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Studies on Behavioural Response of freshwater fish, Tilapia (*Oreochromis mossambicus*) exposed to synthetic pyrethroid cypermethrin

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Abstract
Behavioural modification is one of the most sensitive indicators of environmental stress and may affect survival of an organism. Behaviour allows an organism to adjust to external and internal stimuli to meet the challenge of surviving in a changing environment and also the adaptations to environmental variables. In the present study, Tilapia exposed to cypermethrin exhibited disrupted school behaviour, localization to the bottom of test chamber and independency (spread out) in swimming, dullness, loss of equilibrium, stop of food intake, erratic and hysteric swimming. An increase in the number of mortalities, with an increase in concentration of the pesticide, was observed. The 96-hour LC50 value of synthetic pyrethroid cypermethrin was found to be 0 .07 µg/L.

Keywords: Stress, Behaviour, Environment, Mortalities and Pesticides

Introduction

Fish are able to uptake different xenobiotics dissolved in water by active or passive processes and retain them in the body. We can detect and document pollutants released into their environment by using fishes. Because their entire body surface is direct constant, contact with the chemical exposure occurs in an aquatic environment. Any change in fish behaviour can also provide important guides for ecosystem assessment. Behaviour allows an organism to adjust to external and internal stimuli to meet the challenge of surviving in a changing environment and also the result of adaptations to environmental variables. Behaviour is a sequence of quantifiable actions, operating through the central and peripheral nervous systems (Keenleyside, 1979) and the cumulative manifestation of genetic, biochemical and physiologic processes essential to life such as feeding, reproduction and predator avoidance (Ramesh Halappa, and Muniswamy David., 2009). Behaviour is a selective response that is constantly adapting through direct interaction with chemical, social and physiological aspects of the environment. This behavioural pattern in concert with morphologic and physiologic adaptations stability provides the best opportunity for survival and reproductive success of organisms. Alterations in the behaviour of fish indicate the deterioration of water quality, as fish are the biological indicator and hence index of environmental suitability and the cost of survival.
Any substances used to control organisms, including insects, water weeds, and plant diseases are toxic to fish. Pesticides usage in agricultural fields to control pests are extremely toxic to non-target organisms like fish and affect fish health through impairment of metabolism, sometimes leading to mortality [Shankar et. al., 2013]. They have been found to be highly toxic not only to fish but also to the other organisms, which constitute the food chain. The contamination of waters by insecticides is known to have ill effects on the growth,

reproduction and survival of aquatic animals. Different concentrations of pesticides are present in many types of waste water and many studies have found them to be toxic to aquatic organisms, especially fish species. Sub-lethal concentrations of pesticides in aquatic environments cause morphological and physiological changes in aquatic organisms and this is more common than mortality (Sancho *et al*., 2003). Behavioural modification is one of the most sensitive indicators of environmental stress and may affect survival (Byrne and O'Halloran,2001).Cypermethrin(CY)[(RS)-alpha-cyano-3-phenoxybenzyl(1RS)-*cis*,*trans*-3-(2,2,-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] is a highly active synthetic pyrethroid insecticide. It is one of the most widely used synthetic insecticides for agricultural and domestic purposes, globally [Crawford et. al., 1981]. In the present study it is used to study the behavioural response of tilapia fish.

Materials and Methods

Healthy and active tilapia fish (*Oreochromis mossambicus*) were procured from the commercial fish farm (K.V.K. Namakkal). The fish were transported to the laboratory and acclimatized for a time period of `10-15 days in the laboratory conditions in plastic aquaria containing dechlorinated water. The fish tanks were well aerated and the physical and chemical parameters were kept nearly constant. Fish were fed twice daily with commercial fish food pellets during acclimatization and test periods. Synthetic pyrethroid cypermethrin 10% EC pesticide was purchased from the market at namakkal. A stock solution of cypermethrin with a concentration of 100µg/L was prepared by diluting commercial cypermethrin 10% EC insecticide in distilled water. From this 0.03, 0.05, 0.07, 0.09 and 0.11µg/L concentrations of cypermethrin pesticide were prepared by diluting distilled water.

The experiments were conducted in six aerated plastic tanks (120 x 40 x 30 cm) each containing 10 fish in 30L of contaminated test solution and tap water. Toxicity test were carried out to determine lethal concentration (LC) following FAO procedure for short-term bioassays and APHA standard procedure. The fish were exposed to dissolved synthetic pyrethroids cypermethrin for a period of 96 hour at varied concentrations of 0. 03, 0.05, 0.07, 0.09 µg/L and 0.11 µg/L. The last tank was left untreated as a control group. All the groups received the same type of food and other conditions were maintained similarly. Mortality was recorded every 24 h and the dead fish were removed when observed, every time noting the number of fish deaths at each concentration up to 96 hours.

To determine the lethal concentration (LC) of cypermethrin for 50% mortality (LC_{50}) several levels of concentration were used as experimental water. The number of dead fish in each replicate was recorded in 24h, 48h, 72h and 96h exposure. The LC_{50} were then determined using the probit analysis and the fish behaviours were observed daily at the same time. Mortality of the fish was recorded every 24 hours and the dead fish were removed.

Results and Discussion

Acute toxicity

Total mortality observed in different treated groups of exposure is presented in Table 1 to 4. An increase in the number of mortalities, with an increase in concentration of the pesticide, was observed. The 96-hour LC50 value of synthetic pyrethroid cypermethrin was found to be 0.07 µg/L. The lethal toxicity study was performed for the concentration of ranging from 0.03 to 0.11µg/L. The study shows that practically there was no mortality noticed up to a concentration of 0.02µg/L. The exposure of fish to 24h, at a concentration of 0.03 µg/L to0.05 µg/L of synthetic pyrethroid cypermethrin show 10% mortality, at a concentration of 0.07µg/L to0.09 µg/L show 20% mortality, while at concentration of 0.11 µg/L 30% mortality was observed (Table 1). The exposure of fish to 48h, at a concentration of 0.03 µg/L show 10% mortality, at a concentration of 0.05 µg/L show 20% mortality, at a concentration of 0.07µg/L show 30% of mortality, at a concentration of 0.09 µg/L show 40% mortality, while at concentration of 0.11 µg/L 50% mortality was observed (Table 2). The exposure of fish to 72h, at a concentration of 0.03 µg/L show 10% mortality, at a concentration of 0.05 µg/L of synthetic pyrethroid cypermethrin show 20% mortality, at a concentration of 0.07µg/L show 30% of mortality, at a concentration of 0.09 µg/L show 50% mortality, while at concentration of 0.11 µg/L 80% mortality was observed (Table 3). For finding LC_{50}, exposure of fish to 96hours, no mortality was observed in the control group. The mortality of fish seemed to increase gradually when the concentration increased. The complete mortality rate was observed in the experiment with 0.11µg/L of cypermethrin (Table – 4). It is a minimum concentration which causes a 100% death. at a concentration of 0.03 µg/L show 20% mortality, at a concentration of 0.05 µg/L show 40% mortality, at a concentration of 0.07 µg/L show 50% mortality, at a concentration of 0.09 µg/L show 80% mortality while at 0.11 µg/L synthetic pyrethroid cypermethrin concentration, 100% mortality was observed.

Table-1: The result of lethal concentration showing the observed number and percentage of mortality of fish Tilapia (*O.mossambicus)* at 24 hr of pyrethroid cypermethrin.

S. No	concentration µg/L.	Number of subjects	Number of death at 24hr.	Percentage of mortality
1	0	10	0	0
2	0.03	10	1	10
3	0.05	10	1	10
4	0.07	10	2	20
5	0.09	10	2	20
6	0.11	10	3	30

Table-2: The result of lethal concentration showing the observed number and percentage of mortality of fish Tilapia (*O.mossambicus)* at 48 hr of pyrethroid cypermethrin.

S. No	concentration µg/L.	Number of subjects	Number of death at 48hr	Percentage of mortality
1	0	10	0	0
2	0.03	10	1	10
3	0.05	10	2	20
4	0.07	10	3	30
5	0.09	10	4	40
6	0.11	10	5	50

Table-3: The result of lethal concentration showing the observed number and percentage of mortality of fish Tilapia (*O.mossambicus*) at 72 hr of pyrethroid cypermethrin.

S. No	concentration µg/L.	Number of subjects	Number of death at 72hr	Percentage of mortality
1	0	10	0	0
2	0.03	10	1	10
3	0.05	10	2	20

4	0.07	10	3	30
5	0.09	10	5	50
6	0.11	10	8	80

Table-4: The result of lethal concentration showing the observed number and percentage of mortality of fish Tilapia (*O.mossambicus*) at 96 hr of pyrethroid cypermethrin.

S. No	concentration µg/L.	Number of subjects	Number of death at 96hr	Percentage of mortality
1	0	10	0	0
2	0.03	10	2	20
3	0.05	10	4	40
4	0.07	10	5	50
5	0.09	10	8	80
6	0.11	10	10	100

Fig 1: Tilapia exposed to cypermethrin exhibited disrupted school behaviour, localization to the bottom of test chamber and independency (spread out) in swimming, motionless at the water surface, followed by drowning to the bottom.

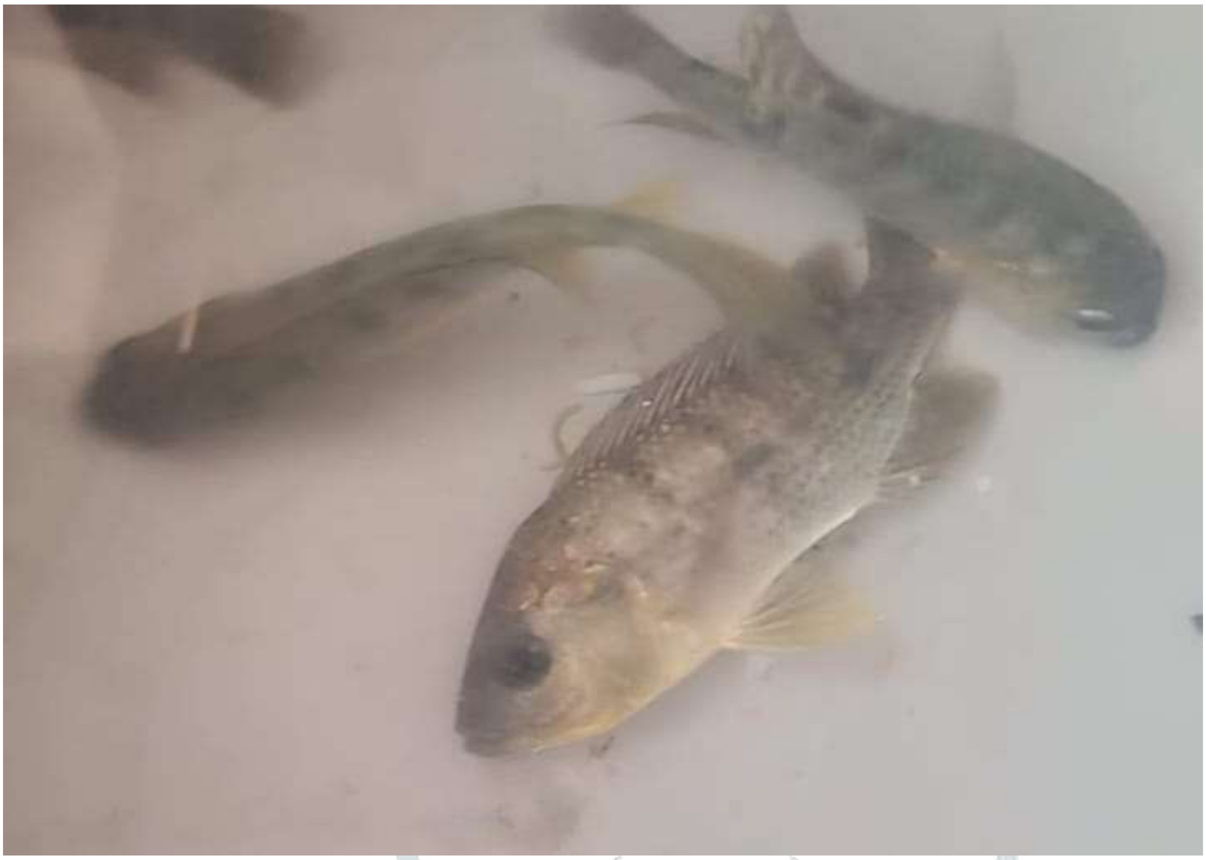

Fig 2: Tilapia exposed to cypermethrin exhibited disrupted school behaviour, localization to the bottom of test chamber and independency (spread out) in swimming, dullness, loss of equilibrium, stop of food intake, erratic and hysteric swimming

Behavioural effects

Several epidemiological and experimental studies have been performed to assess the health risks associated with cypermethrin exposure and measured cypermethrin level in the blood and urine of the pesticides sprayers and exposed individuals [Liao et.al., 2011]. Cypermethrin has been identified as one of the important constituent pesticides associated with human health risks [Liao et al., 2011]. In the present study, the control fish were active for feeding and alert to slightest disturbance with their well-synchronized movements. The behaviour did not significantly vary within the control groups. Fish exposed to synthetic pyrethroid cypermethrin exhibits behavioural abnormalities, such as non random swimming, loss of equilibrium, hyperactivity, increased surface activity, opercular activity, spread out in swimming followed by loss of coordination, localization to the bottom of test chamber and mucus formation. At the beginning of exposure, fish were found to be healthy and very active. During the experiment, they tried to avoid the test water for sometime by swimming fast, jumping, and other random movements in treated groups. Subsequently, fish moved to the corners of the test chambers, which can be viewed as an avoidance behaviour of the fish to synthetic pyrethroid cypermethrin. Similar behavioural patterns were noticed by Rahman *et al*. (2002) on *Anabas testudineus* and *Barbodes gonionotus*, and Haque *Puntius gonionotus*. Holmstedt, 1963; Habig and DiGiulio, reported that due to the toxicity effects of the pesticide, the fish gradually lost equilibrium, became paralyzed and finally settled down to the bottom of the aquarium and remained at the same place till death. At higher concentrations, fish expressed erratic swimming with jerky movements, along with hyper-excitation. They also secreted excessive amounts of mucus, which covered the buccal cavity, body, and gills. Fish became pale as intensity of body colour decreased. Under such conditions, efficiency of oxygen uptake decreased considerably, which was manifested as enhanced breathing rate along with more frequent visits to surface water for gulping fresh air. Eventually, there was loss of balance, exhaustion, and lethargy owing to respiratory incumbency. At last, they assigned a vertical position with mouth near the water surface and tail in downward direction to gulp the air. Soon, they settled down passively at the bottom of the tank; after a while, their bellies turned upward

Cypermethrin is a class II synthetic pyrethroid pesticide and crosses the blood brain barrier and exerts neurotoxicity in the central nervous system and also induces motor deficits. Pyrethroids, including cypermethrin, extend the opening of sodium channels in the central nervous system leading to hypo-polarization and hyper-excitation of the neurons [Eells and Dubocourch, 1988]. Short-term neurotoxicity caused by cypermethrin is primarily mediated through hyper-excitation of the central nervous system [Narahashi et. al., 1992]. Additionally, cypermethrin induces neurotoxicity by modulating the level of gamma-amino-butyric acid (GABA) [Manna et al., 2005]. Furthermore, cypermethrin-mediated neurotoxicity is contributed by its ability to induce free radical generation [Kirby, et al., 1999]. Since oxidative stress critically contributes to the nigrostriatal dopaminergic neuro-degeneration. Tilapia exposed to pyrethroid cypermethrin exhibited irregular, erratic and darting swimming movements and loss of equilibrium followed by hanging vertically in water. The above symptoms may be due to inhibition of acetylcholine-esterase (AChE) activity leading to accumulation of acetylcholine (ACh) in cholinergic synapses ensuing hyper-stimulation. Since, inhibition of AChE activity is a typical characteristic of synthetic pyrethroid cypermethrin. Similar observations made by Hülya *et al.* (2006) in the sentinel freshwater fish, *Oreochromis niloticus* sub-lethal exposure to diazinon. Ismail et al., (2014), observed neurotoxic effects like lateral side movement, loss of balance, movement in circular form with jerks and increased operculum activity in Labeo rohita exposed to chlorpyrifos (Halappa and David, 2009). Exposed fish Cyprinus carpio in a toxic media containing chlorpyrifos and visualized erratic, irregular and darting swimming movements, loss of equilibrium, hyper excitability and ultimately sinking of the fish to the bottom (Canvas and Konen, 2008) had found a different swimming pattern like circular, spiral and upside down swimming in fish O. niloticus followed by domoic acid injection and the symptoms were found to disappear after 3 hours of exposure. The primary molecular mechanism of action of the pyrethroid cypermethrin pesticides is inhibition of AChE activity, a widely distributed serine esterase. ACh is the primary neurotransmitter in the sensory and neuromuscular systems in most species. Activity of the AChE system is vital to normal behaviour and muscular function and represents a prime target on which some toxicants can exert a detrimental effect. Disrupted shoaling behaviour, easy predation, gulping air and swimming at the water surface (surfacing phenomenon) were observed on the day of exposure to sub-lethal concentrations of synthetic pyrithroid cypermethrin.

During the study period, the behavioural changes and reactions of **Tilapia (*Oreochromis mossambicus*)** were observed to different concentrations of the synthetic pyrethroid cypermethrin.. The first reactions of treated fish were observed within five minutes at the concentrations of 0.11µg/L. Several abnormal behaviours such as restlessness, sudden quick and rolling movement, swimming to the back (at higher doses) were observed. Finally, the affected fish became very weak, settled at the bottom and died. The number of dead increased with increasing concentration. Disrupted shoaling behaviour, easy predation, gulping air and swimming at the water surface were observed on the day of exposure to sublethal concentrations of synthetic pyrethroid cypermethrin.. This situation continued intensely throughout the test periods, Ural and Simsek (2006), suggesting that gulping of air may help to avoid contact of toxic medium and to ease respiratory stress. Surfacing phenomenon i.e., significant preference of upper layers in exposed groups may be due to elevated demand for oxygen during the exposure periods (Katja *et al.*, 2005). Similarly, gulping air, swimming at the water surface (surface phenomenon), loss of equilibrium, change in body colour, increase secretion of mucus and irregular swimming activity were observed by (Kumar et al., 2015) in fish Clarias batrachus after exposure with copper sulphate. Naserabad et al, (2015) reported faster opercular activity, jerky moments, erratic swimming and protrusion of eyes in goldfish after treatment with malathion. Of all, the phenomenon of easy predation is one of the most serious damage caused by a pollutant on sensitive species like fish, which ultimately decide the survival of a species in a given ecosystem. Caudal bending was noticed in 0.09 µg/L and 0.11 µg/L concentrations with time and persisted which greatly retarded the normal swimming pattern. The extent of caudal bending was pronounced in the highest toxicant concentration (0.11 of 96 h LC50). 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). Bending of the caudal portion may be a fact that is the thinnest structure of the caudal region and due to paralysis of caudal musculature because of AChE activity inhibition. Leaning of fish indicates reduced feeding behaviour and diversion of fish metabolism towards adaptability to the toxic media. Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. Thus, synthetic pyrethroid cypermethrin induced behavioural responses and affected morphological features.

Hyperextension of fins, dullness in body colour and fish body became lean towards abdomen and tilapia under stress were observed with time and concentration in experimental periods. Intermittently, some of the fish sank to the bottom with their least opercular movements. Feeding preferences were affected and consumption of food in fish was impaired and reduced drastically. This was more pronounced in 96 hr of sub-lethal exposure periods. Fish slowly became lethargic, restless and secreted excess mucus all over the body. Intermittently some of the Tilapia were hyper excited resulting in erratic movements. An excess secretion of mucus in fish forms a nonspecific response against toxicants. Mucus also forms a barrier between the body and the toxic medium, to minimize its irritating effect, or to scavenge it through epidermal mucus. Rao (2006) made similar observations following RPR-V (a novel phosphorothionate insecticide, 2-butenoic acid-3- [diethyl phosphinic thionyl] ethyl ester) exposure to euryhaline fish, *Oreochromis mossambicus.* The present study strongly supported different researchers who have observed similar results in different fish species in response to various toxicants. Hassain et al., (2015) found excessive mucus secretion, change in pigmentation, muscle fasciculation, and respiratory distress in Catla catla in response to dimethoate.

CONCLUSION

The behavioural examination revealed that cypermethrin affects behaviour of fish including erratic movement, inequality movement, increasing of opercular activity and moving to water surface to take some air. Even at low concentration of 0.03 µg/L, fish exhibited an irregular movement and an increase in their visit to the water surface. It will be supposed that all this behaviour were a result from the mode of action of cypermethrin as a neurotoxin that subsequently caused fish to be excited, anxious and an unbalanced movement. We can conclude that the long-term exposure of fish to pesticides (including insecticides), means a continuous health hazard for the population. So, the human population is at high risk by consuming these toxic fish.

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