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Effect of Sub-Lethal Concentrations of Endosulfan on Hematological and Serum Biochemical Parameters in the Carp *Cyprinus carpio*

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Blood is a patho-physiological reflector of the whole body, so blood parameters are important in diagnosing the structural and functional status of the animal exposed to the toxicant. Endosulfan ($C_9H_6C_{16}O_3$) is a broad-spectrum cyclodiene pesticide that is widely used in agriculture for the control of foliar pests of food crops. Contamination of aquatic bodies with this pesticide is reported to cause mass mortalities of fish and other non-target organisms (Vandyk and Greeff 1978). Even very low concentrations of endosulfan are known to affect the behavior, growth, reproduction, haematological and biochemical parameters of fish (Chandraseker and Jayabalan 1993; Ramanujam and Mohanty 1997; Rangaswamy 1999; Rangaswamy and Padbhanabha Naidu 1999; Saravanan and Harikrishnan 1999; Kumar and Patri 2000; Saravanan et al. 2000). So far little or no research information is available on the effect of sub-lethal dosages of endosulfan on the haematology of fish. Therefore in the present investigation an attempt has been made to study the effect of endosulfan on alterations in haematological and serum biochemical parameters of *Cyprinus carpio* with particular reference to the concentration of the pesticide and duration of exposure.

MATERIALS AND METHODS

Specimens of *C. carpio* (10-12 cm length and 18-20 gm weight) were procured from a fish culture pond at Thandava village (Visakhapatnam district, South India) and were acclimatized to laboratory conditions for one month. They were kept in large holding tanks of 1000 liters capacity during the acclimatization period. Water was changed daily and fish were fed ad libitum with commercially available carp food twice a day.

Technical grade endosulfan (97% pure) was obtained directly from the manufacturers (Jayalakshmi fertilizers Tanuku). For experimental purposes stock solutions of 1 gram in 1 liter of tap water were prepared, from which different concentrations required for the present study were made.

After acclimatization fish were selected at random and transferred to fibre tanks measuring 56 × 45 × 36 centimeters filled with stored tap water. Tap water stored for eight to ten days was used for experimental studies to nullify the effect of chlorine present in the water. Experiments were designed to evaluate LC_{50} values, acute and sub-acute toxicity levels. Median tolerance limit (LC_{50}) of endosulfan to fish was estimated by exposing five batches of fish (10 per batch) to different concentrations of the pesticide and by recording 96 hour mortality rate at each concentration. The recorded data was analyzed using Probit analysis (Finney 1971). Another set of twenty

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fish were placed in two fibre tanks of 50 liters capacity filled with 40 liters of water (ten fish per tank) and were exposed to acute toxicity levels of the pesticide (10 ppb) for 96 hours. A third group of forty fish distributed in four fibre tanks (ten fish per tank) filled with 40 liters of water were exposed to sub-acute toxicity levels (5 ppb) for a period of 30 days. Simultaneously a common control group of 20 fish were maintained in two tanks under similar conditions. During the experimental period fish were fed ad libitum, water was changed daily and the toxicant was renewed. Fish under acute toxicity studies were sacrificed at the end of the exposure period (96-hours) where as those under sub-acute toxicity levels were sacrificed at regular intervals of 2, 7, 14 and 30 days post-exposure. At the end of each experiment blood was collected from the exposed and control fish (by cardiac puncture) in EDTA rinsed vials and analyzed for various haematological parameters following standard procedures (Blaxhall and Daisley 1972). Immediately after collecting the blood half of the blood was utilized for analyzing haematological parameters and the other half was used for separating serum. Serum was separated by centrifuging the blood at 3000 rpm for 10 minutes and was stored at 4 ^{0}C. This was later used for biochemical analysis to estimate glucose, total serum proteins, albumin, globulin and enzyme activities of GOT (glutamate oxaloacetate transaminase) and GPT (glutamate phosphate transaminase) employing commercially available kits (Span Diagnostics Ltd., SURAT, INDIA).

Analysis of variance (one way ANOVA) was employed to compute statistical differences between sample means. Group comparisons were made using the Least Significant Difference test. A probability of $P \leq 0.05$ was used to indicate significance.

RESULTS AND DISCUSSION

Probit analysis of data collected on percent mortality at 96-hour exposure to different concentrations of the pesticide revealed the median tolerance limit (LC_{50} value) to be at 22 ppb.

Exposure of *C. carpio* to acute (10 ppb) and sub-acute (5 ppb) concentrations of endosulfan resulted in certain abnormal behavioral changes like hyper-excitability, fast swimming activity, increased frequency of coughing and yawning, and profuse secretion of mucus. The effect was more pronounced in fish exposed to acute toxicity levels than in fish exposed to sub-acute toxicity levels and also during the early phase of exposure. They slowly regained their normal behavior at a later stage but such activities were always remained higher than the controls till the end of the experiment.

Data collected on specific haematological parameters is given in Table 1. It was evident from the data that exposure of *C. carpio* to acute levels (10 ppb, 96 hr) and sub - acute levels (5 ppb, 30 days) of endosulfan induced several changes in haematological parameters. Erythrocyte counts, haemoglobin percentage and haematocrit values of the exposed fish decreased steadily when compared to those from controls. At 30-days post-exposure there was a slight recovery in the total RBC counts and haemoglobin percentage. ANOVA revealed variations in these parameter at different days post-exposure to be highly significant ($P \leq 0.05$). Further analysis to test the significant differences between pairs of means employing the Least Significant Difference test indicated the differences to be highly significant from the controls at 5% level ($P \leq 0.05$). It was observed that differences in means are more significant at 7-day and 14-day post exposure from the controls but no significant difference was observed between these two means.

A reduction in haematological values, indicated anaemia in the exposed fish. The anaemic condition may be due to inhibition of erythropoiesis, haemosynthesis, osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in the haematopoietic organs. Earlier studies on the toxicity of pesticides like sumithion (an organophosphate compound) and sevin (a carbamate compound, commercial name for carbaryl) have shown a decrease in RBC counts, haemoglobin concentration, and haematocrit values at the 48 hour LC_{50} concentration (Koundinya and Ramamurthy 1980). Similar decreasing trends in haematological parameters resulting in macrocytic anaemia in *Heteroneustus fossilis* exposed to zinc were reported by Goel and Kalpana (1985). Natarajan (1981) was of the view that reduction in RBC counts, haemoglobin concentration and haematocrit values resulting in hypo-chronic anaemia could be attributed to the deficiency of iron and decreased utilization of haemoglobin synthesis. The decreased levels of haematological parameters observed during the present study in *C. carpio* might have been caused due to the destruction of mature erythrocytes resulting in reduced RBC levels. On the other hand total leukocyte counts increased steadily and were much higher than the controls from the 14th day of exposure till the end. A slight reduction in total leukocyte values was observed immediately after exposure (2 days post-exposure). Differences between means were found to be significant at the 5% level ($P \leq 0.05$). Increased levels of total leucocyte counts (TLC) observed during the present study in the endosulfan exposed fish may be due to an increase in the population of neutrophils and lymphocytes. Similar observations were made in other fish (James and Sampath 1996), which may be a consequence of tissue damage or injury (Mc Leay and Brown 1974) or greater sensitivity of neutrophils to changes in environment (Mahajan and Dheer 1979).

Total serum proteins, albumin, and globulin levels as well as serum glucose showed a decreasing trend with increased time and concentration (Table 2). There was a significant decrease in total serum protein and glucose levels of exposed fish when compared to controls. Concurrent with reduction in protein values in exposed fish was a corresponding decline in GOT and GPT enzyme activity values. These values were significantly different from controls ($P \leq 0.05$). The fluctuations of chemical constituents (glucose, protein, albumin, globulin, GOT, GPT) in the serum of exposed *C. carpio* were also found to be sensitive to the levels of pesticide concentration and duration of exposure period. Reduction in serum glucose levels after pesticide exposure appears to be caused by hypoxic conditions leading to an excess utilization of stored carbohydrates. Decreased protein and globulin levels may be attributed to stress-mediated mobilization of these compounds to fulfill an increased demand for energy by the fish, to cope with detrimental conditions imposed by the toxicant. Fish under stress mobilize proteins to meet energy requirements needed to sustain increased physical activity (Patel and Parmer 1993; Sievers et al. 1995). Reports on the reduction of lipid and protein levels in tissues of invertebrates after exposure to insecticide suggest that these compounds are mobilized for energy production (Ranjit Singh and Padmalatha 1996). A similar reduction in protein levels was noted during the present study where exposed fish exhibited hyperactivity and abnormal swimming behavior particularly during the early phase of exposure.

The results of the above study indicate the highly toxic nature of endosulfan on fish, exposed fish show that the fish are very sensitive to the presence of even minute quantities of endosulfan and are under severe metabolic stress. This study also shows the significance of haematological parameters in assessing pesticide hazards to fish.

Table 1. Changes in haematological parameters of *Cyprinus carpio* exposed to sub-acute concentrations of endosulfan (5 ppb). Values indicate range (Mean + SD).

Parameters	0 – Day	2 – Day	7 - Day	14 - Day	30 – Days
TEC $\times10^6$	1.0 - 1.5 1.2 ± 0.2	0.03- 1.5 0.7 ± 0.3	0.3 - 0.6 0.4 ± 0.1	0.2 - 0.6 0.3 ± 0.1	0.3 - 0.8 0.7 ± 0.3
TLC $\times10^4$	22.7 - 48.8 35.3 ± 6.6	21.3 – 3 28.6±5.6	24.9-57.2 43.9 ±11.9	38.8-87.7 53.1± 16.3	63.2-163.5 117.5±44.7
Hb gm/dl	1.9 – 4 2.8 ± 0.6	1.9 - 3.2 2.5 ± 0. 4	1.5 - 2.3 1.9 ± 0.3	0.7 - 1.7 1.0 ± 0.3	1.5 - 3.1 2.2 ± 0.6
HCT %	10 – 20 15 ± 3.8	8 - 14 12.1± 1.9	6 - 11 8.5 ±1.7	4 - 11 8.7 ± 2.5	4 - 14 7.4 ± 4.1

Table 2. Changes in serum biochemical parameters of *Cyprinus carpio* exposed to sub-acute concentrations of endosulfan (5 ppb).

Parameters	0 - Day	2 - Day	7 - Day	14 – Day	30 – Day
Protein Gm/dl	1.8 - 4.7 3.1 ± 0.8	0.1 - 1.2 0.5 ± 0.3	0.9 - 1.9 1.3 ± 0.4	0.6 - 2.3 1.3 ± 0.6	0.2 – 0.9 0.4 ± 0.2
Albumin Gm/dl	0.3 - 0.9 0.5 ± 0.2	0.3 - 0.7 0.4 ± 0.1	0.3 - 0.9 0.5 ± 0.2	0.3 - 0.8 0.5 ± 0.1	0.2 - 0.5 0.3 ± 0.1
Globulin Gm/dl	1.5 - 4 2.5 ± 0.8	0.08 - 0.5 0.2 ± 0.1	0.3 - 0.9 0.6 ± 0.3	0.1 - 0.8 0.5 ± 0.3	0.03 - 1.1 0.3 ± 0.4
Glucose Mg/dl	40.9 - 88.3 68.5 ± 18.6	35.8 - 79.5 56.1 ± 20.6	21.8 - 50.3 34.5± 12.8	20.7 - 59.9 38.7± 10.9	22.3 - 69.7 33.8± 16.0
GPT KA units	113.0- 466.7 209.1±172.2	381.9 - 454 414.5± 32.2	NE	31.4 - 82.8 64.0 ± 28.0	59.4 - 90.6 76.9 ±15.2
GOT KA units	132.4- 233.2 196.7 ± 55.8	76.9 - 164 165 ± 78.9	NE	70.1-214.4 138.5±47.7	74.0 - 21.7 83.6± 27.6

NE: not estimated

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