ± 2.25<br>23.03 | 16.50 ± 2.34<br>26.92 | 18.50 ± 2.48<br>44.19 | 15.83 ± 2.30<br>0.00 | 15.00 ± 2.24<br>6.25 | 20.00 ± 2.58<br>10.07 |
| 0.0125 ppm | - | 17.17 ± 2.39<br>39.25 | 18.33 ± 2.47<br>41.00 | 19.67 ± 2.56<br>53.31 | 21.67 ± 2.69<br>36.89 | 23.50 ± 2.80<br>46.87 | 25.50 ± 2.91<br>40.34 |

a = mean of 6 individuals ± S.E.
b = percent increase in clotting time

## 3) RESULTS AND DISCUSSION

The results of the present studies are summarized from **Table 1 to Table 4**. Protein concentration (34.38%) and Blood density (15.55%) were reduced in experimental fishes after exposure to 0.0125 ppm alphamethrin for 30 days (**Table 1 and 2** respectively). Bleeding time (43.29%) and clotting time (53.31%) were also increased in experimental fishes after exposure to 0.0125 ppm alphamethrin for 30 days (Table 3 and Table 4 respectively). These changes were greater and quicker with increase in the dose of alphamethrin.

**Table no. 5, 6, 7 and 8** for ANOVA of protein concentration, blood density, bleeding time and clotting time respectively shows that the data are significant for these concentrations of alphamethrin.

The behavioral response of *Channa punctatus* towards alphamethrin was totally dependent on concentration and period of exposure. When *Channa punctatus* was exposed to high concentrations of alphamethrin, secretion of mucus from the body was observed. They showed hyper-excitability, increased aerial excursion and increased opercular movements. Fishes often showed jerky movements and tried to jump out of the aquaria. Subsequently fish became lethargic progressively and they lost their balance. Ultimately, the fish sank to the bottom and died [21].

Data presented here show that exposure to alphamethrin caused significant changes in protein concentration of *Channa punctatus*. Depletion in tissue protein in fishes exposed to some pesticides was reported by several workers [22, 23, 24].

**Table 5:- ANOVA for protein concentration due to the effect of alphamethrin on *Channa punctatus***

| SOV | DF | SS | MS | F | $F_{0.05}$ |
|---|---|---|---|---|---|
| Concentration | 3 | 2768.82 | 922.94 | 0.0856 | 3.16 |
| Days | 6 | 1049.52 | 174.92 | 0.0162 | 2.66 |
| Error | 18 | 193914.32 | 10773.017 | | |
| **Total** | 27 | 197732.66 | | | |

**Table 6:- ANOVA for blood density due to the effect of alphamethrin on *Channa punctatus***

| SOV | DF | SS | MS | F | $F_{0.05}$ |
|---|---|---|---|---|---|
| Concentration | 3 | 0.0139 | 0.0046 | 0.0030 | 3.16 |
| Days | 6 | 0.0015 | 0.0002 | 0.0001 | 2.66 |
| Error | 18 | 27.4359 | 1.5242 | | |
| **Total** | 27 | 27.4513 | | | |

**Table 7: ANOVA for bleeding time due to the effect of alphamethrin on *Channa punctatus***

| SOV | DF | SS | MS | F | $F_{0.05}$ |
|---|---|---|---|---|---|
| Concentration | 3 | 116.763 | 38.921 | 0.0637 | 3.16 |
| Days | 6 | 139.146 | 23.191 | 0.0379 | 2.66 |
| Error | 18 | 10989.862 | 610.5478 | | |
| **Total** | 27 | 11245.771 | | | |

**Table 8: ANOVA for clotting time due to the effect of alphamethrin on *Channa punctatus***

| SOV | DF | SS | MS | F | $F_{0.05}$ |
|---|---|---|---|---|---|
| Concentration | 3 | 142.734 | 47.3578 | 0.1139 | 3.16 |
| Days | 6 | 28.1409 | 21.3568 | 0.0513 | 2.66 |
| Error | 18 | 481.0914 | 415.6161 | | |
| **Total** | 27 | 751.3057 | | | |

SOV = source of variance, DF = degree of freedom, SS = sum of square, MS = variance, F = ratio between variance of source and error, $F_{0.05}$ = value of F at 0.05 level of significance

## 4) DISCUSSION

Pyrethroids are reported to be highly toxic to fish because these compounds are strongly absorbed by the gills even at very low concentration in water due to their high lipophilicity [16]. Madsen et al [17] also reported that fishes are affected upon exposure to low levels of pyrethroids. Hill [18] observed that high toxicity to fish in water without particulate matter caused adverse changes in productivity of aquatic ecosystem. The haematological parameters have been effectively used as sensitive diagnostic indicators of pyrethroid poisoning. The present investigations show that prolonged exposure of *Channa punctatus* to alphamethrin in water induced a variety of anomalies in its hematology [19, 20].

These workers reported that the pesticidal stress influences the conversion of tissue protein content in to soluble fractions reaching in the blood for utilization. The reduction in the protein concentration in the present findings is in agreement with the findings of earlier workers.

The clotting time of blood in *Channa punctatus* was found increased after exposure to alphamethrin which might be due to thrombocytopenic effect. Hougie [25] and Lone and Javaid [26] demonstrated that fish with disease or organophosphate toxicosis show increased bleeding time and clotting time.

The findings of the present study indicate that the alphamethrin is capable of causing significant alterations in protein concentration, blood density, bleeding time and clotting time in *Channa punctatus*. Though the fish were

apparently healthy and mortality was not observed, but their entire physiology was disturbed due to the accumulation alphatamethrin in their body.

The results of the present investigations show that the entire physiology of *Channa punctatus* was disturbed and they were under stress during alphamethrin exposure. This may be due to accumulation of residue of alphamethrin in blood and various other tissues of this fish.

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