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Behavioural responses and acute toxicity of *Clarias batrachus* to synthetic pyrethroid insecticide, λ- cyhalothrin

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Abstract

Toxicity tests are an important component for assessing the impact of chemicals on aquatic ecosystems because they indicate toxic effects of chemicals. In aquatic toxicity tests, groups of selected organisms are exposed to test materials under defined conditions to determine potential adverse effects. Bioassay tests were conducted on fresh water air breathing fish *Clarias batrachus* to evaluate the acute toxicity of synthetic pyrethroid λ- cyhalothrin insecticide by determining their LC_{50} after 24, 48, 72 and 96 h exposure. The safe concentration of this biocide was calculated on the basis of LC_{50} for 96h. From the results obtained it was observed that the LC_{50} values of 96 h were found lowest among all the exposure periods. The 96 h LC_{50} values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specific conditions. Susceptibility of catfish, *Clarias batrachus* to lethal effect of synthetic pyrethroid was found to be duration and concentration dependent as mortality was increased with an increase in its concentration in the present study. Behavioural characteristics are obviously sensitive indicators of toxicant effect. Many chemical contaminants target specific physiological systems and exert their effects on behavior. Fish in toxic media exhibited irregular, erratic swimming movements, hyper excitability, loss of equilibrium spiraling, loss of balance, rapid respiration, light discoloration, surfacing, and gulping air. The present investigation was designed to elucidate the acute toxic effect of λ-cyhalothrin insecticide on the toxicity and behavior of air breathing fish *Clarias batrachus*.

Keywords: λ- cyhalothrin toxicity, LC_{50}, behavior, *Clarias batrachus*

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Introduction

Insecticides are extensively used to protect agricultural crops against the damages caused by pests. The injuries of insecticides to aquatic environments are fatal. The significant increase of chemical emissions in the water resources has led to deleterious effects for aquatic organisms (Livingstone, 2001; Matsumoto et al., 2006).

Pyrethroids belong to the most commonly used pesticides worldwide. Their massive expansion is a threat to the natural environment including the aquatic medium. Although pyrethroids are rapidly degraded in soil and plants, they are extremely toxic to fish because of high sensitivity to them. Pyrethroids are synthetic analogues of the natural pyrethrins, extracts of the ornamental *Chrysantemum cinerariae folium* and its related species. Synthetic pyrethroid insecticides are derived from natural compounds (the pyrethrins) isolated from the *Chrysanthemum* genus of plants . These compounds include tetramethrin, resmethrin, fenvalerate, permethrin, cypermethrin, λ-cyhalothrin, and deltamethrin. All of these are used extensively in agriculture. They exhibit low mammalian toxicity insecticidal action and photostability with low volatility and persistence. They are broad-spectrum insecticides with tremendous effect to kill insect. Although they were acutely toxic to fish, very few accidental poisoning occured because they were not registered for aquatic use and they seldom had enough persistence to reach water from normal application (Di Giulio RT, Hinton DE, 2008).

Pyrethroids are divided into type I and type II, based on their structure, chemical and neurophysiological properties and toxicological action. Type I pyrethroids are without a cyano moiety at the α-position (i.e. permethrin, bifenthrin, allethrin, tetramethrin, resmethrin, phenothrin, bioresmethrin, etofenprox, prallethrin, tefluthrin), while type II pyrethroids have an α-cyano moiety at the benzylic carbon of the alcohol portion of the ester (i.e. cypermethrin, cyfluthrin, deltamethrin, cyphenothrin, flumethrin, cycloprothrin, fenvalerate, fluvalinate). Type II pyrethroids are more effective (Svobodova Z et al. 2008).

Structure of λ Cyhalothrin:

λ cyhalothrin belongs to the chemical family synthetic pyrethroid insecticide, with a chemical name (S)-α-cyano-3- = phenoxy benzyl(z) –, the IUPAC name (IR,3R) -3- (2-chloro-3,3,3-trifluoro propenyl)-2,2- = dimethyl cyclo propane carboxylate and(R)-α-cyano-3-phenoxybenzyl(Z)-1s,3s)-3-(2-=chloro-3,3,3,-ifluoropropenyl)-2,2- dimethyl cyclo propane carboxylate.

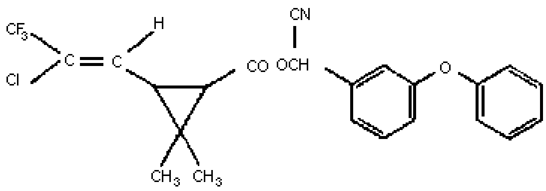

Figure 1 Chemical structure of λ-Cyhalothrin
Molecular formula: $C_{23}H_{19}ClF_3NO_3$

Currently available formulations of pyrethroids are oil based emulsifiable concentrates (EC). The emulsifiable formulation keeps the pyrethroids in solution longer compared to the technical chemicals. Piperonyl butoxide is commonly added to pyrethroid products to enhance the toxic effects of the active ingredient. Piperonyl butoxide inhibites a group of enzymes, which are involved in pyrethroid detoxification (Werner I, Moran K.2008).

The insecticide λ-cyhalothrin was procured from the local market and used. It is manufactured by Syngenta Crop Protection Private Limited, Mumbai. Karate (Lambda cyhalothrin) is a contact residual and stomach acting insecticide with repellency properties. It is a novel pyrethriod insecticide currently known as syngenta. These are neurotoxic agent most probably acting through the central nervous system to cause nerve disruption.. It is readily absorbed from the gastrointestinal tract, by inhalation of dust and fine spray mist and only minimally through intact skin. It is a contact and systemic insecticides widely used to control the insects such as Jassids, Bollworms, Thrips of cotton, Fruit as well as shoots borer of Leaf folder, vegetables, stem borer, Gall midge of Rice and green leaf hopper. Pyrethroids are predominant class of insecticides. Their widespread use represents an increasing threat of water pollution. This insecticide is frequently used by the farmers in and around Madurai, Tamil Nadu, India to intensify the agricultural and aquaculture practices.

Behavioural modification is one of the most sensitive indicators of environmental stress and many affect survival (Olla et al., 1983; Byrne and O'Halloran, 2001).Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment: (Little and Brewer 2001). Recent development in ecotoxicology has been shown that observations of behaviour can constitute a sensitive approach in sub lethal toxicity monitoring.. Behavior allows an organism to adjust to external and internal stimuli in order to best meet the challenge of living in a changing environment. Most significantly, alterations in behavior represent an, whole-organism response. These altered responses, in turn, may be associated with reduced fitness and survival, resulting in adverse consequences at the population level (Bridges 1997). Hence the object of the present study was to examine the impact of acute exposure of λ-cyhalothrin insecticide on toxicity and behavior changes in *Clarias batrachus.*

Materials and methods

Freshwater air-breathing fish *Clarias batrachus*(Family; Clariidae, Order; Siluriformes) were procured from local sources. This species was selected for bioassay because of some ecotoxicological characteristics such as wide distribution, availability throughout the year, easy maintenance under laboratory conditions and commercial importance.

Survival studies on the fish of 2.5± 0.5g were carried out. The feeding was stopped 24 h prior to the exposure period. Fish specimens were subjected to prophylactic treatment by batting twice in 0.05% potassium permanganate (KMnO4) for two minutes to avoid any dermal infections. The fishes were then acclimatized for one week under laboratory conditions in semi static systems. The test solutions were replaced at every 24 h. Dead animals were removed immediately from the medium. For each bioassay test, a control was used. Experiments were conducted using Triplicates.

Determination of sub-lethal concentrations:
Toxicity tests were conducted in accordance with standard methods (APHA, 1992). The stock solution of lambda cyhalothrin with a concentration of .1 ml per 10 litre was prepared in distilled water and the desired degree of concentrations was prepared. Based on the progressive bisection of intervals on a logarithmic scale, log concentrations were fixed after conducting the range finding test. The fish were starved for 24 h prior to their use in the experiments as recommended by storage to avoid any interference in the toxicity of pesticides by excretory products. After the addition of the toxicant into the test tank with 10 litres of water having fifteen fish, mortality was recorded after 24, 48, 72 and 96 h. Another set of 10 fish were also simultaneously maintained in tap water (0.00 mg/l) as the control. The experiment was set in triplicate and fish were not fed during the experimentation as recommended by (Ward and Parrish (1982) and Reish and Oshida (1987). Exposure time was 96 h after which mean mortality from a particular dose and its replicate was calculated. A fish was considered dead when it did not respond after gentle touching with a glass rod; dead fish were removed from the tank immediately. Fish behavior was noticed during the exposure. The median lethal concentration (LC_{50}) of the test pesticides was calculated from the data obtained in acute toxicity bioassays following the probit analysis method as described by Finney (1971).

Acute Toxicity: The median tolerance limit of any pollutant is meant as an elementary guide in the field of toxicology (Ward and Parrish, 1982). Without reference to the median tolerance limit,no information on sublethal effects can be deduced (Patin, 1982).

Hours	LC_{50} Value (µg/l^{-1})
24	0.350
48	0.234
72	0.122
69	0.092

Table.1. LC $_{50}$ values of *Clarias batrachus* exposed to lambda cyhalothrin in acute toxicity test.

From the results of mortality readings the 96 h LC_{50} value and 95% confidence limits for λ-cyhalothrin based on probit analysis was found to be **0 .09µg/l^{-1}** (Table .1) The lower and upper lethal confidence limits indicated a range between **0 .038µg/l^{-1} to 0.134 µg/l^{-1}.** The LC_{50} values and exposure period showed a direct relationship .The LC_{50} values obtained for λ-cyhalothrin were **0.350 µg/l^{-1}, 0.234µg/l^{-1} , 0.122µg/l^{-1}, and 0.092µg/l^{-1} for 24, 48, 72 and 96 h** respectively. From the above results it was observed that the LC_{50} values of 96 h were found lowest among all the exposure periods. The 96 h LC_{50} values provide a useful means of comparing the relative acute lethal toxicity of specific toxicants to organisms under specific conditions.

Behavioural observations:

All pesticides induce toxic stress in form of behavioral changes in the fish. The control fish behaved in a natural manner i.e., displayed uncoordinated behavior. At the initial exposure, fish were alert, stopped swimming and remained static in position in response to sudden changes in the surrounding environment. They were alert to the slightest disturbance, but in the toxic environment fishes tried to avoid the toxic water with fast swimming and jumping. Observations from the bioassay revealed that fishes exhibited increase in stress as evidenced by slow and uncoordinated movement. Faster opercular activity was observed as surfacing and gulping of air.

Figure.1 Right: control left: toxicant exposed fish showing signs of discoloration

of the skin, caudal bending, blood clots and pronounced mucous secretion over the gill with belly turned upwards at the time of death.

Opercular movements increased initially in all exposure periods but decreased later steadily. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escaping phenomenon. A copious amount of thick layer of mucus was found deposited in the buccal cavity and gills. The discolouration of the skin was more pronounced. The fish progressively showed signs of tiredness and lost positive rheotaxis. Fishes spiraled at intervals and engulfed the air through mouth before respiration stopped. Soon they settled at the bottom of the tank, and after some time died with their mouth and operculum wide opened.

Discussion

Median lethal concentration (LC_{50}) is the most widely accepted basis for acute toxicity test. It is the concentration of a test chemical,whichkills 50 % of the test organism in a particular length of exposure, usually 96 h.

Toxicity data for a variety of pesticides such as organophospate, organochlorine, carbamide and pyrethroid pesticides have been reported for number of fish species by various authors.Acute toxicity of fish species exposed to type II pyrethroids λ -cyhalothrin for 96 h vary among fishes. for example in *Brycon amazonicus* it is 6.5 µg l^{-1}, (F.D. DeMoraes et al., 2013) in *Gambusia affinis* as 1.10 µg l^{-1} (Güner 2009), in *Brachydanio rerio* as 1.94 µg l^{-1} (Wang et al. 2007), in *Clarias batrachus* as 5.0 µg l^{-1}(Kumar et al. 2011).

Examining cypermethrin toxicity to other aquatic organisms, the work of Clark et al. (1987) reported the cypermethrin 96-h LC_{50} for grass shrimp *Palaemonetes pugio* as 0.016lg/l. The LC $_{50}$ values of cypermethrin for *C. fasciatus* at 96h was 0.006 mg/L.(Shailendra Kumar Singh 2010.), the 96h LC $_{50}$value of profenofos on *Catla catla* as 0.0079ppm (A. Maharajan et.al 2013.), 96h LC_{50} value of cypermethrin on Juvenile African Catfish *Clarias gariepinus* as 0.063mg/l. (Ayoola S.O and Ajani E.K 2008) have also been reported. The 96 h LC_{50} of λ-cyhalothrin in *mosquito fish* was 1.107 ug/L(Guner, U. (2009) under laboratory conditions.

The differences in the literature LC_{50} values in the sensitivity of different fish species to pesticide exposures seems to be influenced by the age of the fish chosen for the toxicity tests. For example, the 96 h LC_{50} value for zebrafish, *Danio rerio*, embryos exposed to λ-cyhalothrin was 0.875 g/L (Xu et al., 2008) compared with 0.5 g/L of 37–52 day old post-hatch *zebrafish* fingerling with same expo-sure times (Wang *et al*., 2007).Time of exposure, method of determination, chemical purity , age and species of fish when taken into consideration., also determines the toxicity of λ -cyhalothrin.

The present investigation reported lower LC $_{50}$ value of 0.092µg/l^{-1} for 96h in the test animal (Clarias batrachus) as compared to previous researchers. However, the present LC $_{50}$ value was less than that of LC $_{50}$ values for 96h by K-cyhalothrin reported in *Clarias batrachus* as 5 ug/L (Kumar, A., 2011) From all the results and observations, it is inferred that toxicity values were not constant for any particular group of fishes and it varies from species to species with alteration in physical and chemical factors. Low concentrations of pyrethroids, as reported in this study, are usual in water environments (Marino &Roco, 2005; Belluta et al., 2010) and they are close to that reported to fish farms in some countries (EMEA, 2003; Haya, 2005).

The fish serves as bio-indicator of water quality and this can be easily judged by morphological, physiological and behavioural changes in an altered environment. When the fish were exposed to the lethal concentration of λ-cyhalothrin they migrated immediately to the bottom of the tank and independency (spread out) in swimming.. The migration of the fish to the bottom of the tank following the addition of λ-cyhalothrin clearly indicates the avoidance behaviour of the fish. Murthy (1987) observed avoidance behaviour in *trout* on exposure to pesticides. Prashanth and Patil (2006) have also observed the avoidance nature by Catla catla on exposure to sodium cyanide.

Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress of the toxicant, results in increased swimming activity and requiring increased expenditure of energy (Murthy, 1987). The disturbance of the schooling behaviour of the fish in treated media indicates that the group hydrodynamic effect of fish would help them to swim within the school has been lost. (Zuyer and Belyayen, 1970). Weis and Weis (1974) have reported that carbaryl has a marked effect on the schooling behaviour of the *Atlantic silverside*.

The erratic swimming of the treated fish indicates loss of equilibrium. It is proved that the region in the brain associated with the maintenance of equilibrium should have been affected..(Rao ,S.and Rao ,C.,1987) Santhakumar and Balaji, (2000) observed exciting and erratic movements in *Anabas testudineus* exposed to monocrotophos. Similar observations were reported in Hiteropneustes *fossilis* exposed to rogor and endosulfan (Sabita and Yadav, 1995).

Fishes exposed to toxicins generally show increased opercular movement. The increased opercular movementmight be due to clearance of the accumulated mucus debris in the gill region for proper breathing as suggested by Carlson and Drummond, (1978).and Prashanth et al., 2005.Monocrotophas exposure has been reported to cause increased opercular movements in *Anabas testudineus* (Santhakumar and Balaji, 2000). The increased opercular gill movements observed is to compensate for increased physiological activity under stressful conditions (Sivakumar and David, 2004).

Loss of positive rheotaxis observed of the fish is a good indication of toxic response. The hyper excitation and abnormal behavior like fin-flickering observed in the insecticidal exposed fish may be due to the irritating effect of the toxicant. Excess mucus secretion all over the body was another change noticed. Mucus also forms a barrier between the body and the toxic medium, to minimize its irritating effect. Rao (2006) made similar observations following RPR-V (a novel phosphorothionate insecticide, 2-butenoic acid-3-[diethoxy phosphinothionyl] ethyl ester) exposure to euryhaline fish, *Oreochromis mossambicus* .Blood clots and pronounced mucus secretion over the gill were also observed, is related to the uptake of water containing pesticide through gill. Aggressive behaviour such as nudge and nip were increased following exposure to the toxic material.

Caudal bending was noticed in the toxicant exposed fish and this greatly reduced the normal swimming pattern. Caudal bending may be a sort of paralysis, which might be due to the inhibition of muscular AChE activity resulting in blockage of neural transmissions. This produces rapid twitching of voluntary muscles followed by paralysis (Ware, 1989; Habig and DiGiulio, 1991). Thus, λ - cyhalothrin not only reduced instinctive behavioral responses but affected morphological features.

The treated fishes also showed fading of their body color Fish were also found striking their heads against the walls of the experimental containers and body color was observed to become light. Marked change in color was observed in the present study which became lighter. It may be due to shifting and degeneration of melanophores and also destruction of mucus cells in the solution as said by various authours. Sriwastawa and Srivastava(1982) also observed change in color of *Cirrhinus mrigala.* Ikpi et al, (2003) observed the loss of skin coloration in *0. niloticus* fingerlings exposed to different concentrations of textile mill effluents..

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Surfacing phenomenon seen in the present study might be to gulp maximum possible air to ease the stress. Rao and Rao(1987) reported this phenomenon to be due to hypoxic condition of the fish Surfacing phenomenon i.e., significant preference of upper layers in the exposed group might be the result of the need for higher oxygen levels during the exposure period (Katja et al., 2005). Atlast the animal became sluggish and all the activities were ceased followed by death.

These behavioural changes can be considered as symptoms of stress on account of the toxicological nature of the environment. These behavioural responses can be used as a tool in biomonitoring programme to monitor ecotoxicity risk of λ - cyhalothrin to the test species. This study has proved convincingly that toxicants are deleterious to natural population of fish. Even though pesticide pollution may not directly kill aquatic organisms like fishes, the sublethal concentration of pesticide in water can adversely affect several vital behavioural patterns of the fish reducing its overall fitness .

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