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EFFECT OF AGRICULTURAL PESTICIDE (CYPERMETHRIN) ON *Labeo rohita*

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Abstract: The effect of cypermethrin pesticide on juvenile rohu (*Labeo rohita*) was determined in captive condition. Live and healthy rohu (average initial weight 21g) were exposed to five concentrations of Cypermethrin 0.63, 0.13, 0.025, 0.005, 0.001 ppm and one control group (no pesticide) with two replications for each treatment. The mean temperature and pH in the experimental tanks were 27.53±0.41ºC and 7.22±0.013 ppt, respectively. The highest growth recorded in control tank was 0.2g per week, where as no significant growths were found in 0.001 ppm and 0.005 ppm concentration. Rohu reared in the 0.13 ppm had the lowest growth. The median lethal concentration values were found at 0.025, 0.005 and 0.001 ppm for 9th, 17th and 20th day, respectively. Behavioral patterns were observed in both lethal 0.63, 0.13 ppm and sub lethal concentration 0.025 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.

Keywords: Cypermethrin, lethal concentration, pesticide, toxic, *Labeo rohita*

Introduction

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)*. *L. rohita* is one of the prime cultured fresh water teleost in Bangladesh. This fish are cultured in pond, gher and also beel by using temporary fencing. 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. Fish are particularly susceptible to a potent and broad pyrethroid pesticide like cypermethrin which is a permethrin analogue and 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 and used to control many pests, including moth pests of cotton, fruit, and vegetable crops.

The pure isomers of cypermethrin 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. It is well known that cypermethrin is extremely toxic to fish and aquatic arthropods

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under laboratory conditions (Bradbury and Coats, 1989). It is also recognized that its toxicity is reduced under field conditions in water bodies with abundant particulate material (Hill, 1989). 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 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 (Stephenson, 1983). cypermethrin is widely used and common pesticides in Bangladesh. The effect of cypermethrin in fish is very much hazardous. The present experiment was conducted to determine the median lethal concentration (LC_{50}) of cypermethrin pesticide on juvenile rohu *(L. rohita*) and to analyze the growth performance and behavioral change of fish under laboratory condition with different concentration of cypermethrin.

Materials and Methods

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 the Fish Physiology Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna.

Animals: The specimens (average weight 21g) were collected from the wild source of Khalishpur beel area and transported to the laboratory in appropriately aerated plastic bags. Which were placed into the maintenance aquarium for about 24 hr for acclimatization, and then the fish were allowed to swim into the aquarium water.

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

Sample preparation: The total body weight of the rohu was taken by using top balance. The fish were released into the experimental tanks in water and kept over night to be adapted with new environment. Aeration through aerator was supplied to the acclimatization.

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 reasonable amount of food is given to the fish.

Experimental design: The experiment was conducted in 12 glass aquaria arranged in two rows on two iron stands. The size of each aquarium was (50× 30× 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 fish. Thermometer was used to record the temperature. The experiment was carried out with five different treatments as 0.63, 0.13, 0.025, 0.005, and 0.001 ppm concentrations of cypermethrin and a control group as no pesticide used. Each concentration had two replicates. All specimens were acclimatized 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 feces and the waste food was eliminated everyday by siphoning. Total the water of the tanks was changed every seven days intervals and was maintained the concentration and total 20L water used for each tank. Present study was performed in two replicates in order to ensure the reproducibility of the results. Randomly divided seventy two fish were equally divided into six treatment groups as follows: In Treatment 1, control group had no pesticide exposure, where as fishes in treatment 2, 3, 4, 5 and 6 had exposure of cypermethrin at a concentration of 0.001, 0.005, 0.025, 0.125 and 0.625 ppm, respectively.

Biological data collection: Each group of fish consisting of twelve (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 immediately. Behavioral changes were followed closely. Total body weight of each rohu was measured and recorded every seven days interval. The body weight was measured by a top balance. The dead fish was removed and used for further experiment for observed of gill and kidney conditions.

Results

The present experiment was conducted to determine the median lethal concentration (LC_{50}) of cypermethrin pesticide on juvenile rohu *(L. rohita*) under laboratory condition with different concentration and exposure duration. The second objective of this experiment was to analyze the growth performance of fish in various concentration of cypermethrin.

Determination of LC_{50}: LC_{50} is a measure of pesticide acute toxicity by concentration. It is the concentration of a chemical at which 50% of the test group dies. It is a time dependent value (i.e., the length of the test may vary) and may be an observed value or, more frequently, one calculated by interpolation or extrapolation. The cumulative percentages mortality of *rohu* was recorded at 24 hours interval against 0.001, 0.005, 0.025, 0.125, and 0.625 ppm concentration of cypermethrin and *rohu* in the control tanks (no pesticides) showed normal behavior and were found to be alive until the end of the experiment. There was no mortality found at 0.001 ppm concentration up to 10 days. At 11th days 8.33 % mortality was found in the 0.001 ppm concentration aquarium. At 4th days 8.33% mortality was found in the 0.005 ppm and the same mortality was found in second days at 0.125 ppm concentration. The maximum mortality was recorded at 100% with 0.125 ppm and 0.625 ppm in the first day. In this concentration fish showed some peculiar behavior and all the fish are died before twelve hours. Within the 20 days the maximum mortality at 66% was found in 17th day at 0.025 ppm concentration. After 20th days no fish are seen to be died in Table 1. The LC_{50} value was measured separately for 0.001, 0.005, 0.125 ppm concentration by plotting the mortality data against the days. The LC_{50} values were found at 0.025, 0.005, 0.001, ppm for 9th, 17th, and 20th day respectively. In the sub-lethal concentration the maximum mortality was recorded in 17th days. After 20 days no mortality are found in the sub-lethal concentration. The fish are adapted itself to that concentration. 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, large fish took water and pesticides than the smaller one. Large fish have large gill and their respiration is 8 also faster than that of the smaller one. Cypermethrin is a neurotoxin; large fish have large lateral line and more nerve than the smaller one. So the large fish was invaded faster than the smaller one.

Table 1. Cumulative mortality percentage of *L. rohita* in different concentration of cypermethrin at different exposer time.

Day	Cumulative mortality rate % at different (Concentration ppm)				
	0.001 ppm	0.005 ppm	0.025 ppm	0.125 ppm	0.625 ppm
1	-	-	-	100%	100%
2	-	-	8.33%	-	-
3	-	-	-	-	-
4	-	8.33%	25%	-	-
5	-	-	-	-	-
6	-	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	-	-	50%	-	-
10	-	25%	-	-	-
11	8.33%		-	-	-
12		33%	-	-	-
13	16.6%	-	-	-	-
14	-	-	-	-	-
15	-	-	-	-	-
16	-	-	-	-	-
17	25%	50%	-	-	-
18	-	-	-	-	-
19	41%	58%	-	-	-
20	50%	-	-	-	-

Behavior observed: Generally fish showed very normal behavior. In these experiment different doses of pesticide used to understand the behavioral change of fish when they were exposed in those doses. The behavioral changes of fish in different concentration of pesticides were determined in different expose time of the experiment were recorded and presented in Table 2.

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

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 and the fish occupied about twice the area than that of the control group. They spread out and appeared to be swimming independently. 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 could be revealed by their 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 operculam. 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 color 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 behaviour 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 (Table 3).

Table 3. Composition of LC_{50} of cypermethrin on different fish and invertebrate species

Species	Group	Lc50 (ppm) 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 0.005,ppm at 17th day 0.001,ppm at 20th day	Present study

Growth of Fish: The Fig.1 shows that the fish in the control group gained its body weight normally. The growth in first two weeks was not high but in the last week it showed the higher growth rate. The fish that are exposed in the 0.001 ppm showed less growth as against the control group. Generally fish lost its energy when they tried to adapt new environment. For that reason their body might not developed as desired. In the 0.025 ppm group the fish did not show higher growth rate. In this group the fish could not adapt itself and many of them died before completing the full experiment (Fig. 4).

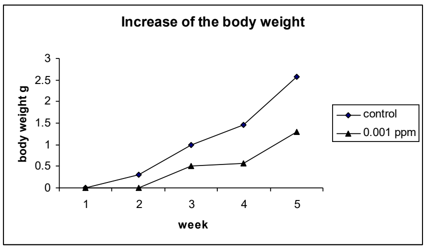

Fig. 1. Weekly gains of *L. rohita* at control and 0.001 ppm cypermethrin tank.

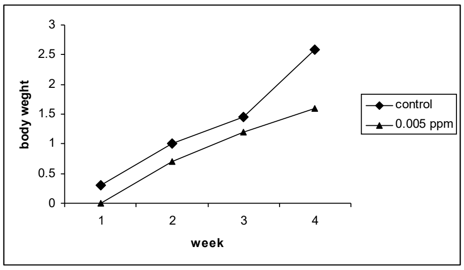

Fig. 2. Weekly gains of *L. rohita* at control and 0.005 ppm cypermethrin tank.

Among the experiment the highest growth was found in the control group of fish. This group of fish showed very normal behaviour. They took food easily. But in different concentration of the pesticides group the fish had loss growth and behavior tried to adapt itself to the new environment. 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. 2, 3).

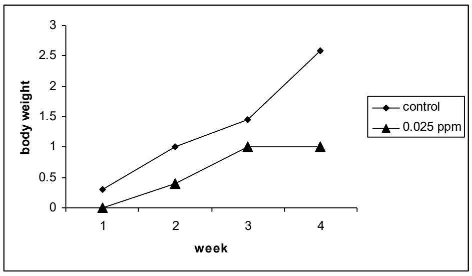

Fig. 3. Weekly gains of *L. rohita* at control and 0.025 ppm cypermethrin tank

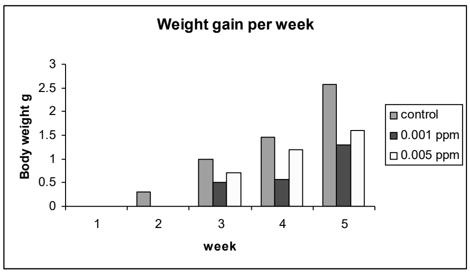

Fig. 4. Weekly gains of *L. rohita* at control, 0.001 ppm, 0.005 ppm and 0.025 ppm cypermethrin tank

In the treatment 4, 0.025 ppm concentration many fish died and the weight did not gained in the last two weeks. In the treatment 1 and 2 of 0.001ppm and 0.005 ppm respectively solution medium the weight were not increased properly. In the present experiment the species are kept into small aquarium and the stocking density was also high. The given food for the fish remained uneaten. In the 30 days of the experiment the body weight were not develop as desired. However, 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.

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 96 hr 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 96 hr LC_{50}. Bradbury and Coats (1989) have reviewed the toxicology of pyrethroids in mammals, birds, fish, amphibia and invertebrates (terrestrial and aquatic) and cited 96h 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* . 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. 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.

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 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, 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.

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, 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). Other workers such as Singh *et al.* (1996), who have also 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 cypermethrin and fish *L. 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

The maximum mortality was found in the 0.63 and 0.125 ppm concentration of cypermethrin. The growth of fish also hamper when they are exposed in cypermethrin medium. The gill, kidney, brain also get stress. If the fish are exposed for long time in the pesticide medium, then different types of biochemical compounds are decreased from the body. So it requires minimum six months to minimize pesticide effect on fish. After six month the concentration that exist in nature are not harmful for fish.

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