Effect of cypermethrin and 2, 4- Dichlorophenoxy acetic acid on histopathological changes in liver of *Clarias batrachus*

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Abstract

The objective of this study was to observe the effect of insecticide Cypermethrin and herbicide 2, 4-D on the Histopathology of liver of Clarias batrachus fish. Cypermethrin and 2,4-D cause severe histopathological changes like increase in size of liver, destruction of architecture of liver, haemorrhage, steatosis, infiltration of inflammatory cells, necrosis of hepatic cells, fatty changes and cloudy swelling of hepatocyte with a severe thick fibrous connective tissue.Thus on the basis of obtain result in the present investigation it can be concluded that 96 hrs. exposure of 80ul of Cypermethrin and 34.64ul of 2,4-D aqueous solution has toxic effect and suggests that exposure of cypermethrin and 2,4-D could cause some level of stress as indicated by changes in the histopathological indices of the fish under consideration. These histopathological observation indicated a strong response to tissue damage exposed to cypermethrin insecticide were higher when compared with 2, 4-D herbicide.

Key Words: *Histopathology, Clarias batrachus Cypermethrin, 2,4-D Toxic, Liver*

Introduction

The term ‘pesticides’ encompasses all chemical materials used for the control of pests. The pesticides chlorinated hydrocarbons and organophosphate are generally used in agriculture crop protection programmed. These pesticides are most toxic and stable chemicals which are not metabolized, degraded or excreted to any degree. Cypermethrin is used broadly in industrial, households and agriculture fields[1] for control of many insect pests which is a broad-spectrum synthetic pyrethroid insecticide[2]. Restricted Use Pesticides may be used and buying only by certified applicators. Cypermethrin insecticide affects various target aquatic organisms through its random use and due to agriculture run-off their entrance into water bodies[3], unfavourably affects fish hematology, metabolism[4], meat quality and fish population[5]. 2, 4-D (2,4-dichlorophenoxyacetic acid) is a systemic herbicide that is used to control many types of broadleaf weeds and agricultural uses include pastureland, soybeans, barley, rice, oats, wheat, corn and sugar cane. The toxicity of 2, 4-D to fish is variable, compare to other form with the ester form of 2, 4-D expressing greater toxicity. 2, 4-D disrupts energy production in mammals[6] and to bio-accumulation in fish it has also been demonstrated[7].

2, 4 D is used in cultivated agriculture, in pasture, forest management, garden and home to control aquatic vegetation. Its function by maintaining high levels of the plant growth and ultimately death. According to[8] early life stages of fish, like larvae and eggs are especially sensitive to contaminant. Aquatic contamination of the pesticides causes acute and chronic poisoning in fish and other organism. The growth and fitness of the fish depending on toxicity, exposure time and concentrations of the chemical substances involved[9]. Study the effect of chemicals on the structural components of the living system is mainly directed by histopathology and the ways in which cells and tissues respond to injury. A chemical acting directly on the cell or most frequently by altering its environment causes chemical cytotoxicity. The cells in turn respond histopathologically by degeneration, inflammation, proliferation and repair.The current investigation was undertaken to investigate **effect of cypermethrin and 2, 4-dichlorophenoxyacetic acid on histopathological changes in liver of *Clarias batrachus.***

Material and Methodology

Experimental animal: Healthy *Clarias batrachus* were used as an experimental animal and it is was collected from local fish market & acclimatized to the laboratory for one week during which they were regularly feed with prawn powder & soya meal.

Test chemical: Insecticide Cypermethrin (80ul/l) and Herbicide 2, 4-Dichlorophenoxyacetic acid (34.64 ul/l) were used as a test chemical. Test fishes were exposed to sub lethal doses for 96 hrs

Experimental design:

In the present investigation experimental fishes were divided into two groups.

1. Control group: - In this group 10 fishes were kept and exposed to normal water.
2. Experimental group: - In this group 40 fishes were exposed to concentration of pesticides (cypermethrin and 2-4 D) solution.

Experimental duration: In both control and experimental group fishes were exposed to maximum 96 hrs.

Autopsy: Fishes of control and experimental groups were sacrificed at 0 hrs. 24 hrs, 48hrs, 72 hrs and 96 hrs. and then processed for Histopathological tests.

Collection of Liver:-Fishes of control and experimental groups was sacrificed and Liver was collected, blotted, weighted and then processed for various experiments.

Histopathological analysis

After 48-72 hours, formalin preserve tissue pieces and washed over night in running tap water , dehydrated in ascending grades of alcohol , cleared in benzene and embedded in paraffin wax (60-62^{0}C melting point), section of 4-6 micron thickness were cut though as Spencer's rotary microtome and stained with haematoxylin and eosin as per the standard procedure recommended by Lillie (1954).

The following special staining method was also use:

- For Fat/Lipid –Oil red O stains[10].
- For glycogen- PAS (Periodic Acid Schiff) stain[10].

Results

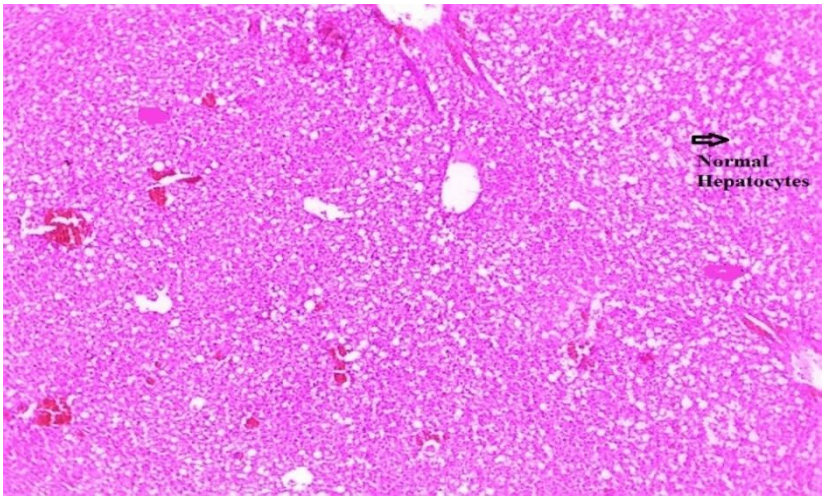
In the present investigation histopathological estimation of liver of control and cypermethrin (insecticide) and 2, 4-D (Herbicide) treated fishes (*Clarias batrachus*) were done. The results of histopathological estimation were summarized and presented by figures (1-9).

Histopathology of liver

Liver of fish is enclosed by a membrane and packed with parenchymal cells, these cells known as hepatic cells. A clear spherical nucleus present in hepatic cells and its structure is roundish polygonal. Large amount of lipid glycogen are present in the cytoplasm.

The histopathological investigation of the fish liver on exposure to the cypermethrin pesticide (80ul/l) and 2,4-D herbicide (34.64 ul/l) at different time interval like 24, 48, 72 and 96 hrs (Fig. 1-9). Cypermethrin and 2,4-D cause severe histopathological changes like increase in size of liver, destruction of architecture of liver, haemorrhage, steatosis, infiltration of inflammatory cells, necrosis of hepatic cells, fatty changes and cloudy swelling of hepatocyte with a severe thick fibrous connective tissue.

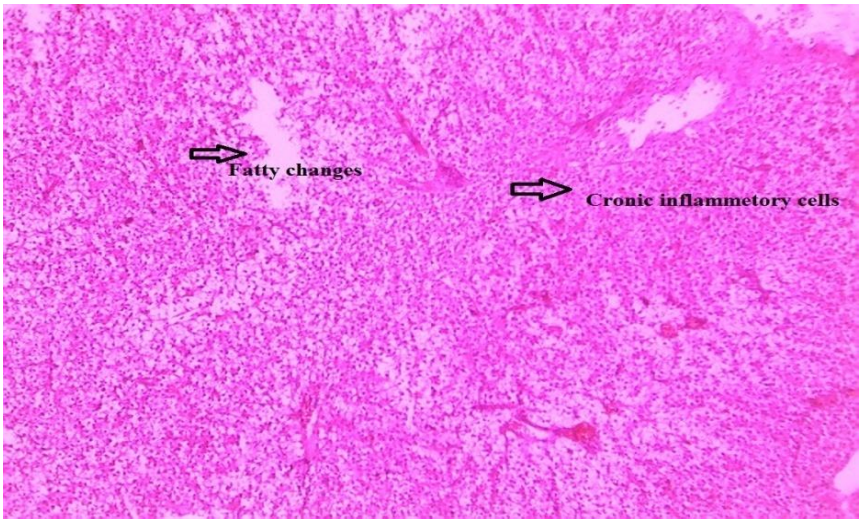
Fig.1: Histopathology of normal (Control) liver of *Clarias batrachus*



10x40 at the simple compound microscope

Section studied shows normal liver parenchyma with normal hepatocytes.

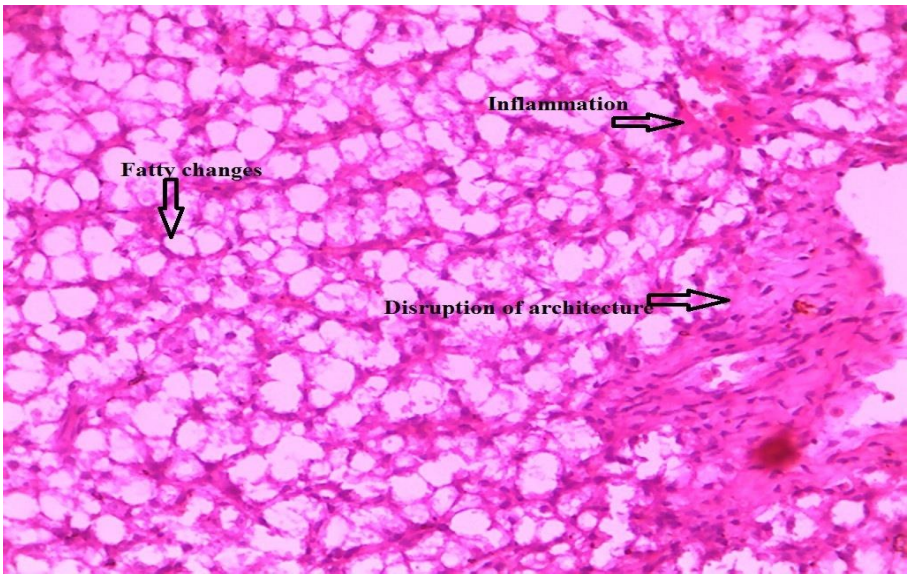
Fig. 2: Histopathological changes in liver at 24 hrs. exposure of cypermethrin



10x40 at the simple compound microscope

Section studied shows liver parenchyma with hepatocytes showing fatty changes and there is chronic inflammatory cells predominantly in the periportal area.

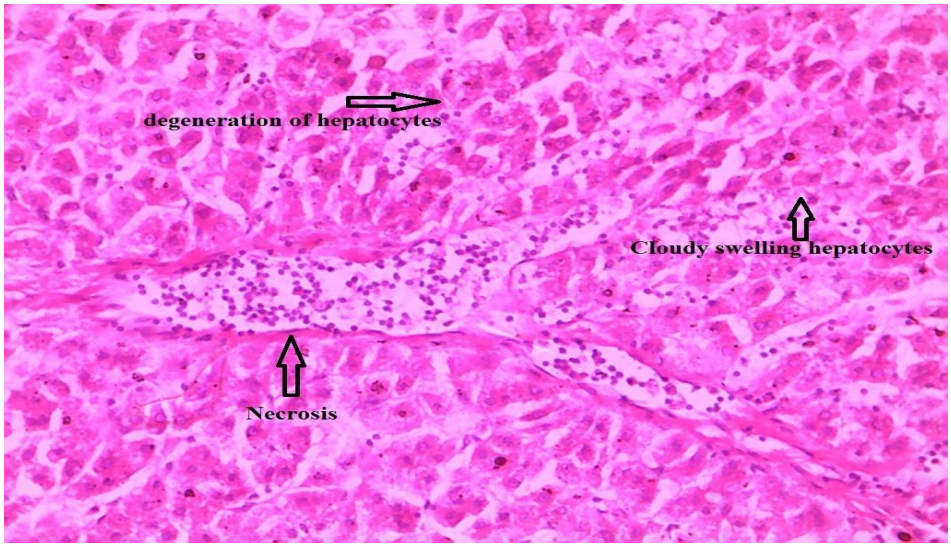
Fig. 3: Histopathological changes in liver at 48 hrs. exposure of cypermethrin



10x40 at the simple compound microscope

Section reveals fatty changes with Sinusoids showing inflammation and Disruption of architecture

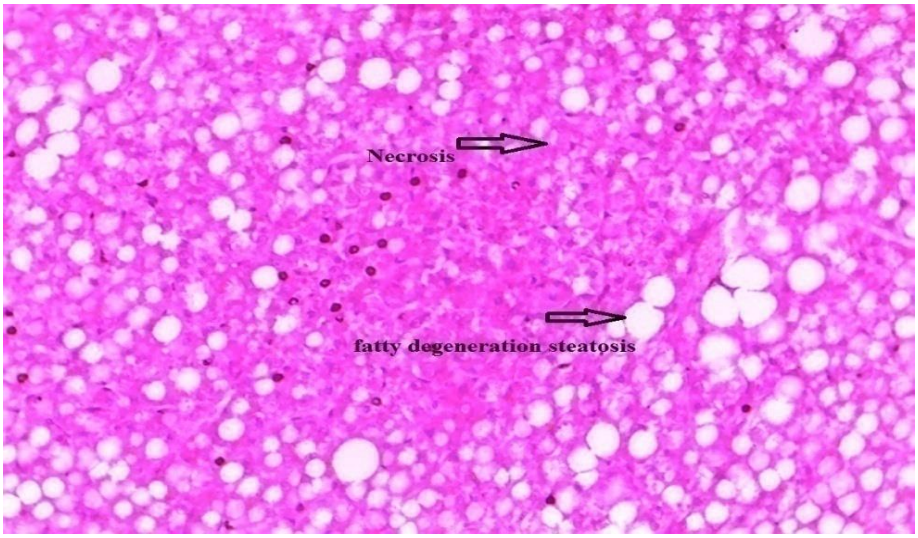
Fig.4: Histopathological changes in liver at 72 hrs. exposure of cypermethrin



10x40 at the simple compound microscope

Section reveals Degeneration of hepatocyte Part sinusoidal inflammation, cloudy swelling hepatocytes. Necrosis

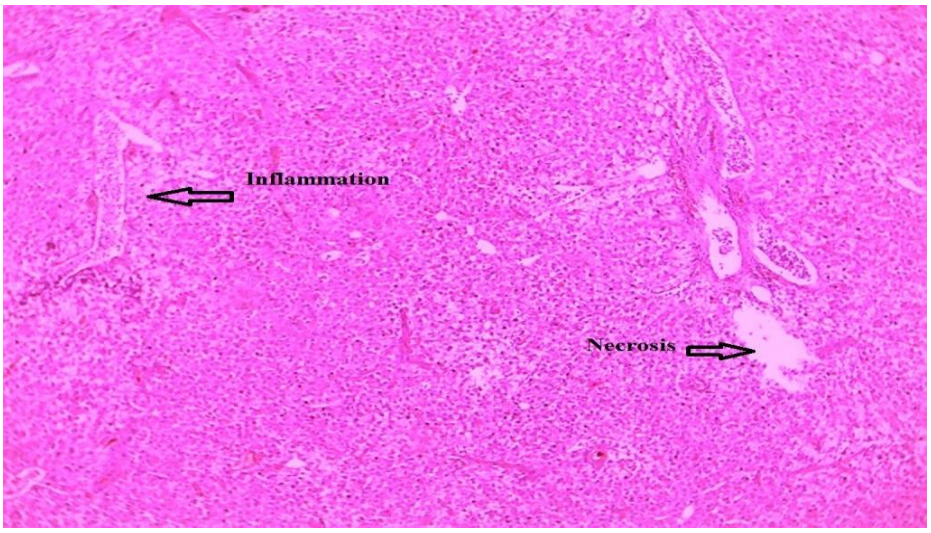
Fig.5: Histopathological changes in liver at 96 hrs. exposure of cypermethrin



10x40 at the simple compound microscope

Section studied shows liver parenchyma with disruption of normal architecture. Hepatocytes show fatty degeneration steatosis, necrosis.

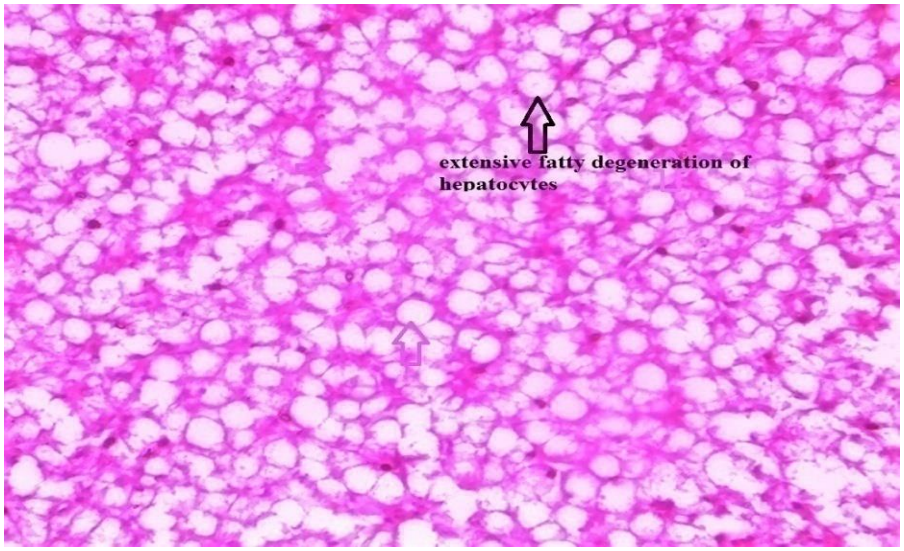
Fig.6: Histopathological changes in liver at 24 hrs. exposure of 2,4-D



10x40 at the simple compound microscope

Section show in most of the hepatocytes. Inflammation of liver cells, necrosis.

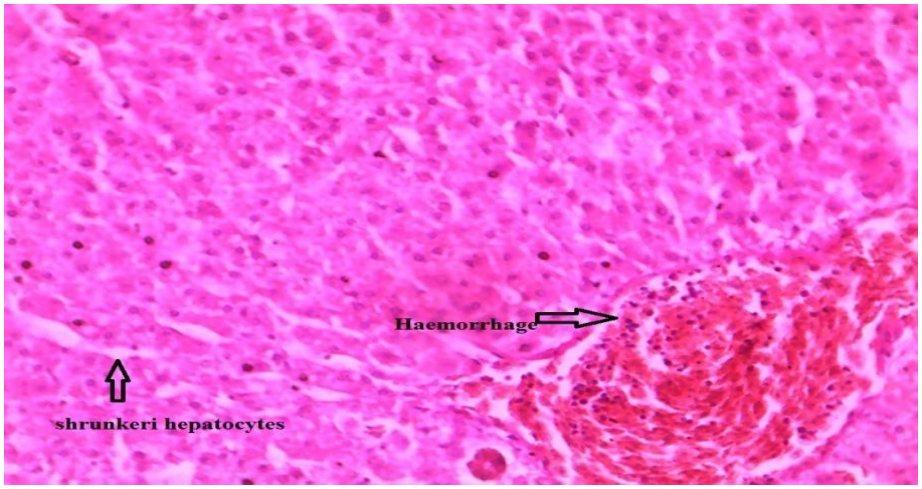
Fig.7: Histopathological changes in liver at 48 hrs. exposure of 2,4-D



10x40 at the simple compound microscope

Section studied shows liver parenchyma with extensive fatty degeneration of hepatocytes showing accurnulation of fat.

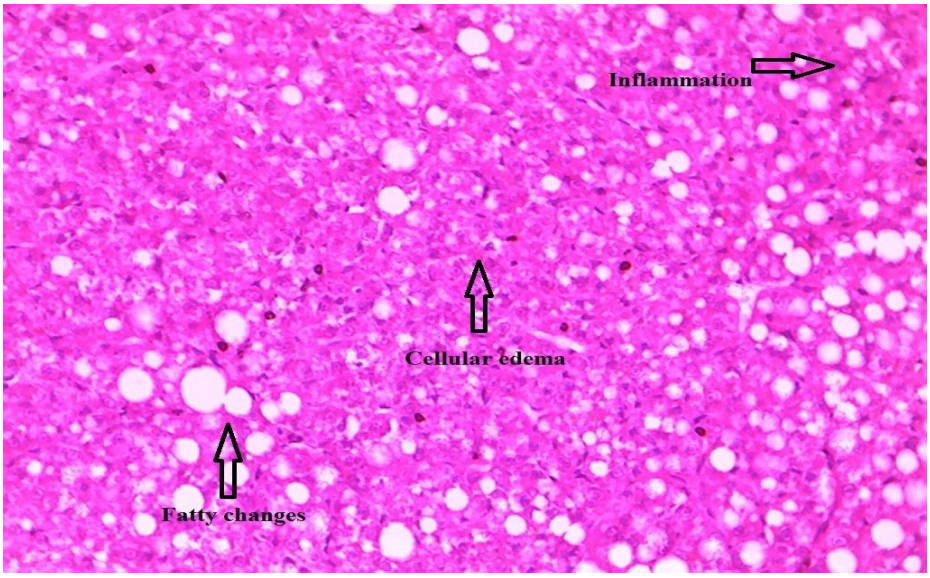
Fig.8 Histopathological changes in liver at 72 hrs. exposure of 2,4-D



10x40 at the simple compound microscope

Section studied shows liver parenchyma with disruption of architecture with loss of cohesion, shrunkeri hepatocytes and Haemorrhage

Fig. 9 : Histopathological changes in liver at 96 hrs. exposure of 2,4-D



10x40 at the simple compound microscope

Section reveals cellular edema and mild inflammation, fatty changes.

These histopathological observation indicated a strong response to tissue damage exposed to cypermethrin insecticide were higher when compared with 2, 4-D herbicide.

Discussion

In the present investigation the histopathological changes of the fish *Clarias batrachus* exposed to 2, 4-D (34.64ul/l) and cypermethrin (80ul/l) in liver were observed. Normal hepatocytes were shown in the liver tissue of fish in the control. The histomorphology of liver exposed to 2, 4 D and cypermethrin showed significant variation from the control group. At different time intervals (24,48,72 and 96 hrs.) liver exposed to 2,4- D and cypermethrin shows destruction of architecture of haemorrhage, steatosis, infiltration of inflammatory cells, extensive fatty degeneration of hepatocytes showing shrinkage of hepatocytes, accumulation of fat, fatty changes, cellular edema, necrosis of hepatic cells, and cloudy swelling of hepatocyte with a severe thick fibrous connective tissue.

Focal necrosis, congestion, congestion and cloudy swelling of hepatocyte in fenvalerate exposed liver of *Cirrhinus mrigala* [11]. In *Labeo rohita* exposed to cypermethrin changes such as disintegration of hepatic mass, hyperplasia and focal coagulative necrosis were found[12]. For the toxicants liver is the detoxification place. To detoxify the toxicant-cypermethrin the hepatic changes suggested mobilization of same kind of defensive mechanism in an endeavour. Through portal circulation the first organ to face any foreign molecule is Liver, which subjected to more damage[13]. Liver which breaks down toxic substances and metabolites of administered substances is an important organ for detoxification. These are the reasons that cause the hepatic cells to damage severely. Evidence of fatty degeneration shown by the liver of the exposed fish which had slightly vacuolated hepatocytes. The excessive work done by the fish causes Necrosis of some portions of the liver tissue because of get rid of the toxicant during detoxification process of its body by the liver. Necrosis of hepatic cells of sinusoids may occur due to the inability of fish to regenerate new liver cells[14]. In teleost fish *Nemachelius denesoni* (Day) exposure to phosphamidon caused highly vacuolated and cloudy swelling and even the damage of the connective tissue in liver[15].A fundamental role played by the Liver in biotransformation, uptake and detoxification of foreign compounds in body and thus a target organ of xenobiotics[16]. It also undergoes different levels of damage as it is one of the most affected organs by contaminants in water[17].

From the results of the present study, it could be suggested that the exposure of catfish, *Clarias batrachus* to cypermethrin (80ul/l) and 2, 4 –D (34.64ul/l) resulted in moderate and severe damage to some organs such as liver. These adverse effects in liver were simultaneously correlated with sever physiological and biochemical changes, these changes making the fish less fit for better survival. Our finding is also well in agreement with the finding of many previous authors.

Conclusion: -

All effects that were observed reduce the general state of health of *Clarias batrachus* fish, It may therefore, be said that a sub lethal concentration may be safe however, it cannot be used indiscriminately. Replacing of these insecticide (cypermethrin) and herbicides (2, 4-D) with less harmful and more biodegradable pesticide is recommended. Also, must take the essential safety measure during the uses of pesticides (utilize the appropriate devices that reduce pollution of environment) to Protection of water quality and wildlife is possible. These

observations will helpful in determine the safe or harmless and safe dischargeable concentration of these Pesticides to protect the fish and fish food organism.

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