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Bhooleshwari Singh
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Shivakumar Magada
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Narendra Kumar Maurya
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Sruthisree C
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Lakshamipathi MT
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

TS Annappaswamy
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Sanjay Chandravanshi
Fisheries College and Research Institute Thoothukudi, Tamil Nadu, India

Amit Singh Kshatri
College of Fisheries Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Bhooleshwari Singh
College of Fisheries, Mangalore, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Histological, accumulation and depuration changes on fish common carp (*Cyprinus carpio* Linnaeus, 1758) when exposed to sublethal concentration of pesticide Acephate

Bhooleshwari Singh, Shivakumar Magada, Narendra Kumar Maurya, Sruthisree C, Lakshamipathi MT, TS Annappaswamy, Sanjay Chandravanshi and Amit Singh Kshatri

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Abstract
Acephate (O,S-dimethyl acetyl phosphoramidothioate), a widely used organophosphorus insecticide, poses significant risks to both human health and the environment. Its metabolite, methamidophos, is highly toxic to aquatic organisms, birds and mammals. This study investigates the acute and sublethal toxicity of acephate in fingerlings of *Cyprinus carpio* through histological evaluation and depuration analysis. Acute toxicity was assessed via 96-hour LC_{50} tests, revealing a lethal concentration of 850.41 ppm. Sublethal toxicity trials were conducted over 28 days using two concentrations: $1/5^{th}$ LC_{50} (170.082 ppm) and $1/10^{th}$ LC_{50} (85.041 ppm). Histological analysis of liver tissues exposed to acephate revealed significant alterations, including hepatocellular degeneration, cytoplasmic vacuolation, necrosis, and pyknotic nuclei. Following 28 days of exposure, the fish were transferred to pesticide-free water to observe depuration and tissue recovery. Gradual histological improvement indicated the potential for tissue regeneration post-exposure. These results highlight acephate's harmful effects on fish health and emphasize the importance of histopathological biomarkers in ecotoxicological assessments. The findings call for stringent regulations and continued research to establish safe pesticide residue limits in aquatic environments.

Keywords: Acephate, bioaccumulation, *Cyprinus carpio*, depuration, histology, lethal toxicity physiology

1. Introduction
The widespread use of organophosphorus pesticides has led to significant negative effects on both terrestrial and aquatic wildlife. The application of insecticides in agriculture, particularly organophosphates such as acephate and its metabolite methamidophos, poses considerable risks to various species. Organophosphates are frequently used as insecticides due to their advantageous properties, such as rapid biological degradation and minimal long-term persistence in the environment. When applied at recommended levels, acephate, a systemic organophosphate insecticide, exhibits residual activity lasting approximately 10 to 15 days. However, its toxic effects on aquatic organisms have raised serious environmental concerns (Jagyanseni *et al*., 2023) [20]. Acephate exposure has been demonstrated to be genotoxic in *Clarias batrachus* (Jagyanseni *et al*., 2023) [20], cytotoxic (Perocco *et al*., 1996) [32], carcinogenic (Carver *et al*., 1985) [7], and mutagenic (Jena *et al*., 1994) [21]. The residues of acephate and other pesticides pose significant threats to critical organs in fish, amphibians, mammals and other aquatic organisms (Kavitha *et al*., 2015) [24]. Pesticides, among the most persistent pollutants in aquatic environments, enter water bodies through various anthropogenic and natural processes. Their toxicity induces substantial histopathological changes in aquatic organisms, adversely affecting development, physiological functions, reproduction, immune response and hemato-biochemical parameters. These histopathological and hemato-biochemical markers serve as crucial indicators of pesticide-induced water contamination (Rohani, 2023) [34]. The indiscriminate application of pesticides in agricultural fields not only targets pests but also adversely impacts non-target organisms, such as fish, by

impairing their metabolic processes and in severe cases, leading to mortality. Large-scale fish kills in aquatic ecosystems have been largely attributed to pollutants such as untreated or partially treated industrial effluents, household waste, heavy metals and pesticides. Research on pesticide toxicity in fish has revealed a broad spectrum of long-term consequences, including oxidative stress, inhibition of acetylcholinesterase (AChE) activity, histopathological damage, developmental disorders, genetic mutations and carcinogenesis (Sabra & Mehana, 2015) [35].

One of the primary drawbacks of pesticides is their harmful impact on non-target species, including humans and beneficial plants, as well as their prolonged persistence in the environment. Several studies have established acephate as a potent neurotoxin that interferes with the nervous system of both insects and vertebrates. Acephate acts by inhibiting acetylcholinesterase, an enzyme essential for the breakdown of the neurotransmitter acetylcholine, leading to overstimulation of cholinergic neurons and, ultimately, paralysis of target organs (Spassova *et al*., 2000) [39]. Furthermore, neurobehavioral and motor neuron expression changes suggest that acephate may exhibit neurotoxic properties in aquatic organisms (Liu & Zhang, 2018) [27]. Given the widespread use and toxicity of acephate, further research is required to assess its environmental impact and establish regulatory measures for its safe application in agriculture.

The loss of native freshwater species has drawn a great deal attention from all around the world, however there are gaps in the explanations of how extrinsic factors contribute to these patterns' physiological mechanisms and provide potential long-term remedies. Although acephate has been used extensively in agricultural and other sectors, little is known about its harmful effects on local freshwater species.

Due to its easy availability, commercial and regional significance, especially in India and eligibility for study as a sample of the economically significant freshwater farmed fish species, *Cyprinus carpio* was chosen for the toxicity research. Acephate's toxicity was examined as a possible hazardous organic pesticide residue on freshwater ecosystems by evaluating the patho-physiological changes in *C. carpio* fingerlings, an aquaculture candidate, and an aquatic animal that was not intended to be exposed to the herbicide.

2. Materials and Methods

Present study was carried out at Fisheries Research and Information Center (Inland) Hebbal, Karnataka Veterinary, Animal and Fisheries Sciences University.

2.1 Test animal

Common carp, *Cyprinus carpio* (Linnaeus, 1758) is a commercially important edible fish, wide geographical distribution and availability throughout the year having great demand. It's economic importance and this fish shows a well adaptive nature with the changing environment. Hence this fish is selected as the experimental model for the present investigation.

2.2 Maintenance of test animals

The fingerlings of *Cyprinus carpio* (Linnaeus, 1758) 8-10 cm size were collected from the Seed Production Hatchery, Hesargatta, Bangaluru and transported in well oxygenated polythene bags and acclimatized into the FRP (Fibre Reinforced Plastic) tanks for 7 days.

2.3 Test chemical

Acephate is an insecticide in the chemical family known as organophosphates insecticide ($C_4H_{10}NO_3PS$) was used to assess the toxicity and their impact on the histological and physiological changes in *C. carpio* (Linnaeus, 1758). Acephate is manufactured by Karnataka Agro Corporation.

2.4 Stock solution

The stock solution was prepared having strength of 50,000 ppm by adding known quantity of Acephate to 1000 ml distilled water. Calculated amount of stock solution was added to water of known volume and mixed thoroughly to arrive at working standard concentrations for the experiment.

2.5 Bioaccumulation Study

2.5.1 Lethal Toxicity Studies

A standard toxicity dose measurement, called Lethal Concentration$_{50}$ (LC_{50}) was measured. This is the concentration of a pesticide that kills fifty percent of a test population of animals within 96 hours period. Experiments were conducted in the laboratory following static renewal bioassay technique based on the standard procedure for the measurement of pollutants toxicity on fresh water organisms (Sprague, 1969 and APHA, 2005). The result was analyzed by using probit method (Finney, 1971) [13].

The Fibre-reinforced plastic (FRP) tanks of 50L capacity were used for bioassay experiment with triplicate. In each FRP tank volume of water were maintained at 40L and the stocking density of test animal were 10 no.s at each tank. After determining LC_{50}, its sub-lethal doses ($1/10^{th}$ and $1/5^{th}$) are used for further studies because environmental contamination will not be generally of the toxic level. The contact of pollutants brings in certain behavioral changes in fish, then alters the physiological and histo-pathological. The very purpose of the present study is to understand the acute and chronic toxicity of acephate to the fish *C. carpio*, the acute and chronic effects of toxicants on various parameters such as physiological behavior, respiratory responses, histopathological and depuration with the alterations produced in the animal on exposure to lethal and sub-lethal concentrations of Acephate are to be found, compared with control.

2.5.2 Sublethal Toxicity Studies

Sublethal effect of acephate was carried out by selecting the one fifth ($1/5^{th}$) and one tenth ($1/10^{th}$) of the acute toxicity values (LC_{50}) obtained during the present study. All the tests were performed in triplicate for a period of 28^{th} days presented. Fish were exposed to two sublethal concentrations for 7^{th}, 14^{th}, 21^{st} and 28^{th} days along with the control. In each 50L of FRP tank, volume of water maintained at 40L and 10 no.s of test animal were exposed at each tank for the experiment. Fishes were fed with commercial floating feed daily once during the test period. The test solution was replenished completely at every 48 hours. Behavioral responses, oxygen consumption rate, food consumption rate, ammonia-N excretion rate and Oxygen: Nitrogen ratios of fishes were studied during the experimental period. The acephate exposed fishes were kept under continuous observation during the experimental periods.

2.6 Depuration phase
Treated fishes were transferred into clear water to removal of toxicants after the completion of 28th days of bioaccumulation phase. Fishes were kept in 50L volume of FRP tank contributing 40L of clear water. Water was changed in alternate days and then the fish were sacrificed isolated tissues like gill, liver and kidney under laboratory conditions for histological studies with the interval of 7th, 14th, 21st and 28th days.

2.7 Histological alteration studies
Histology has been proved useful giving relevant data concerned with tissue damaged, prior to the external symptoms of abnormalities. The microscopic studies of different fish tissue before and after the effect of toxicants, identify the water contamination by toxic substance. In the present work, histological study of vital organs Gill, Liver and Kidney has been undertaken for any structural damage caused as a result of exposure to the toxicants used. The histology has been done by Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology by Luna, 1979.

3. Results and Discussion
The 96 h LC_{50} values of Acephate for *C. carpio* was found to be 850.41 ppm is shown in Fig.1.

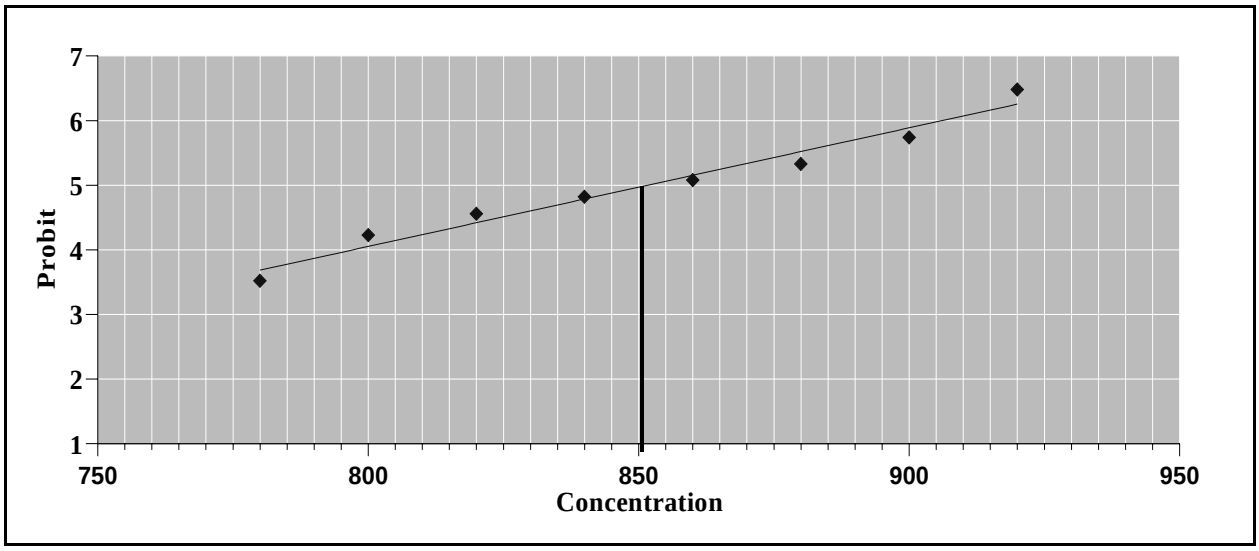

Fig 1: Graphical representation of 96 hr LC_{50} of pesticide Acephate concentration in fingerlings of *Cyprinus carpio*.

Similar findings about the acute toxicity of the organophosphate insecticide Acephate on *Puntius sophore* were also found by Gavit and Patil (2016) [16]. Finney's approach was used to carry out the static bioassay trials (Finney, 1971) [13]. Acephate was administered to the fish at different concentrations, with LC_{50} values of 1762 ppm, 1509 ppm, 1281 ppm and 1117 ppm at 24, 48, 72 and 96 hours, respectively. Because these values are so important in determining toxicity levels, the current study begins with the determination of Acephate 96-hour LC_{50} in the freshwater fish *C. carpio*. Satish and Sravani *et al*. (2018) [37], examined Acute toxicity and behavioural abnormalities are brought on by the organophosphate insecticide acephate in *Channa punctata*. The LC_{50} values for Acephate were determined using pilot studies that lasted 24 hours, 48 hours, 72 hours and 96 hours. Moreover, from these observations they could found the sublethal concentration of Acephate was 910 mg/mL. The fish were then subjected to pesticide for 24 hours, five days and ten days to see if there were any hematological alterations. For comparison, an Acephate control group was maintained throughout the trial.

3.1 Exposure period
In recent study, *C. carpio* fingerlings were treated to acephate during a sub-lethal experiment at doses of 1/5th of the LC_{50} (170.082 ppm) and 1/10th of the LC_{50} (85.041 ppm), respectively. In addition to the control groups, tests were conducted over intervals of 0th, 7th, 14th, 21st and 28th days. A similar study conducted by Habib *et al*. (2019) [19] reported that the lethal concentration (LC_{50}) of Sevin 85 SP for *Batasio tengana* was 25.91 ppm, with a 95% confidence interval of 16.82 to 34.59 ppm. Their findings also indicated a gradual decline in LC_{50} values over time, accompanied by an increase in mortality rates with higher pesticide concentrations. According to Karra *et al*. (2015) [23], the LC_{50} value was defined as the concentration of the test chemical that caused 50% mortality of the total fish population across different exposure durations (24, 48, 72 and 96 hours). The study reported LC_{50} values of 3.0, 2.5, 2.3 and 2.1 mg/L for 24, 48, 72 and 96 hours, respectively. The findings indicate a progressive decline in LC_{50} values with increasing exposure time, aligning with the log concentration trend from 24 to 96 hours.

3.2 Histological changes when exposed to Bioaccumulation phase
Histology of gills exposed to acephate at 1/10th and 1/5th concentrations, where result showed no differences in the Control group. The gill structure was weakened after 28 days of exposure. In 1/10th concentration of acephate, epithelial hyperplasia (HP), edema (ED), increased in size and shrinkage of primary lamellae (SPL), and damage on lamellae (DL) were found. In 1/5th concentration of acephate, damaged cells (DC), severe damage to the lamellae region, disorganization of lamellae (DL), lamellar fusion (LF), lamellar tangiectasis (LT), hyperplasia (HP), filament cartilage (FC) and edema (ED) were observed (Fig. 2).

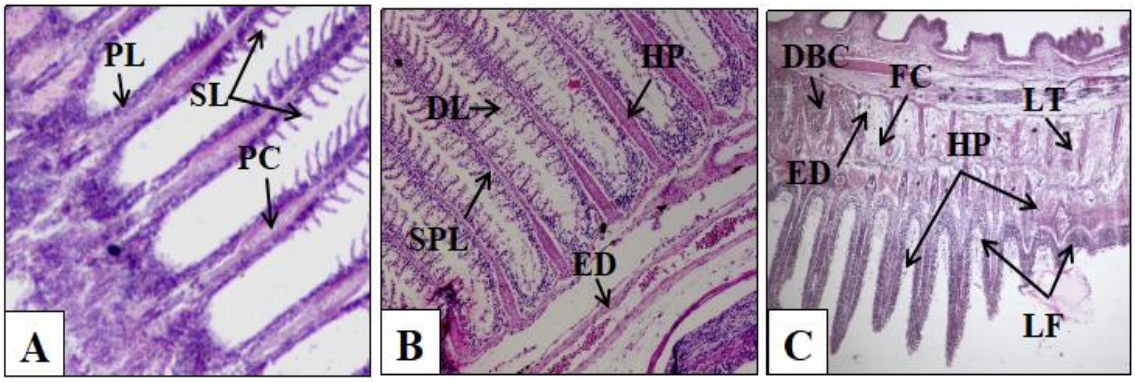

Fig 2: Magnified *Cyprinus carpio* gills were subjected to acephate concentrations of 1/10th and 1/5th for 28 days during the accumulation phase. On the 28th day A (Control), B (1/10th) and C (1/5th).

There was no significant difference in control group, only sinusoid (S) and small hemorrhages observed in liver of fish. Increased cytoplasmic vacuolization (CV), bleeding melanomacrophage (M) aggregation, with significant vacuolization (V), hemorrhages (HM) infiltration of blood (IB) to create blood clots (BC) were detected after 28 days of exposure to 1/10th and 1/5th concentrations of acephate. Fish exposed to a 1/5th (170.082 ppm) concentration of acephate suffered more severe liver damage than those subjected to a 1/10th (85.041 ppm) concentration (Fig. 3).

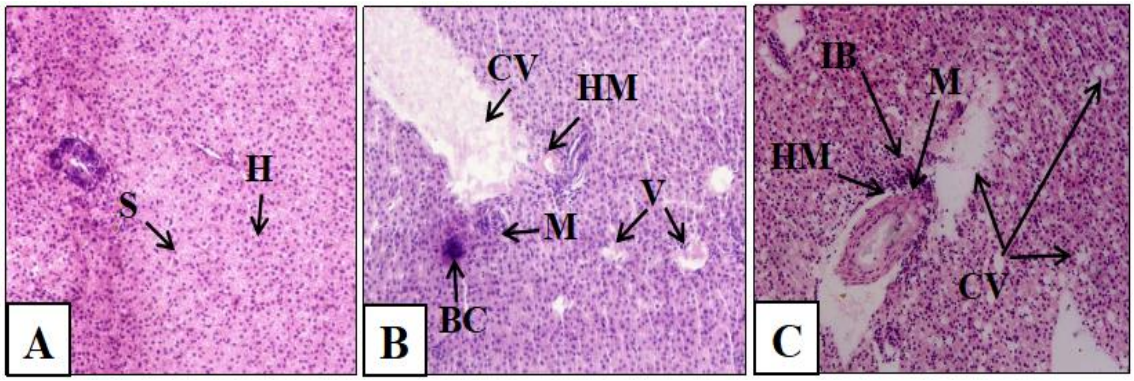

Fig 3: *Cyprinus carpio's* magnified liver after being subjected to acephate concentrations of 1/10th and 1/5th for 28 days during the accumulation phase. On the 28th day A (Control), B (1/10th) and C (1/5th).

The kidneys displayed deteriorated areas such as, glomerular edoema (Ed), bowmens space (BS), blood vacuole (BV), congestion of hematopoietic tissue (CHt), blood infiltration (BI), a few vacuolations (V) and clumping of injured blood cells (CB), piknotic necrosis (PN) on the 28th day of sub-lethal exposure while small blood clot (BC) and normal glomerular tubes (G) were observed in control group (Fig. 4).

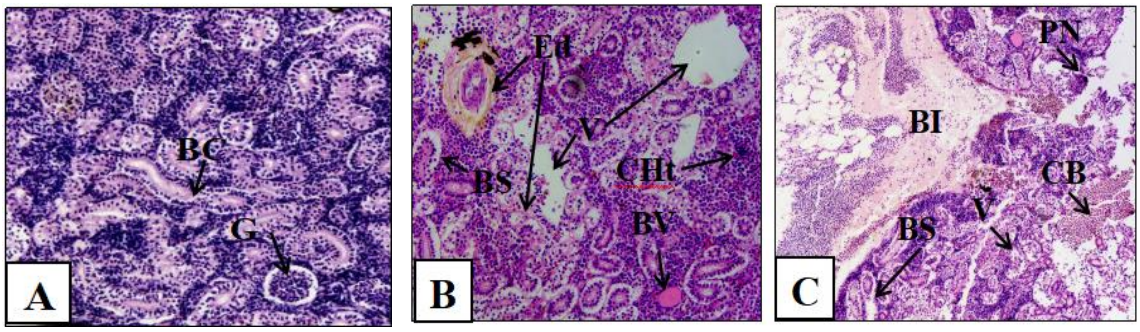

Fig 4: Magnified Kidney of *Cyprinus carpio* exposed to 1/10th and 1/5th concentration of Acephate for 28 days of accumulation phase. A (Control), B (1/10th) and C (1/5th) on 28th day

The gills enormous surface area makes them directly susceptible to pollutants. Oxidative stress brought on by excessive ROS production triggers the up-regulation of antioxidant defenses. An important organ that facilitates the efficient biotransformation of xenobiotics is the liver. The same study findings by using malathion were in line with previous research that found fish treated with showed signs of necrosis, lipidosis vacuolization, an increase in macrophage aggregates, and eosinophilic granular cells. the insecticides malathion and paraquat, respectively studied by Fernandes *et al*. (2006) [12], Elezaby *et al*. (2001) [9], With greater concentration and length time, the liver demonstrated more severe alterations in this investigation.

The common carp, *C. carpio* L., has a high survival rate and quick growth, making it a great candidate for aquaculture in East Asia. Using *C. carpio* as a model, the effects of

trichlorfon on stress, neuroendocrine components, and antioxidant enzymes were investigated (WHO, 1992) [44]. Concluded that the acetylcholinesterase (AChE) activity, the antioxidant response, and the median lethal concentration (LC_{50}) of acephate in the liver, gills and spleen of *Synechogobius hasta* after 96 hours. At 24 hours, 51.36 hours, 48 hours, 72 hours and 96 hours, the LC_{50} was 60.83 mg/L; at 48 hours, 47.07 mg/L and at 72 hours, 40.13 mg/L. The increased MDA levels suggested that one of acephate's main adverse effects could be ROS-induced damage, but the decreased MDA levels suggested that the antioxidant levels produced could quench the excess free radicals generated. It should be noted that acephate was largely absorbed by fish due to its high-water content. The effect of a designed grade of organophosphate insecticide, Acephate, on amylase activity was discovered by Bhilave and Kulkarni (2016) [3]. The fish were given high and sublethal doses of 3000 ppm for 96 hours, as well as 1/10th and 1/20th doses for 30 days. In the freshwater fish *Labeo rohita*, acephate has deadly and sub-lethal toxic effects on amylase activity. The aquatic environment is greatly impacted by acute and long-term acephate exposure, and *Labeo rohita* amylase activity is significantly decreased. It refers to a disruption in the usual digesting process, which has impacted the health of the fish. Li *et al*., (2008) [26] found that the 48-hour LC_{50} of acephate in *Apocyclops borneoensis* and *Tigriopus japonicus*, respectively, was 216.3 mg/L and 76.54 mg/L. The 12 h LC_{50} of acephate on *Paramecium caudatum* and *P. bursaria*, according to Lou *et al*. (2008) [28], was 3.19 and 2.90 mg/L, respectively. As a result, acephate toxicity (LC_{50} value) varied greatly depending on the species.

3.3 Histological changes after Depuration phase

After a sublethal experiment, fingerlings of *C. carpio* were exposed to clean water for 28 days. With these replications on the 0th, 7th, 14th, 21st and 28th days, along with the control groups. The water was replaced after 48 hours and the fish were fed with supplementary feed. In 1/10th concentration of acephate, on 56th day the changes were observed in gill that, primary gill lamellae (PL), secondary gill lamellae (SL), epithelial capillary (EC) and pillar cells (PC) were recovered. Where in 1/5th concentration of acephate the changes were observed that reduced hypertrophy (RHT) and reduced hyperplasia (RHY), recovered swollen secondary lamellae (Fig.5).

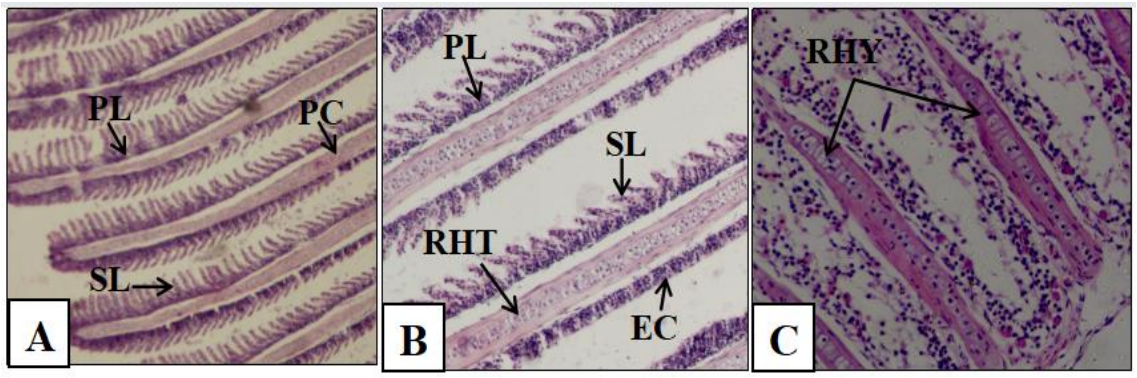

Fig 5: Magnified Gill of *Cyprinus carpio* exposed in Clear water for 28 days of the depuration phase. A (Control), B (1/10th) and C (1/5th) recovery observed on 56th day of exposure

Among this on the 56th day in fish liver, the size and number of big cytoplasmic vacuoles reduced (CV) and reduced hemorrhages (RHM), Small vacuolization (SV), normal hepatic cells (H) and sinusoid (S) were detected at a 1/10th concentration of acephate. Reduced vacuolization, melanomacrophage aggregation (RM) and the cytoplasmic vacuolization (CV), reduced haemorrhage (RHM) was observed in 1/5th concentration of acephate (Fig.6).

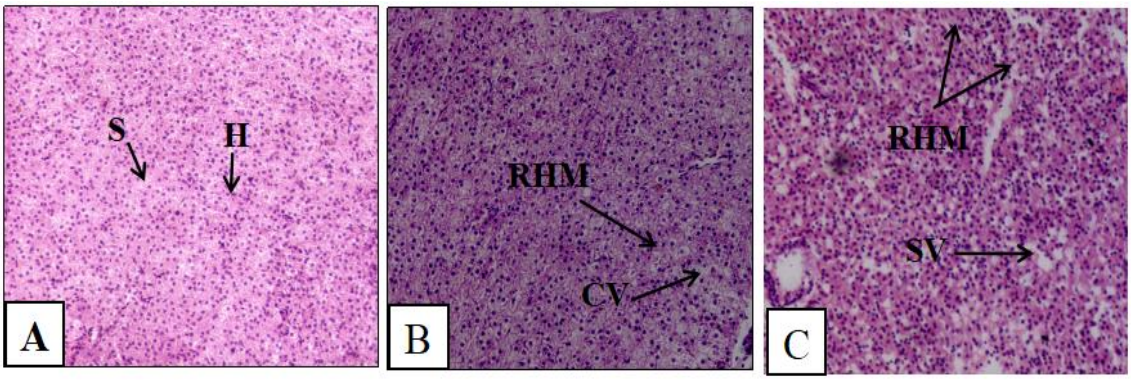

Fig 6: Magnified Liver of *Cyprinus carpio* exposed in Clear water for 28 days of depuration phase. A (Control), B (1/10th) and C (1/5th) recovery observed on 56th day of exposure.

The kidneys showed normal bowmens capsules (BC) and glomerulus tubule (G) in control group and also detected the less glomerular necrosis, vacuolation and expanded glomeruli without a large gap in the cup, blood infiltration (BI) and coagulation were also reduced on the 56th day, there were changes in vacuole formation (Reduced vacuole, RV) and glomerular enlargement with 1/10th and 1/5th acephate concentrations in fish tissue organ (Fig. 7).

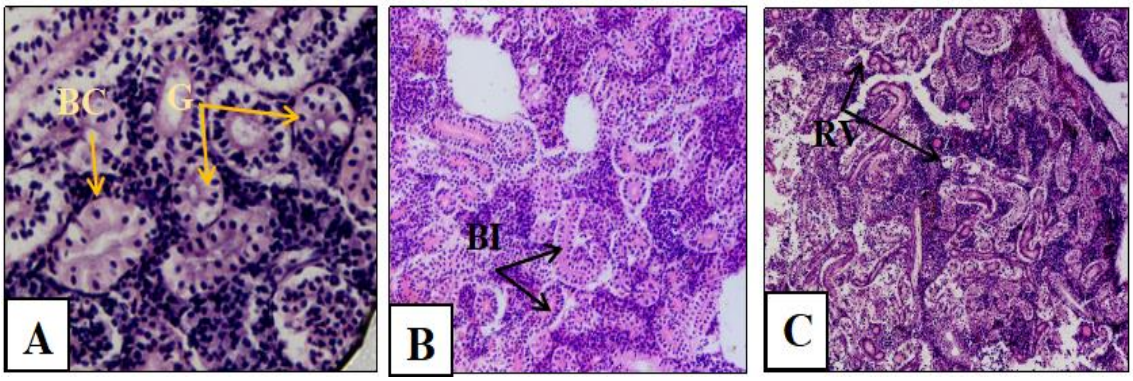

Fig 7: Magnified Kidney of *Cyprinus carpio* exposed in Clear water for 28 days of depuration phase. A (Control), B ($1/10^{th}$) and C ($1/5^{th}$) recovery observed on 56^{th} day of exposure

The inherent complexity of depuration stems from a range of interdependent variables that impact animal behavior. This complexity leads some authors to conclude that efforts to depurate contaminated shellfish and fish are impractical, primarily due to the associated economic burden and the extended periods of toxin retention common in numerous species. Observations of histopathological lesion reversal in the liver and kidneys across numerous studies are consistent with the biochemical alterations identified in the same fish organs during a prior investigation (Guzmán *et al*., 2014) [18]. Although the tilapia's liver and kidney continued to experience oxidative stress following three days of depuration, as evidenced by changes in catalase activity, glutathione (GSH) content and lipid and protein oxidation, their overall reaction was adaptive. The first organ to fully recover from the lesions caused by Cylindrospermopsin (CYN) after three days of depuration during the study period was the gills (Guzmán *et al*., 2017) [17].

Certain histological abnormalities were still visible in the liver of fish that had been depurated for three days as opposed to the kidney, which may be explained by the fact that only the liver's proteins continued to undergo oxidation after seven days. This trend is consistent with the findings of Jiang *et al*. (2009) [22], who found that malachite green was extensively and densely distributed in the kidney and liver excretory tissues of three common freshwater fish and that its depuration happened more swiftly in the kidney than in the liver of any fish. This suggests that the toxicant may be better detoxified in renal excretory tissues. Given that the results showed the organism's ability to recover from them through a depuration process, the histopathological abnormalities discovered in this study may generally be seen as a stress manifestation against acephate exposure and a general compensating mechanism. These deficits declined after depuration, reflecting a distinct pattern in each organ, which indicated why some took longer to recover.

4. Conclusion

The vulnerability of different fish species to high pesticide concentrations in water varies. The size, age, sex, genetic features of the fish, water quality, physicochemical parameters and insecticide formulations all influence the acute toxicity of insecticides. Many other studies have confirmed that pesticide accumulation in vital tissues and blood causes behavioral changes in fish. In this study the most affected organ was liver>gills>kidney and fast recovery has found in gills>kidney>liver. The behavior of the fish and the physiological parameter has also changed their normal properties when exposed to acephate and after 28 days test animals have been exposed to clear water for 28 days the test animals, their behavior and physiological parameters has slightly recovered. That clearly showed fish can recover after accumulation of pesticide acephate when exposed in clear water. The study's findings made it abundantly evident that exposure to acephate at sublethal levels at $1/10^{th}$ and $1/5^{th}$ of the lc_{50} concentrations is toxic to fish organisms and causes changes in physiological and histological indices in *c*. *Carpio* fingerlings. On the other hand, the outcomes of the depuration phase demonstrated that organisms could recover, indicating that *cyprinus carpio* reactions were reversible in the majority of indices when exposure was discontinued. The findings of this study can be used as a tool in bio-assessment to monitor toxicity risks of acephate pesticide.

5. Disclaimer (Artificial Intelligence)

Authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

6. Acknowledgements

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7. Ethical Statement

The experiment was authorized by Karnataka Veterinary Animal and Fisheries Sciences, University, Bidar, Karnataka (575002), India and the fish species examined in this study are not protected under the Wildlife Protection Act, 1972 (most recent amendment in 2013), Government of India.

8. Competing Interest

The authors state that none of the work described in this study could have been influenced by any known competing financial interests or personal relationships.

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