RESEARCH ARTICLE

Static bioassay and behavioural responses of cypermethrin on freshwater teleost, Labeo rohita

MOHINI GADHIA AND RAKESH PRAJAPATI

ABSTRACT...... Static renewal bioassay test was conducted to determine the toxicity of pyrethroid Cypermethrin (10% EC) insecticide on a freshwater teleost (Labeo rohita). Fishes were exposed to various concentrations of Cypermethrin for 96 h and the per cent mortality was recorded. The LC₅₀ value was found to be 0.06 ppm. Behavioural changes were observed in fish exposed to lethal concentrations exhibiting erratic and darting movements with imbalanced swimming.

KEY WORDS...... Labeo rohita, Cypermethrin, Behavioural response

INTRODUCTION..............................................

The pervasive use of pesticides in agriculture, public health and forestry ultimately leads to the contamination of aquatic biotopes posing a great threat to the environment (Visweswaraiah et al., 1975). Elucidations of the action of pesticides in non-target organisms form a thrust area in the field of toxicology due to serious effects and persistent nature of pesticides. Metabolic and pathological dearrangements on exposure to pesticides are well documented.

Due to the increasing regulatory restrictions on organophosphate pesticides, pyrethroid pesticides have replaced the organophosphates for many residential and agricultural uses. Urban use of pyrethroids has increased significantly due to professional pest control and retail sales for home usage (Lydy, 2005). Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, such as moth pests of cotton, fruit and vegetable crops, including structural pest control, landscape maintenance, for residential and garden use. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed which evidenced that Cypermethrin is extremely toxic to fish at very low concentrations and to aquatic invertebrates (Sarkar et al., 2005). Fish sensitivity to pyrethroid may be explained by their relatively slow metabolism and elimination of these compounds through excretion. Labeo rohita is one of the prime cultured fresh water teleosts in polyculture and of great economic importance. Hence, the present study is a contribution to the assessment of aquatic toxicity (bioassay) and behavioural responses of Cypermethrin pesticide to freshwater fish L. rohita.

RESEARCH METHODS.................................

L. rohita fingerlings weighing 8 ± 2 g and average length of 9 ± 1 cm were collected from the Krishna Fisheries, Sayan, Surat and acclimatized to laboratory condition for 15 days in large glass tanks previously washed with potassium permanganate to free the walls from any microbial growth. Fishes were fed with rice bran mixed with oil cake in the ratio 2:1 every day. Healthy fishes were exposed in batches of ten to various concentrations (0.02, 0.04, 0.06, 0.08 and 0.10 ppm) of Cypermethrin with 50 L of water to at least three replicates for each concentration alongwith a set of control experiment. Replacement of the water medium was followed by the addition of the desired dose of the test compound. Cypermethrin (10% EC) was obtained from Heranba Industries Limited, Vapi,
Gujarat, India. Fishes were aerated to prevent the hypoxic condition of the medium. Feeding was stopped before 24 hrs of bio-assay test.

Tests were carried out to find out the median tolerance limit (TLm) of the fish to Cypermethrin for 96 hrs using the method of APHA (1995), probit analysis method of Finney (1971) and the mortality was recorded at 24, 48, 72 and 96 hrs. The median tolerance limit at which 50 per cent mortality occurred was noted and also calculated. A set of control fishes was maintained to compare the results with experimental fishes. During this experiment the behavioural responses were also critically observed.

Physico-chemical characters (temperature, pH, dissolved oxygen, total hardness, total alkalinity) of water were monitored according to methods described by Trivedy and Goel (1984).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads:

Bioassay test :

The minimum concentration at which 0 per cent mortality of Labeo rohita recorded was 0.02 ppm and the maximum concentration where 100 per cent mortality recorded was 0.10 ppm (Table 1 and Fig. 1). Table 1 shows that there was minimum mortality (30 %) of fishes observed with 0.04 ppm of exposure whereas death of all fishes took place at the end of 96 hrs of treatment with 0.10 ppm. Low rate of death of fishes was noted during the initial period upto 48 hrs. The exposure of L. rohita to Cypermethrin treatment was most critical at 72 hrs and 96 hrs (Table 1). Lethal concentration (0.06 ppm) was found with 50 per cent mortality of fishes (Table 1 and Fig. 1).

Behavioural responses :

When the fishes were exposed to various concentrations of Cypermethrin, they moved immediately to the bottom of the tank. The movement of fishes was observed to be disrupted at 24 hrs itself. Fishes spread out and occupied twice the area than that of the control group. Irregular, erratic and darting movements followed by imbalanced swimming activity were observed. The frequency of surfacing phenomenon was greater after 48 hrs of exposure. The swimming behaviour was sudden, rapid, spurt of forward movement. The fish progressively showed signs of tiredness and weakness at 72 hrs. On the last exposure period with higher concentrations (0.04, 0.06, 0.08 and 0.10 ppm) showed that the animals lost their equilibrium and response, to external stimuli such as touch by drowning to the bottom and correspondingly died with their mouth and operculum wide opened by the end of 96 hrs. Change in colour of the gill lamellae was found with thick coating of mucus in dead fishes.

### Table 1 : Mortality of L. rohita to Cypermethrin

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration of cypermethrin (ppm)</th>
<th>No. of fishes exposed</th>
<th>24 hrs</th>
<th>48 hrs</th>
<th>72 hrs</th>
<th>96 hrs</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.02</td>
<td>10</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>2.</td>
<td>0.04</td>
<td>10</td>
<td>00</td>
<td>00</td>
<td>01</td>
<td>02</td>
<td>30</td>
</tr>
<tr>
<td>3.</td>
<td>0.06</td>
<td>10</td>
<td>00</td>
<td>00</td>
<td>02</td>
<td>03</td>
<td>50</td>
</tr>
<tr>
<td>4.</td>
<td>0.08</td>
<td>10</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>03</td>
<td>60</td>
</tr>
<tr>
<td>5.</td>
<td>0.10</td>
<td>10</td>
<td>01</td>
<td>01</td>
<td>03</td>
<td>05</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2 : Physico-chemical characters of control water and treated water

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Control water</th>
<th>Treated water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Temperature (°C)</td>
<td>29 ± 2</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>2.</td>
<td>pH</td>
<td>7.8 ± 0.2</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>3.</td>
<td>Dissolved oxygen (ppm)</td>
<td>5.73</td>
<td>5.48</td>
</tr>
<tr>
<td>4.</td>
<td>Total hardness (ppm)</td>
<td>85.13</td>
<td>85.74</td>
</tr>
<tr>
<td>5.</td>
<td>Total alkalinity (ppm)</td>
<td>151.62</td>
<td>152.34</td>
</tr>
</tbody>
</table>
Physico-chemical characters of water:

Physico-chemical characters (temperature, pH, dissolved oxygen, total hardness and total alkalinity) of water from control and treated aquaria were found to be unchanged (Table 2).

Acute chemical toxicity is generally determined as a TLm-96 hrs. Present results show that Cypermethrin is very toxic even at lower concentration. Lethality in the present study is comparable to the few published studies for all species exceeded this concentration. This could be attributed to the inability of the L. rohita. The acute toxicity treatments showed strong negative effects on survival as pesticide concentration increased. This suggests dose-dependent lethality. The varying degrees of mortality reported in this study is consistent with the report of David and Philip (2005) who reported that differences in an organism’s biological adjustment and behaviour response to no change in water chemistry. Behavioural characteristics are obviously sensitive indicators of toxicant’s effect. It is necessary, however to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural system.

The movement of the fish to the bottom of the tank following the addition of Cypermethrin clearly indicated the avoidance behaviour of the fish which was reported by Murthy (1987) in trout. The opercular movement of the fish ceases immediately following exposure to Cypermethrin. The decrease in opercular movement and corresponding increase in frequency of surfacing of fish clearly indicated that fish adaptively shifted towards aerial respiration (by obtaining atmospheric oxygen) and the fish tried to avoid contact with the pesticide through gill chamber (Santhakumar et al., 2000).

The increased ventilation rate by rapid, repeated opening and closing of mouth and opercular was observed in the present study. This could be due to accumulation of mucus in the gill region for inhibiting proper breathing.

The surfacing phenomenon of fish observed under fenvalerate exposure was due to hypoxic condition of the fish as reported by Radhaiah and Jayantha (1988). Cypermethrin concentrations changes in ventilation elevated rate of metabolism noticed in the fish after exposure to the pesticide and help the fish to avoid contact with poison and fight against stress. Chronic exposure of finfish to aroclor was found to induce surfacing phenomenon of fish and was explained by Drummond et al. (1986).

In the present study, the abnormal changes in the fish exposed to higher concentration of Cypermethrin were time dependent. However, the normal behaviour of the fish after on exposure to sub lethal concentrations indicated its adaptability to the sub-lethal concentration due to long term exposure of Cypermethrin. Philip et al. (1988) suggested that the fish behaviour indicated that the fish was adapted to a compensatory mechanism to derive energy during pyrethroid toxicosis. Hence, this type of study can be useful to compare the sensitivity of the various species of aquatic animals and potency of chemicals using LC50/TLm values and to derive safe environmental concentration by which there is no lethality and stress to the animals. Behavioural changes are physiological responses shown by the animals are often used as the sensitive measure of stress syndrome in the organism experiencing.

LITERATURE CITED


INTRODUCTION

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. As a result of the pollutants' transport from industrial and agricultural areas into the environment and their chemical persistence, many freshwater ecosystems are faced with spatially alarming high levels of xenobiotics chemicals (Brack et al., 2002; Diez et al., 2002). Due to the increasing regulatory restrictions on organophosphate pesticides, pyrethroids pesticides have replaced organophosphates for many residential and agricultural uses. Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, such as moth pests attacking cotton, fruit and vegetable crops, including structural pest control, or landscape maintenance. This has resulted in its discharge into the aquatic environment and consequently several laboratory studies have been performed which have shown that cypermethrin is extremely toxic to fish and aquatic invertebrates at very low concentrations. Fish sensitivity to pyrethroids was explained by David et al. (2003) through their relatively slow metabolism and elimination of these compounds.

Gill is a vital organ for respiratory and osmoregulatory functions in fishes. Respiratory distress is one of the beginning symptoms of pesticide intoxication (Ortiz et al., 2002). Substantial interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish. Tissue's structural changes in fishes exposed to a sublethal concentration of Cypermethrin are a functional response of fishes which provides information on the nature of the Cypermethrin. Any change in the gill of fish indicates the deterioration of water quality; since fish are biological indicators of water quality, the present study was undertaken to evaluate the aquatic toxicity of Cypermethrin with special emphasis to histopathology of gills from freshwater well known edible teleost, L. rohita exposed to sublethal concentration (0.03) of Cypermethrin.

RESEARCH METHODS

Healthy Labeo rohita fingerlings were procured from the freshwater farm situated in Sayan, Surat district. They were acclimatized for a maximum period of 15 days in the laboratory condition. Fingerlings measuring 9 ±1 cm in length and 8 ±2 g in weight were exposed to sublethal concentration...
(0.03 ppm) of Cypermethrin for a period of 8 days. The gill pathology was examined after 1, 2, 4 and 8 days. The gills were removed carefully from control and treated aquaria, fixed in Bouin’s fluid and processed for microtomy at 5?m. Sections were stained with haematoxylin, eosin, mounted in DPX and observed under the microscope (Kapoor, 1976).

**RESEARCH FINDINGS AND ANALYSIS**

The gill of normal fish is made up of filaments of primary lamellae (PL) prearranged in twice rows. Secondary lamellae come up from these filaments. The secondary lamellae (SL) are lined by a squamous epithelium; each gill consisted of a primary filament and secondary lamellae (Fig. 1).

In the present study the observed changes was the excessive mucus secretion at the end of first day of experiment (Fig.2). Lamellar fusion, damages like discontinuity and dilation in central cord (DCC), degeneration of secondary gill lamellae were observed at the end of second day of experiment (Fig.3). With the end of fourth day fusion of gill filaments (FGF) due to separation, necrosis of gill epithelium, abridged and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae (FSL) (Fig. 4) and necrosis in the primary lamellae were well marked. Besides there were changes in vacuolization and lamellar aneurysm (LA) were also observed in significant at the end of fourth day (Fig.5 and 6). All the changes were increased after day by day and the end of 8th day of experiment's gill lost their identity. Structure of gill was observed totally damaged at end of experiment.

Histopathological changes in the gill of fishes due to pesticides and other contaminants have been reported earlier (Dutta et al., 1993). According to Leino et al. (1987) the gill of
pearl dace and fathead minnows from environmentally polluted Canadian lakes exhibited various cellular, histological and histopathological changes which contributed to problems related to respiration and acid-base balances. The severe damage in rupture of the gill epithelium resulted in hypoxia and respiratory failure. Roy and Datta (1991), reported slight hyperplasia of gill epithelium in *Pineaus monodon* exposed to Gusathion, a commonly used organophosphate. They also reported that provocative alterations of lamellar epithelium and hyperplasia in the gills of freshwater major carp *Cirrhinus* *mrigala* (Hamilton) during 48 hrs exposure to sublethal dose of Malathion. These findings support the results of present study adding that alteration in gill in the form of lamellae epithelium and hyperplasia due to exposure of fish to different pesticides.

Additionally, similar changes were observed in *L. rohita* exposed to Monocrotophos and Fenvalerate (Tilak et al., 2001) and to Cypermethrin (Veeraiah, 2001).

In fish exposed to 0.03 ppm Cypermethrin concentration (8 days) in the present study, the major changes in gills were thickening of the primary lamellar epithelium and fusion of secondary lamellae. All these lesions may damage respiratory function. Filament cell propagation reduce the inter lamellar space and may cause a complete lamellar fusion reducing the total surface area for gas exchange reported by Nowak, (1992). Otherwise, they increase in the distance of the water-blood barrier which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake and if the responsible agent is not removed, can lead to the damages in central cord, discontinuity in central cord, dilation in central cord.

**Fig. 5 and 6 :** Change in vacuolization and lamellar aneurysm

**LITERATURE CITED**


