Effect of agricultural pesticide (Cypermethrine) on *Labeo rohita*

By

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Effect of agricultural pesticide (Cypermethrine) on *Labeo rohita*

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DECLARATION

The results submitted in this project thesis are entirely of the candidate's own investigations and no part of the results has been accepted for any degree, nor is it being concurrently submitted for any degree

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Abstract

The effect of cypermethrine pesticide on juvenile rohu (*Labeo rohita*) was determined in captive condition in the fish physiology laboratory of Fisheries and Marine Resource Technology Discipline of Khulna University from September 15 to October 20, 2009. Live and healthy Rohu were exposed to five concentrations of Cypermethrine 0.625, 0.125, 0.025, 0.005, 0.001 ppm and a control group (no pesticide) with two replications for each treatment. The mean tank water temperature and pH of water in the experiment tanks were 21.53±0.41°C and 7.22±.013 respectively. The average initial body weight of the experimental Rohu was 20.965 g, 21.4 g, 20.55 g and 16.25 g in the control, 0.001 ppm, 0.005 ppm, and 0.025 ppm concentration tank respectively. The highest growth was recorded in control tank 0.2 g per week, whereas no significant growth was found in 0.001 ppm and 0.005 ppm concentration. Rohu reared in the 0.125 ppm had the lowest growth. The percentage of mortality of the test animals was varied depending on the concentration of pesticide and exposure time. The median lethal concentration values were found at 0.025, 0.005, 0.001, ppm for 9th, 17th, and 20th day respectively. Behavioral patterns were observed in both lethal (0.625, 0.125 ppm) and sub lethal concentration (.025 ppm). *L. rohita* in toxic media exhibited erratic and darting movements with imbalanced swimming activity, which might be due to the malfunctioning of neurotransmitters, followed by hyper and hypo opercula activity, loss of equilibrium, and mucus secretion all over the body were observed. The structure of the gill lamellae broken and colure of the gill turn into brown. Kidney in the dead fish was damaged and found some jellylike substance.

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Introduction

Fish is considered one of the major components of the aquatic habitats and a good source of protein for human. Fish are finned, aquatic, cold blooded vertebrates with gills. They share a body plan similar to that of humans and other vertebrate (with a backbone) animals. Fish breath oxygen, feed, moves, reproduce, and sense their surroundings like other vertebrate animals, but they do so in water. Over 20,000 known species of vertebrates are fish. Ninety percent of these belong to the Class Osteichthyes, or bony fish. Most of the other ten percent belong to the Class Chondrichthyes, or cartilaginous fish, the sharks, rays, and skates, all of which have a skeleton of cartilage. The third class of fish is called the Agnatha ("without a jaw"), a primitive group of mostly extinct fish, with only two living forms, the lampreys and the hagfish. Fins give fish mobility, stability, and maneuverability. Fins can be paired or unpaired. The dorsal and anal fins are unpaired fins that act as keels to prevent the fish from rolling. The caudal or tail fin, also unpaired, helps to propel and maneuver the fish. Pelvic and pectoral fins are always paired and these permit the fish to steer, stabilize, and stop. Paired fins that are maneuverable and flexible provide better control at slow speeds, whereas fins that are rigid act as steering planes for fast swimming fish. The fins and the body of bony fish are covered with skin that contains thousands of tiny scales (Hill, 1989).

Growth of a fish is marked in annual rings on each scale, and scales vary in size and shape characteristic of each species of fish. Therefore, examination of a fish scale can tell an investigator not only which species of fish produced the scale but also the age of that particular fish. Mucous covers the skin and scales of bony fish, which is why fish feel slimy, but the mucus is extremely important because it protects the fish from disease, predators, parasites, and sunburn. The skin of cartilaginous fishes, however, is quite different, without mucus but instead thick and tough like armor, covered with tiny impervious scales. The scales of sharks and their relatives are called denticles (like teeth), and the skin feels like sandpaper (Devlin & Nagahama, 2002).

The most popular among carp species for food in Bangladesh is Rohu, *Labeo rohita*. It is a fast growing fish in rivers and even in ponds and other close water bodies but has a comparatively slower growth rate than catla, *Catla catla*. *Labeo rohita* is one of the prime cultured fresh water teleost in Bangladesh. This fish are cultured in pond, gher, and also bill by using temporary fencing. Most of the rivers of Bangladesh are used as the matting place of this fish. During the hatching period they inter into the shallow water area of bill, khal, and swamp.

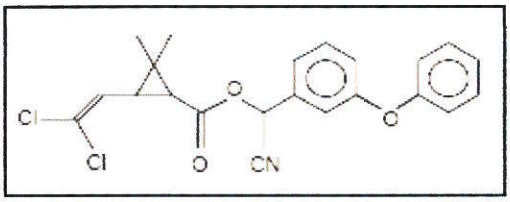
The pesticides sprayed on different crops are subsequently drained off to different water bodies, where the fishes live and breed. The indiscriminate use of pesticide in modern agriculture, forestry, and sanitation has caused different types of damage to non-target organisms.

Most of the pesticides are chemicals used in agriculture for the control of pests, weeds or plants, diseases. Pesticides commonly called pests and chemical used to control harmful organisms in everyday life of modern men. Pesticides can be defined as a large variety of chemical compounds that are used to kill or prevent the undesirable living organisms in agriculture purpose for higher productivity, free from diseases and to improve quality of the products (Sattar, 1985). Unfortunately their misuses have resulted in environment pollution especially for water. It affects on the aquatic fauna, specially fishes, crabs and shrimps. Pesticides can enter water sources through drift, runoff, soil erosion, leaching, and occasionally, accidental or deliberate release can kill fish. Sometimes indirect effects of pesticides on fish may be found which are created by dissolved oxygen depletion and causes the death of fish. The most obvious effects of pesticides on fish are direct effects of acute poisoning. Generally pesticides use in the cropland to control the insect which are very much harmful for the crop. These pesticides are washed away into the river, canal, and bill by the rain water. Pesticides are directly mixed with water and pollute the water. These pesticides make different types of problem in fish directly kill fish or long term effect.

Fish are particularly susceptible to cypermethrin. Cypermethrin is a synthetic pyrethroid and a permethrin analogue. Cypermethrin is a synthetic compound primarily used as an insecticide. It acts as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. Cypermethrin is a synthetic pyrethroid insecticide used to control many pests, including moth pests of cotton, fruit, and vegetable crops. It is also used for crack, crevice, and spot treatment to control insect pests in stores,. Technical cypermethrin is a mixture of eight different isomers, each of which may have its own chemical and biological properties. Cypermethrin is light stable. It is available as an emulsifiable concentrate or wet table powder. (Carriquiriborde, *et al.*, 2007.)

The pure isomers of cypermethrine are colourless crystals; the technical material is a viscous yellow-brown semi-solid. The melting points are 60-80°C. Cypermethrin is soluble in water at 21°C, 0.01-0.2 mg/l; in hexane at 20°C, 103 g/l; in acetone, cyclohexane, ethanol, xylene and chloroform. Cypermethrin is quite stable at temperatures under 220°C; photodecomposition has been observed in field tests, with no reduction in biological performance. The optimum stability occurs at pH 4 while decomposition occurs under alkaline conditions. (Chapman & Harris, 1978).

The common name of Cypermethrin is Cypermethrin (ISO, BSI and BPC) IUPAC name is (RS)-ý-cyano-3-phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. Molecular formula is $C_{22}H_{19}C_{12}NO_3$. Molecular weight is 416.32.



cypermethrin

(+/-) alpha-cyano-(3-phenoxyphenyl) methyl (+)-cis,

trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

The median lethal concentration (the concentration that kills 50 percent of a population of test animals; LC_{50}) for most of the fish is less than 5 ppb (WHO, 1989). This is partly because fish are unable to break down pyrethroids as efficiently as mammals and birds and partly because fish nervous systems are particularly sensitive to pyrethroids (Bradbury & Coats, 1989). Cypermethrin also causes sub lethal effects in fish. These include an increase in cholesterol at 1/5 of the LC_{50} and abnormal levels of blood sugars at concentrations as low as 1/10 of the LC_{50} (Philip *et al.,* 1995) Cypermethrin bioconcentrates in fish. Bioconcentration factors (the ratio between the concentration in fish tissue and the concentration in the water in which the fish is living) in rainbow trout range from 180 to 438 depending on water type (Muir *et al.,* 1994)

The effect of Cypermethrine also found in other animals. If exposed to cypermethrin during pregnancy, rats give birth to offspring with developmental delays. In male rats exposed to cypermethrin, the proportion of abnormal sperm increases. It causes genetic damage: chromosome abnormalities increased in bone marrow and spleen cells when mice were exposed to cypermethrin. Cypermethrin is classified as a possible human carcinogen because it causes an increase in the frequency of lung tumors in female mice.

This group of chemicals acts primarily on the basal ganglia of the central nervous system, causing repetitive nerve action through prolongation of sodium permeability during the recovery phase of the action potential of neurons (Chapman& Harris, 1978). Cypermethrin is toxic to the nervous system. Symptoms of exposure include dizziness, nausea, headaches, and seizures. It also suppresses the immune system, inhibiting the formation of antibodies to disease-producing microbes. Toxicity of this chemical depends on the size of fish, species and temperature (Stephenson, 1983).

It is well known that cypermethrin is extremely toxic to fish and aquatic arthropods under laboratory conditions (Bradbury and Coats, 1989), and it is also recognized that its toxicity is reduced under field conditions in water bodies with abundant particulate material (Hill, 1989). For example, a 12-fold increase of the LC_{50}at 96 h was observed for the fish *Cnesterodon decemmaculatus* exposed to cypermethrin in stream-water when compared to laboratory waters. Furthermore, no lethal effects were observed after insecticide exposure under field-use conditions (Carriquiriborde *et al.,* 2007). However, concern exists about the possible sublethal effects induced by this pyrethroid, particularly taking into account that cypermethrin spraying events mainly occur during the breeding season of many fish species. Cypermethrin has also been reported to exhibit in vitro antiestrogenic activity (Tyler *et al.,* 2000). Estradiol is a key hormone in ovarian differentiation in many fish species (Devlinand & Nagahama, 2002; Strüssmann and Nakamura, 2002), therefore the potential capacity of cypermethrin to act as an endocrine disruptor in vivo should be taken into consideration. Moreover, a recent study reported that in vivo waterborne exposures to cypermethrin affected gonadotrophic cells, gonads, and concentration of steroids in the plasma of the catfish, *Heteropneustes fossilis* (Singh and Singh, 2008).

Cypermethrin is very highly toxic to fish and aquatic invertebrates. The LC_{50} (96-hour) for cypermethrin in rainbow trout is 0.0082 mg/L, and in bluegill sunfish is 0.0018 mg/L (Stephenson, 1983). Its acute LC50 in *Daphnia magna*, a small freshwater crustacean, is 0.0002 mg/L. Cypermethrin is metabolized and eliminated significantly more slowly by fish than by mammals or birds, which may explain this compound's higher toxicity in fish compared to other organisms . The half-lives for elimination of several pyrethroids by trout are all greater than 48 hours, while elimination half-lives in birds and mammals range from 6 to 12 hours. The bioconcentration factor for cypermethrin in rainbow trout was 1200 times the ambient water concentration, indicating that there is a moderate potential to accumulate in aquatic organisms. Elimination of half of the accumulated amount of the compound took nearly eight days. After 14 days 70 to 80% of the material had been eliminated from the organisms (Stephenson, 1983)

Cypermethrine is widely used and common pesticides in Bangladesh. The effect of cypermethrine in fish is very much hazardous. Cypermethrine is extensively used in sundarban forest for capturing fish.

Objective of this study:

- To know the lethal effect of cypermethrin on freshwater fish, *Labeo rohita* juvenile by of LC_{50}.
- To observe behavioral changes during exposing to cypermethrine.
- To analyze the growth performance of fish in various concentration of cypermethrine.

Review of literature

Bradbury and Coats (1989) have reviewed the toxicology of pyrethroids in mammals, birds, fish, amphibia and invertebrates (terrestrial and aquatic) and cited 96-h LC50 cypermethrin toxicity as 2.2 μg/L for *Tilapia nilotica*, 0.9-1.1 μg/L for carp (*Cyprinus carpio*), 1.2 μg/L for brown trout (*Salmo trutta*), 0.5 μg/L for rainbow trout (*Salmo gairdneri*), and 0.4 μg/L for *Scardinius erythropthalmus*.

Davit *et al.* (2004) studied the effect of the sub-lethal concentration of cypermethrin on the metabolic profile of *Cyprinus carpio*. They reported a decrease in soluble proteins, while amino acids and activities of protease, aspartate and alanine aminotransferase increased with the administration of the toxicant. They interestingly noted that ammonia content decreased but urea and glutamine increased with the dosage.

Das and Mukherjee (2003) examined the Sub-lethal exposure of cypermethrin (1/10 and 1/50 of 96-h LC50 at 0.139 ppm) altered the\ biochemical, haematological parameters and enzymes of organs tissue and exerted stress on *Labeo rohita* (Indian major carp) fingerlings. Plant extracts may be useful in counteracting some of these effects.

Moore and Waring (2001) reported even low levels of cypermethrin in the aquatic environment having a significant long-term effect on Atlantic salmon populations through disruption of reproductive functions. They studied the effect of cypermethrin on olfactory mediated reproductive endocrine function where pheromones released by reproductively mature female salmonids are detected by the olfactory systems of the males and that this results in an increase in the levels of plasma reproductive steroids and expressible milt. Salmon milt and ova were mixed in the presence of sub-lethal concentrations of cypermethrin and the subsequent development of the embryos was monitored. Fertilization was reduced in the cypermethrin exposed group.

Philip *et al.* (2002) examined the carbohydrate metabolism of *Labeo rohita* subjected to sub-lethal concentration of cypermethrin under in vitro conditions. They reported that the fish adopted a compensatory mechanism to derive energy during pyrethroid toxicosis.

Polat *et al.* (2002) found the 48-h LC50 value of beta cypermethrin in male guppies is 21.4 μg/L.

Stephenson (1983) has compiled 96-h LC50 of cypermethrin on species as follows; 2.8 μg/L for rainbow trout (*Oncorhynchus mykiss*), 1.2 μg/L for fathead minnow and 0.93 μg/L for *Pimephales promelas* (juvenile).

Wend *et al.* (2003) investigated the effect of cypermethrin upon the composition of crustacean. rotifer, periphyton and phyhoplankton communities and observed that the

Materials and Method

The experiment was conducted to determine the LC 50 of cypermethrin and the comparative growth rate of rohu juvenile in different concentration of the pesticide in respect to control from 15 September to 20 October 2009 in the Fish Physiology Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University.

3.1 Animals:

The fish, (*Labeo rohita*) were obtained from the wild source of Khalishpur bill area. The specimens (average weight 20g and length 15 cm) were transported to the laboratory in appropriately aerated plastic bags. The plastic bags were placed into the maintenance aquarium for about 24 hours for acclimatization, and then the fish were allowed to swim into the aquarium water.

3.2 Chemicals:

Cypermethrin was selected as the test chemicals and was collected from local market in Khulna city. Cypermethrin is marked in Bangladesh by Mosko marketing company. The trade name the pesticide is Cypermethrin 10 EC. It contains 100 ml active ingredient of 1liter.

3.3 Sample preparation:

The total body weight of the Rohu was taken by using top balance. The samples were released into the experimental tanks in water and kept over night to be adapted with new environment. Additional air was supplied to the acclimatization tanks with aerator.

3.4 Feed:

During the acclimation period, the fish were not fed. Two days after the fish were fed to commercial diet. A definite quantity of food was given to know how much they can take everyday. No foods remain uneaten and a measurable amount of food is given to the fish.

3.5 Experimental design:

The experiment was conducted in 6 glass aquaria arranged in two rows on two iron stands. The size of each aquarium was 50 cm x 30 cm x 30 cm. At first all the aquaria were cleaned with water and filled with 30 liter tap water. The water was supplied to the tanks by PVC pipe from internal supply water. In each aquarium, one aerator was set to provide sufficient dissolved oxygen to the test animals. Thermometer was used to record the temperature.

The experiment was carried out with five different concentrations (treatments) (0.625, 0.1250, 0.025, 0.005, and 0.001 ppm) of cypermethrine and a control (no pesticide). Each concentration had 2 replicates. All specimens were acclimated for 24 hours with tap water from the start of the experiment. In each aquarium, twelve individuals of juvenile sample were released for one month exposure time. The faces and the waste food was eliminated everyday by siphoning. The water of the tanks was changed every seven days intervals and was maintained the concentration.

Present study was performed in two replicates in order to ensure the reproducibility of the results. Seventy two fish were randomly divided into six groups as follows:

- Group I (n = 12), the control group, which was no pesticide exposure.
- Group II (n =12), fish exposed cypermethrin at a concentration of 0.001 ppm.
- Group III (n = 12), fish exposed cypermethrin at a concentration of 0.005ppm.
- Group IV (n = 12), fish exposed to cypermethrin at a concentration of 0.025 ppm.
- Group V (n = 12), fish exposed to cypermethrin at a concentration of 0.125 ppm.
- Group VI (n =12), fish exposed to cypermethrin at a concentration 0.625 ppm.

3.6 Biological data collection:

Each group of fish consisting of 12 individuals were selected at random and placed into aerated test chambers. After 24 hours of adaptation, different concentrations of cypermethrin were added to the experimental aquaria. Mortality was assessed everyday and continuing at the end of the experiment. Dead individuals were removed

In the experiment two different types of fish are taken for the test. Among the test the larger fish died first then the smaller one. More often the large fish took more water and took more pesticides than the smaller one. Large fish have large gill and their respiration also faster than the smaller one. Cypermethrine is a neurotoxin; large fish have large lateral line and more nerve than the smaller one. So the large fish attacked faster than the smaller one.

4.2 Behavior: Generally fish shows very normal behavior. But if there may originate any external stimuli fish change it's behave to adapt itself in the foreign environment.

Table 2: Behavior of fish shows in different concentration of test chemical in respect of time.

Behavior of fish	Day at different concentration				
	0.001	0.005	0.025	0.125	0.625
Migration to the bottom	-	-	2nd	1st	1st
Schooling behavior disrupted	-	-	9th	1st	1st
Spread out and swimming independently	-	-	-	1st	1st
Irregular, erratic and darting movements	-	-	4th	1st	1st
Escaping phenomenon	4th	10th	3rd	1st	1st
Repeated opening and closing of the mouth and opercula	-	5th	3rd	1st	1st
Hyperextension of all fins	-	7th	4th	1st	1st
Excitement	-	-	3rd	1st	1st
Corkscrew pattern swimming behavior	-	-	3rd	1st	1st
Sudden, rapid, non-directed spurt of forward movement	-	5th	3rd	1st	1st
Signs of tiredness	-	7th	4th	1st	1st
Barrel-rolled or spiraled at intervals	-	-	6th	1st	1st
Engulfed the air through mouth	-	-	7th	1st	1st
Mouth and operculum wide opened	-	16th	14th	1st	1st
Colour of the gill lamellae from reddish to light brown	28th	17th	10th	1st	1st

In these experiment different doses of pesticide used to understand the behavioral change of fish when they exposed in that doses. The behavioral changes of fish in different concentration of pesticides are determined in different expose time of the experiment and are recorded. (Table-2)

When the fish were exposed to the lethal concentration of cypermethrin, they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted in the first day itself and the fish occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. Irregular, erratic and darting movements followed this with imbalanced swimming activity. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escaping phenomenon. The frequency of surfacing phenomenon was greater on the second day of exposure wherein the fish frequently come to the water surface. Respiratory disruption was observed in the normal ventilating cycle (cough, yawn) with a more rapid, repeated opening and closing of the mouth and opercular coverings. Partially extended fins and singlewide opening of the mouth and opercula coverings accompanied by hyperextension of all fins were found and the fish was in a state of excitement on the third day. The swimming behaviour was in a corkscrew pattern rotating along horizontal axis and followed by `s` jerk, partial jerk, sudden, rapid, non-directed spurt of forward movement (burst swimming). The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. On the 4th day they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom.

They often barrel-rolled or spiraled at intervals and engulfed the air through mouth before respiration ceased. The fish eventually died with their mouth and operculum wide opened. A change in colour of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was seen in dead fishes.

In sub lethal treatment, 0.025 ppm the schooling behavior of the fish was slowly disrupted during the first day. The ventilation rate was increased, but hyperactivity, excitement, hyperventilation etc., were not influenced on exposure to the sub lethal concentration of cypermethrin at 5 and 10 days. Further, the fish at 15 days of exposure exhibited free, normal swimming and active feeding

4.3 Growth of fish: *Labeo rohita* is one of the first growing fresh water species in the world. Large pond size and proper food make a suitable condition for the growth of this fish. The growth of this fish is directly related to the size of the water body. Large water body makes this specie easy to move and help to gain better body weight in compare to the smaller one.

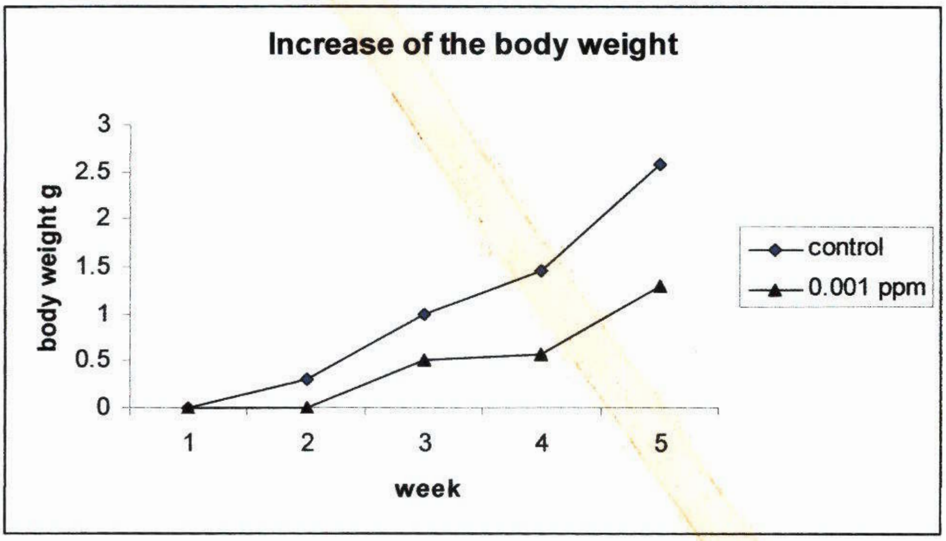


Fig -2: Weight gains of *Labeo rohita* every week interval at control and 0.001 ppm tank.

The figure shows that the fish in the control group gain its body weight normally. First two week the growth is not so high but in the last week it shows the higher growth rate. The fish that are exposed in the 0.001 ppm shows less growth than compare to the control group. (fig -2).

Generally fish lost its energy when they try to adapt foreign environment. For that reason their body may not develop as desired.

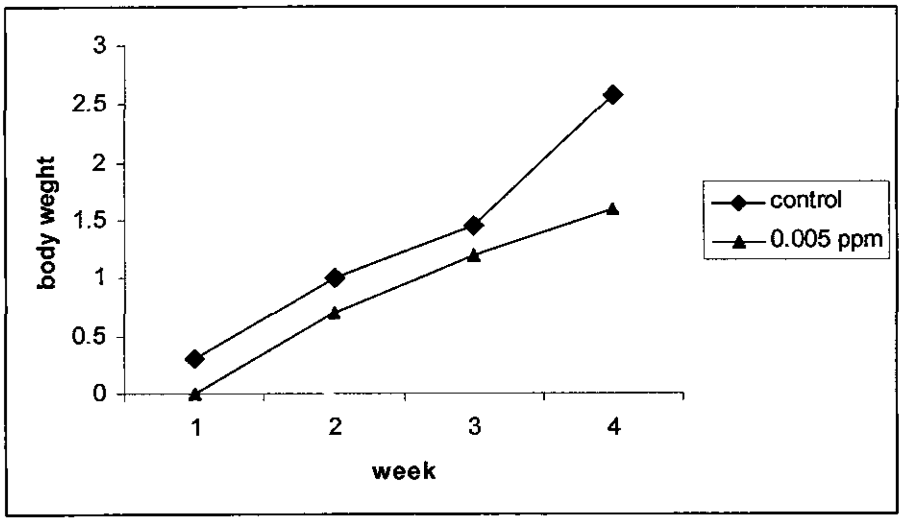


Fig-3: Weight gains of *Labeo rohita* every week interval at control and 0.005 ppm tank.

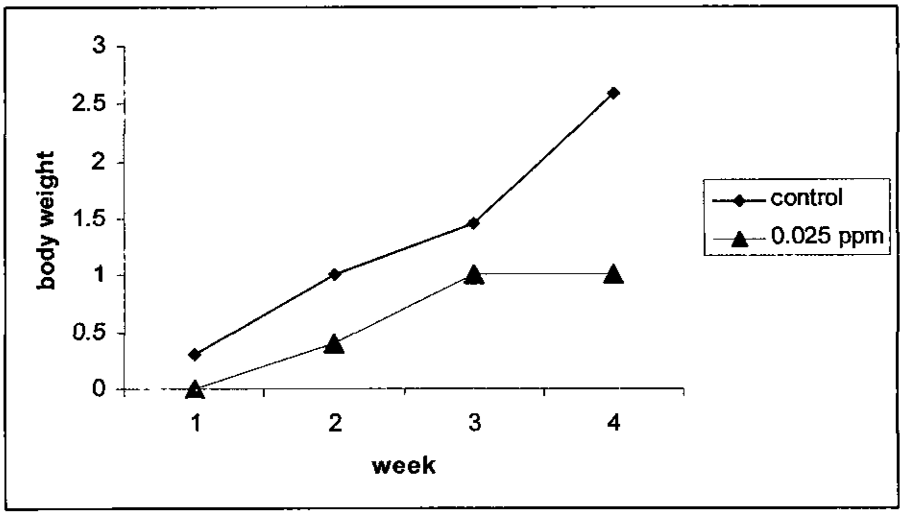


Fig-4: Weight gains of *Labeo rohita* every week interval at control and 0.025 ppm tank.

In the 0.025 ppm group the fish were not showed the higher growth rate. In this group the fish can not adapt itself and many of them died before completing the full experiment. (fig-4).

Among the experiment the highest growth was found in the control group of fish. This group of fish showed very normal behavior. They took food easily. But in the different concentration of the pesticides group the fish are try to adapt itself to the new environment and the sufficient growth may not found. The feed that are given were remained uneaten. In the concentration of 0.001ppm and 0.005 ppm solution the body wait increased in respect of time (Fig-3).

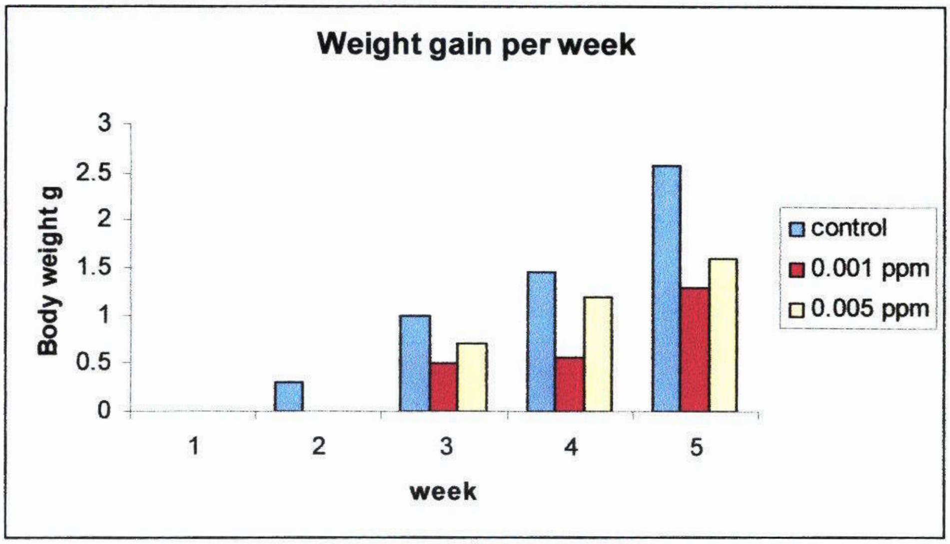


Fig-5: Weight gains of *Labeo rohita* every week interval at control, 0.001 ppm, 0.005 ppm and 0.025 ppm concentrated tank.

The weight of the fish in control group which has no pesticide increase in a regular basis but the other groups of fish, whose are exposed in several concentration of pesticides medium are not increase as like the control group. In the medium of 0.025 ppm concentration many fish are died and the weight not gained in the last two weeks. In the medium of 0.001ppm and 0.005 ppm solution medium the weight were not increased properly.

In the present experiment the species are kept into small aquarium and the stocking density is also high. The given food for the fish remains uneaten. In the 30 days of the experiment the body weight were not develop as desired.

In the control tank the fish showed better growth compare to the pesticide tank. The movement of the fish was also normal and this group of fish has taken food normally in compare to the pesticide group.

4.4 Gill and kidney structure:

Generally the gill of the fish is reddish or pink in colour. when they stay as a live condition and no breaking is found in the gill. Cypermethrine is a neurotoxins and it attack on the nerve of the fish. The fish try to repeat its opening and closing of the mouth and opercula in that situation the structure of the gill lamellae broken and coloure of the gill may turn into brown. In this experiment when fish are died they are taken to test out for the gill and kidney.

Kidney: In this experiment the size of the fish was under 5 cm. The actual structure of kidney is not found properly. Kidney in the dead fish was damaged and found some jellylike substance.

Discussion

Pyrethroids are highly toxic to insects and have been widely used in the past few years in agriculture. Although they are not persistent in the environment, their acute toxicity to fish is high. The acute test for a long time has been a major component in toxicity testing. In which acute chemical toxicity is determined as a 96hr LC_{50} value however the environmental significance of death of individuals after short term exposure to high concentration is questionable. In contrast to this our results shows cypermethrin is very toxic even at lower concentration (0.125 ppm) for 96hr LC_{50}.

Bradbury and Coats (1989) have reviewed the toxicology of pyrethroids in mammals, birds, fish, amphibia and invertebrates (terrestrial and aquatic) and cited 96-h LC_{50} cypermethrin toxicity as 2.2 µg/L for *Tilapia nilotica*, 0.9-1.1 µg/L for carp (*Cyprinus carpio*), 1.2 µg/L for brown trout (*Salmo trutta*), 0.5 µg/L for rainbow trout (*Salmo gairdneri*), and 0.4 µg/L for *Scardinius erythropthalmus*.

Table: A composition of LC_{50} of cypermethrine on different fish and invertebrate species.

species	group	Lc50 (ppb) at 96 hrs	source
Cyprinus carpio	Fish	0.9	Sukla *et al.*, 1998
Salmo trutta	Fish	1.2	
Salmo gairderi	Fish	0.5	
Scardinus erythropthalamus	Fish	0.4	
Tilapia nilotica	Fish	2.2	
Panaeus duorarum	Shrimp	0.036	Hill, 1985
Mysidopsis bahia	Crustacean	0.005	
Uca pugilator	Crab	0.2	
Labeo rohita	Fish	0.025,ppm at 9th day	Present study
		0.005,ppm at 17th day	
		0.001,ppm at 20th day	

Sub-lethal exposure of rohu to cypermethrin produced appreciable changes in the biochemical, enzymatic and haematological parameters. In case of *Salmo trutta* the LC_{50}

value was 1.2 ppb at 96 hrs and for shrimp *Panaeus duorarum* the LC_{50} value is 0.036 ppb at 96 hrs. In the present experiment the LC_{50} value was 0.025 ppm at 9^{th} day, 0.005 ppm at 17^{th} day, 0.001 ppm at 20^{th} day which was different from this experiment.

Lethality in the present study is comparable to the few previously published studies that exist, but that LC_{50}'s for all species exceeded this concentration. This can be attributed to the inability of the *L.rohita* to withstand and metabolize the cypermethrin intoxication. The acute toxicity treatments showed strong negative effects on survival as pesticide concentration increased. This suggests dose-dependent survival and concentration graded lethality (Table 1). The varying degree of mortality reported in this study is consistent with the report of David *et al.,* (2000). World Health Organization, (1989) reported that differences in an organism's biological adjustment and behavior response to change in water chemistry.

Behavioral 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 migration of the fish to the bottom of the tank following the addition of cypermethrin clearly indicates 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 indicates that fish adaptively shifts towards aerial respiration (by obtaining atmospheric oxygen) and the fish tries 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 coverings accompanied by partially extended fins was observed in the present study. This could be due to accumulation of mucus in the gill region for proper breathing (David, *et al.*, 2000).

The surfacing phenomenon of fish observed under cypermethrin exposure might be due to hypoxic condition of the fish as reported by (Radhaiah and Jayantha, 1988).
The increased surfacing during the initial periods of exposure to cypermethrin concentrations suggests an elevated rate of metabolism. Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to the pesticide and these activities 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 as pointed out by (Drummond, *et al.* 1986). Acute respiratory distress, degeneration of hepatocytes in perportal zones can imply the influence of toxic compounds in the digestive tract. The biochemical changes in liver profile can relate to hepatocytes damage (Sarkar, *et al.* 2005).

Aggressive behaviour such as nudge and nip were increased following exposure to the toxic material. Orientation and locomotors patterns were found to be involved in most aspects of fish behaviour such as migration, mating, courtship and feeding, which were altered under cypermethrin stress and observations in the present investigation are consistent with the earlier reports (Sarkar, *et al.* 2005) on exposure to environmental contaminants.

In sub lethal treatment, the schooling behavior of the fish was slowly disrupted during the first day itself. The ventilation rate was increased, but hyperactivity, excitement, hyperventilation etc were not influenced on exposure to the sub lethal concentration of cypermethrin on day 1 and day 5. Further the fish exposed to 10 and 15 days exhibited balanced swimming and active feeding.

The hyper excitability of the fish invariably in the lethal and sub lethal exposure to cypermethrin may be attributed to the hindrance in the functioning of the enzyme acetylcholine esterase enzyme (AChE) in relation to nervous system as suggested by many authors (Deva, 2000). It leads to accumulation of acetylcholine, which is likely to cause prolonged excitatory postsynaptic potential. This may first lead to stimulation and later cause a block in the cholinergic system. In most cases changes were more pronounced during a lethal exposure compared to sublethal exposure period.

In the present study the abnormal changes in the fish exposed to lethal concentration of cypermethrin are time dependent. However, the normal behaviour of the fish at 10 and 15 days on exposure to sub lethal concentrations indicates its adaptability to the sub lethal concentration due to long term exposure of cypermethrin. The fish behavior indicates that the fish has adapted to a compensatory mechanism to derive energy during pyrethroid toxicosis as suggested by (Philip, *et al.* 1988). Hence this type of study can be useful to compare the sensitivity of the various species of aquatic animals and potency of chemicals using LC_{50} values and to derive safe environmental concentration, by which there is no lethality and stress to the animals.

Fish species are sensitive to enzymic and hormone disruptors. Chronic exposure to low levels of pesticides may have a more significant effect on fish populations than acute poisoning. Doses of pesticides that are not high enough to kill fish are associated with subtle changes in behavior and physiology that impair both survival and reproduction (Kegley, *et al.* 1999). Biochemical changes induced by pesticidal stress lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism (Murty, 1986) Liver, kidney, brain and gills are the most vulnerable organs of a fish exposed to the medium containing any type of toxicant (Jana, *et al.* 1987) The fish show restlessness, rapid body movement, convulsions, difficulty in respiration, excess mucous secretion, change in color, and loss of balance when exposed to pesticides. Similar changes in behavior are also observed in several fishes exposed to different pesticides (Haider & Inbaraj, 1986).

Proteins are mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy (Umminger, 1977). Behavioural responses of fish exposed to sublethal concentration of cypermethrine showed that they were under stress condition. During stress condition, fish needed more energy to detoxify the toxicants and to overcome stress. Since fish have a very little amount of carbohydrates, the next alternative source of energy is protein to meet the increased energy demand. The depletion of protein fraction in liver, muscle and gonad tissues may have been due to their degradation and possible utilization of degraded products for metabolic purposes. Other worker such as, (Singh *et al.* 1996), have reported that the decline in protein constituent in different fish tissue exposed to sublethal concentrations of insecticides. Furthermore, any obstruction in RNA synthesis may also affect protein level as it plays an important role in protein synthesis. In the present experiment the body weight may decrease due to adapt the fish from such types of foreign environment.

Investigations have been shown that changes in carbohydrate and nitrogenous metabolism in fish induced by the stress occurred by pesticide-induced hypoxia. These changes include depletion of proteins, glycogen and pyruvate stores from fish tissues such as liver and muscle (Laul *et al.,* 1974). An elevation in free amino acids and protease activity was found due to pesticide-induced hypoxia (Sambasiva Rao, 1999). Black (1958) reported an elevation in lactic acid level in liver, muscle and blood and suggests that an uncontrolled entry of lactic acid into the tissues interferes with internal mechanisms, which maintain the acid-base balance. Lactic acid may also reduce the affinity of hemoglobin for both oxygen and carbon dioxide, diminishing the oxygen-carrying capacity of blood.

The insecticide cypermethrine and fish *Labeo rohita* were selected for study because the former is used often in field and the latter is an important paddy fish of Indian capture fishery.

Conclusion

Fish is a cold blooded vertebrate. Fish can tolerate a narrow range of salinity, pH and alkalinity changes. When fish are exposed in such areas which are not favorable for its body, they are try to survive and try to adapt in the foreign environment. When they can not tolerate they are died and if any can adapt themselves they are attacked by different types of disease. In the foreign environment fish loss its energy and they can not grow out as the desired growth rate. Bangladesh is agricultural country. Every year a huge amount of pesticides is used to protect the croups from the insects. These pesticides are wased out from the agricultural land to the river, canal, and ponds. The effects of these pesticides are yet not studied. In the present experiment the effect of cypermethrin on *Labeo rohita* is an example for other fresh water fish species. The concentrations of cypermethrine which are taken to examine the effect were 0 .625 ppm, 0.125 ppm, 0.025 ppm 0.005 ppm and 0.001 ppm. The maximum mortality was found in the 0.625 and 0.125 ppm concentration of cypermethrine. The growth of fish also hamper when they exposed in cypermethrine medium. The gill, kidney, brain also get stress. In the wild condition the effect of pesticide is not so much harmful but the growth is not found as desire. If the fish expose long time in the pesticide medium different types of biochemical compound are decrease in the muscle. The protein level is decreased and the construction of the muscle is damaged. In the present experiment the water body is so small in compare to the stocking but if we compare the growth rate of fish in different media to the control, the growth is high in the control group. Cypermethrin is a broad-spectrum insecticide. In addition to killing the insects that are the target of a particular treatment, it can also reduce populations of insects and other arthropods that are economically desirable because they prey on unwanted insects or are useful pollinators. While not usually considered beneficial insects in an economic sense, many aquatic insects are important components of aquatic ecosystems. In khulna region cypermethrine is directly used for capturing fish in Sundarban forest. It may hamper the non target species which are beneficial for ecosystem.

The time it takes to reduce the concentration of a pesticide in an organism or medium by half, through transport, degradation or transformation is called half life. The half life of this pesticide is 18 days. The chemicals that are taken to conduct this experiment contain 100 ml active ingredient of 1liter. So the concentration of this chemical is 100 ppm. 10 ml of this chemical are mixed with 10 liter of water and sprayed in the crop land. In the crop land the concentration is 1 ppm. So in the nature the concentration of this chemical is 1 ppm. After 18 days later the concentration is 0.5 ppm and again after 18 days the concentration is 0.25 ppm. So it required minimum six months to minimize its effect on fish. After six month the concentration that exist in nature are not harmful for fish.

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Appendix

Table-1: The total body weight of fish at 0.001 ppm concentration and date:

Total body weight of each fish on day 15/09/09	Total body weight of each fish on day 22/09/09	Total body weight of each fish on day 29/09/09	Total body weight of each fish on day 6/10/09	Total body weight of each fish on day 13/10/09
50	50	50.5	51	52
10	10	10	10.5	11
35	35	35	-	
10	10	-	-	-
65	65	65.5	66	67
75	75	75	75.5	76
15	15	15	15.5	16
20	20	20	-	-
15	15	15.5	16	16.5
25	25	25.5	-	-
35	35	35	35.5	36
20	20	-	-	-

Table-2: The total body weight of fish at 0.005 ppm concentration and date:

Total body weight of each fish on day 15/09/09	Total body weight of each fish on day 22/09/09	Total body weight of each fish on day 29/09/09	Total body weight of each fish on day 6/10/09	Total body weight of each fish on day 13/10/09
55	55	55.5	56	56
65	65	65	65.5	66
10	10	10	-	-
65	65	-	-	-
50	50	50.5	-	-
20	20	21	21.5	22
20	20	-	-	-
10	-	-	-	-
15	15	15.5	-	-
20	20	20	21.5	22
20	20	-	-	-
15	15	16	16.5	17

Table-3: The total body weight of fish at 0.025 ppm concentration and date:

Total body weight of each fish on day 15/09/09	Total body weight of each fish on day 22/09/09	Total body weight of each fish on day 29/09/09	Total body weight of each fish on day 6/10/09	Total body weight of each fish on day 13/10/09
55	-			
60	60	60	60	61
10	10	-	-	-
10	-	-	-	-
10	-	-	-	-
10	10	-	-	-
20	20	-	-	-
15	15	-	-	-
15	15	15	16	16
15	15	15	-	-
30	30	30	31	31
20	20		-	

Table-4: The total body weight of fish at control and date:

Total body weight of each fish on day 15/09/09	Total body weight of each fish on day 22/09/09	Total body weight of each fish on day 29/09/09	Total body weight of each fish on day 6/10/09	Total body weight of each fish on day 13/10/09
60	60.2	60.5	62	62.5
20	20.5	21	21.3	22
100	100.2	101	102	102
20	20	20.5	21	23
15	15	16	17	18
30	30	32	32.5	33
20	-	-	-	-
10	-	-	-	-
20	-	-	-	-
20	-	-	-	-
20	-	-	-	-
20	-	-	-	-