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COMPARATIVE STUDY OF ACUTE AND SUB-CHRONIC TOXICITY IMPACTS OF CHLORPYRIFOS AND CYPERMETHRIN ON ADULT ZEBRAFISH (*DANIO RERIO)* ON BIOCHEMICAL, HISTOLOGICAL AND ACCUMULATION LOAD RESPONSES

S. Alis Rega, G. Sasikala*,
Department of Zoology, Psgr Krishnammal College for Women, Coimbatore-641 004, Tamil Nādu, India.
Corresponding Author email ID: gsasikala@psgrkcw.ac.in,

Abstract
The toxicological effects of Chlorpyrifos (0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate) and Cypermethrin (cyano-(3-phenoxy phenyl) methyl] 3-(2,2-dichloroethenyl)-2,2 dimethyl cyclopropane-1-carboxylate) on adult zebrafish (specimens exceeding six months of age) were examined in the current investigation. Selected adult male zebrafish were randomly and the acute and chronic toxic were exposed. The mortality rate of zebrafish under laboratory conditions up to 96h period. The toxicity tests showed the 96-h LC_{50} values as 0.5, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 mg/l for Chlorpyrifos (CPS) and Cypermethrin (CP) respectively. Biochemical, Histopathological, and bioaccumulation loads were determined. The findings indicated that CPS and CP could swiftly accumulate within the physiological structure of fish during acute exposure, demonstrating a dose-dependent relationship. Furthermore, following a sub-chronic exposure period of 28 days, damage to the intestine and liver was noted. The organ impairment and metabolic disruptions induced by CPS and CP may contribute to the inhibition of zebrafish development. The objective of this investigation is to ascertain and compare the acute and sub-chronic toxicities of chlorpyrifos and cypermethrin pesticides

Keywords: Danio rerio, Acute toxicity, Sub-chronic toxicity, Biochemical, Histopathological effects

1. Introduction

Pesticide application has become an essential element in the realm of agricultural productivity. The increased utilization of pesticides has generated notable advancements in agricultural output, leading to a reduction in grain loss during storage and an overall improvement in human well-being. On a global scale, approximately 3 billion kilograms of pesticides are employed annually, accumulating to a budget of roughly 40 billion USD. Nevertheless, pesticide use may result in unwanted residues, thereby presenting a potential hazard of food contamination and pollution of the environment and living tissues. These substances can disperse beyond the treated agricultural areas, subsequently impacting non-target organisms within the wider ecological framework. Such exposure directly influences various levels of biological entities. Fish, for instance, experience alterations in their physiological characteristics, encompassing histology, haematology, defense mechanisms, and behaviour, when exposed to sub-lethal

levels of such pesticides [1].

Zebrafish are employed as model organisms for the present study owing to their established role as model organisms [2] in the domain of developmental toxicology research, as well as their endorsement by the International Organization for Standardization (ISO) and the Organization for Economic Co-operation and Development (OECD) [3]. The completion of the zebrafish genome sequencing is approaching, thereby offering a promising pathway for augmenting its utility as a tool for investigating the complexities inherent in developmental processes that bear resemblance to human physiology. Furthermore, their substantial clutch sizes and small body dimensions contribute to the reduction of resources and expenses necessary for the execution of experiments.

Chlorpyrifos as an organophosphorus pesticide has been extensively studied; it is widely utilized across the globe, and its usage has been linked to the potential onset of acute cholinergic crisis. Moreover, chronic exposure to this pesticide has been associated with various behavioral changes, including alterations in attention span, memory, and perception [5]. The occurrence of suicidal poisoning related to organophosphorus pesticides is particularly prevalent in rural regions [6]. The global impact of acute organophosphorus poisoning on human health is a significant concern, resulting in over 1,00000 deaths annually [7,8]. Moreover, beyond their cholinergic activities, organophosphorus pesticides are known to provoke oxidative stress [9], interfere with metabolic pathways [10], and lead to various organ dysfunctions, including hypoxia and inadequate tissue perfusion in both the liver and the heart [11].

In the hepatic system, protrude elicits structural modifications at the microscopic scale, in addition to biochemical, metabolic, and mitochondrial dysfunctions, which are observable through alterations in hepatic biomarkers such as serum aminotransferase levels and both direct and indirect bilirubin concentrations [12,13]. Furthermore, exposure to organophosphates has been correlated with increased mortality rates in Apis mellifera and Daphnia, deterioration of macroinvertebrate ecosystems, and a potential reduction in the growth rates of ichthyological populations [14,15]. Consequently, it is crucial to enhance comprehension of the effects and mechanism of action of organophosphates to assess the risks precisely when exposed to organisms and populations. Cypermethrin (CP), a synthetic pyrethroid of type II, is frequently employed as an environmental and animal health agent due to its substantial toxicity to insects. The application of cypermethrin extends to the management of ectoparasites within the realm of veterinary medicine [16] as well as the regulation of agricultural and indoor pest populations. Furthermore, cypermethrin possesses a notable tendency to bioaccumulate in various tissues, with a particular affinity for the central nervous system (CNS), attributable to its lipophilic characteristics. An increased concentration of cypermethrin within cerebral tissues precipitates the emergence of neurobehavioral toxicological effects. Cypermethrin is positioned among the quintet of leading pesticides on a global scale, representing 17% of the international insecticide market, and is extensively utilized across diverse sectors, including agriculture, aquaculture, forestry, industry, and domestic environments [17,18]. Consequently, as a result of such widespread utilization, cypermethrin is commonly detected in aquatic environments at concentrations that vary from 1 to 2.81 $\mu g\ L^{-1}$ [19]. Moreover, the concentration of cypermethrin can escalate to as high as 9.8 $\mu g\ L^{-1}$ in the water column and 194 $\mu g\ ^{L-1}$ in runoff water originating from agricultural zones [20,21]. A multitude of studies has substantiated that

cypermethrin demonstrates significant acute toxicity to both fish and invertebrate species [22,23]. The objective of this investigation is to ascertain and compare the acute and sub-chronic toxicities of chlorpyrifos and cypermethrin pesticides.

2. Materials and Methods

2.1 The preparation and Dilution of Chemicals

The subsequent chemical compounds and reagents: 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate and cyano-(3-phenoxy phenyl) methyl] 3-(2,2-dichloroethenyl)-2,2 dimethyl cyclopropane-1-carboxylate were acquired from Sri Precision Chemicals, located in Coimbatore, Tamil Nadu, India. The working concentrations of each respective compound were meticulously prepared by accurately measuring from the stock solution in 1L of dechlorinated tap water (designated as fish medium) to achieve final concentrations (0.5 mg/l) at eight distinct concentrations along with a control.

2.2 Maintenance of Zebrafish

Adult, zebrafish (>6-month-old) were procured from Bhuvana Aquarium, Coimbatore Tamil Nadu, India, and transferred to the laboratory within 40 minutes. The guidelines established by the Organization for Economic Co-operation and Development (OECD) were duly adhered to when carrying out the necessary protocols for the welfare of the zebrafish, as outlined in the relevant publication [24] concerning the testing of chemicals.

In summary, approximately five hundred (500) adult male zebrafish, each exceeding six months of age, were housed and acclimatized in a 550-liter aquarium tank filled with 500 liters of dechlorinated tap water, which was appropriately outfitted with a Bio-Foam Filter and a thermometer. The subjects were provided with brine shrimp salt (Artemia) in the morning and desiccated flake food in the afternoon, daily for seven days (comprising 48 hours for settling and an additional 7 days for acclimatization, totalling 9 days). An assessment of the fish was conducted to detect any indications of illness and to evaluate their overall health status. The aquatic environment was partially renewed every other day. The zebrafish were maintained at a temperature of 28 ºC and a pH level of 7.0, with a light-to-dark photoperiod ratio of 14:10 hours. The aquarium was subjected to continuous aeration, except during feeding periods, ensuring that the dissolved oxygen concentration remained above 4.0 mg/l. Throughout the pesticide exposure phase, deceased specimens were promptly extracted, and mortality rates were documented daily to ascertain the acute toxicity threshold of LC50 for the fish over 96 hours.

2.3 Acute Toxicity Test

For this experimental study, adult male zebrafish exceeding six months of age were meticulously selected from maintenance aquarium tanks. All experimental trials were conducted over 96 hours. The toxicity assessments were executed by subjecting ten individuals of fish per treatment to specified test concentrations (0.5, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 mg/l) of each compound, namely Chlorpyrifos (CPS) and Cypermethrin (CP), in five liters of dechlorinated tap water. The fish were categorized into three distinct groups: the first group served as a control without any treatment, while the second and third groups were subjected to varying concentrations of the CPS insecticide and CP pesticide (ranging from 0.5 to 0.40 mg/l).

The mortality rate was assessed after the 96-hour exposure period utilizing Finney's Probit Analysis LC50 determination method, with daily observations documented throughout the testing duration. A secondary analysis involved the utilization of tissues for biomarker evaluation, employing ten fish per treatment group. All treatment and control groups were maintained under controlled conditions of 25˚C and pH 7.0. Each treatment group underwent replication three times, with ten fish allocated per replicate. Consequently, upon reaching the 96-hour exposure mark, the cumulative mortalities, and the 96-hour lethal concentration (LC50) values for each tested compound were ascertained. Throughout the experimental investigation, all fish were withheld from feeding.

2.4 Sub-chronic toxicity test

A cohort of approximately 70 healthy, adult male zebrafish, each exceeding six months of age, was meticulously selected for the purposes of this experimental study. The semi-static exposure methodology was employed over a duration of 28 days, adhering closely to the established protocol outlined in [25]. The concentrations of CPS and CP, specifically 0.025 and 0.015 mg/l, respectively, for each test compound utilized in this investigation were determined subsequent to the completion of the acute toxicity assessment. These concentrations were strategically chosen based on one-tenth of the 96-hour LC50 values as reported in [26]. Upon conclusion of the experimental procedures, the fish within each group were humanely euthanized by immersion in ice water for a duration of 20 seconds, followed by necropsy; furthermore, 10 specimens from each group were allocated for histopathological analysis of the intestinal and hepatic tissues. The remaining 10 specimens from each group were designated for biochemical assay analysis. At the culmination of each exposure interval, the muscular tissue of the zebrafish was subjected to homogenization at a 1/10 (w/v) ratio with respect to a cold physiological saline solution of NaCl (0.86%), utilizing a mortar and pestle; subsequently, the homogenate was centrifuged for 10 minutes at 8000 revolutions per minute at a temperature of 4°C, with the resulting supernatant being utilized for biochemical analytical procedures.

Histopathological evaluations were conducted following a 28-day exposure period, during which the hepatic and intestinal tissues of zebrafish (10 specimens for each concentration group) were excised and subsequently preserved in a 10% neutral buffered formalin solution at 4°C for a 24-hour period. Following the fixation process, the hepatic tissues underwent dehydration via a sequential series of ethanol concentrations, hyalinization in xylene, and embedding in paraffin wax at a regulated temperature of 56°C. Subsequently, the paraffin blocks were precisely sectioned to a thickness of 4 μm. The resulting sections were affixed onto glass slides and stained employing hematoxylin and eosin (H&E) through the utilization of an H&E Staining Kit.

3. Results

3.1 Impact of Pesticides on Ichthyological Behavior:

In the current investigation, the control specimens exhibited a high level of activity during feeding periods and demonstrated an acute awareness of minimal disturbances, characterized by their well-coordinated movements. The behavioral patterns remained relatively consistent across the control groups; thus, these findings serve as a baseline for the entire experimental framework. The repercussions of chlorpyrifos (CPS) and chlorpyrifos intoxication manifested as symptoms including asphyxiation, hyperactivity, disorientation, disrupted shoaling behavior, the piping phenomenon (aerial gulping), and surface swimming (surfacing

phenomenon), with the fish consistently seeking refuge in the corners of the experimental chambers from the onset of exposure to sublethal concentrations of chlorpyrifos. This condition persisted with increasing severity throughout the trial, as documented in the observations of [27]. Fish frequently remained at the substrate level with their mouths agape prior to succumbing (Table 1).

Table. 1 Effect of Chlorpyrifos and Cypermethrin on the Behavioral Patterns of *Danio rerio*

Pesticides	Asphyxia	ιying on the substratum	nconsistent swimming	pening of oral and gills	Sluggish movements	Earthward movement	bble before death
CPS	+++++	++++	+++	++++	++++	+++	+++
CP	++++	+++++	+++++	++++	+++	+++++	++++

The augmentation or reduction in the magnitude of behavioral parameters is denoted by the presence of the (+) symbol. Conversely, the (-) symbol signifies the existence of normative behavioral conditions.

3.2 Determination of acute toxicity:

The mortality response and correlation of selected ichthyological species to a spectrum of pesticide concentrations were illustrated in Figure 1. An escalation in the frequency of mortalities was recorded concomitant with the augmentation of CPS and CP concentrations. The control group exhibited no recorded instances of mortality. In the instances of CPS and CP, a dose-responsive elevation and a time-responsive diminution in the mortality rate were observed as the duration of exposure extended from 24 to 96 hours; specifically, the median concentration was diminished. A statistically significant difference ($P < 0.05$) was identified among the LC50 values acquired at varying exposure durations. At the 96-hour mark, the median lethal concentrations were documented as 0.25 mg/l for CPS and 0.15 mg/l for CP. Complete mortality of Danio rerio was noted (Fig 1) at a concentration of 0.40 mg/l of CPS after 96 hours, and at a concentration of 0.30 mg/l of CP, with a statistically significant distinction. An overarching comparison of the evaluated pesticides from a toxicity perspective indicated that CP was highly toxic, even at minimal concentrations, resulting in mortality in zebrafish. Conversely, CPS was determined to be the least toxic compound relative to the other substances tested.

In the present study, a static acute toxicity assay was conducted to determine the LC50 values of Chlorpyrifos and cypermethrin on zebrafish at time intervals of 24, 48, 72, and 96 hours, with the upper and lower confidence limits specified in Table 2. Control mortality was observed to be non-existent throughout the experiment (96 hours), whereas higher concentrations such as 0.25 and 0.30 ppm led to documented mortality before the completion of the 24 hours. The lethal concentration at which 50% of zebrafish exhibit mortality (LC50 values) for chlorpyrifos and cypermethrin at the specified intervals of 24, 48, 72, and 96 hours are recorded as 0.38, 0.27, 0.19, and 0.14 µg/l, and 0.81, 0.70, 0.18, and 0.12 µg/l, respectively. The median lethal concentration for chlorpyrifos after a 24-hour exposure period is established at 0.38 ppm, while for cypermethrin, it is quantified at 0.81 ppm; for the 96-hour exposure duration, the values are determined to be 0.14 ppm for chlorpyrifos and 0.12 ppm for cypermethrin, thus indicating a significant positive correlation between mortality rates and both the concentration of chlorpyrifos and the duration of exposure.

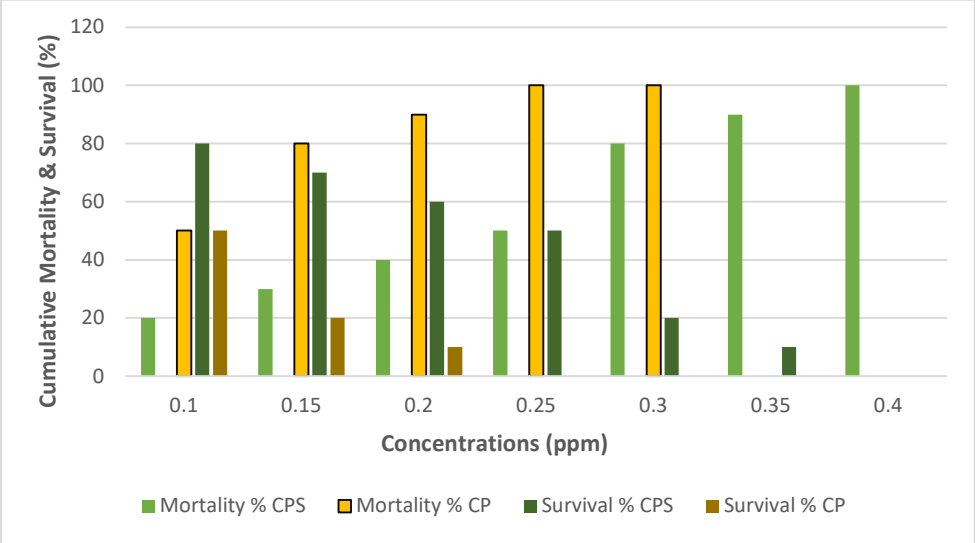

Fig 1: Mortality and Survival of Acute Toxicity Test at various CPS and CP concentrations

Table 2. Acute Toxicity (LC_{50}) of Chlorpyrifos and Cypermethrin in Zebrafish at different time intervals

Pesticides	Values	24 Hours	48 Hours	72 Hours	96 Hours
CPS	LC_{50} (µg/l)	0.39	0.28	0.20	0.16
	LCL-UCL (95%)	0.31-0.63	0.28-0.35	0.18-0.23	0.15-0.9
CP	LC_{50} (µg/l)	0.81	0.70	0.18	0.12
	LCL-UCL (95%)	0.38-22.70	33-41.70	0.14-00.27	0.10-00.14

3.3 Bioaccumulation of toxicants in the entire physiological system of *Danio rerio*

In the context of the acute toxicity evaluation (LC50 96h), *Danio rerio* specimens were subjected to diverse concentrations of chlorpyrifos (CPS) and cypermethrin (CP) to assess the bioaccumulation phenomena throughout their comprehensive biological system. The findings demonstrated that the concentrations of the toxicant in CP (5.8 ± 0.014 mg/l) resulted in a markedly higher degree of bioaccumulation in comparison to CPS (1.63 ± 0.014 mg/l), as delineated (Table 3). Values are articulated as a mean ± standard deviation (SD).

Table. 3 Bioaccumulation factor (BCF) of CPS and CP exposure to the body tissues of zebrafish during 96h.

Pesticides	LC_{50} (µg/l) 96 h	Bioaccumulation
CPS	0.16	1.63 ± 0.014
CP	0.12	5.8 ± 0.014

3.4 Effects of toxicants on Protein and Lipid contents in zebrafish tissues

In accordance with the findings pertaining to sub-chronic toxicity, zebrafish were subjected to varying concentrations of (CPS and CP), with assessments conducted on the entire body of the fish over a duration of 28 days.

The protein concentration within the muscle tissue exhibited a statistically significant decline ($p<0.01$) with the escalation of toxins from both CPS and CP, particularly noted at elevated pesticide concentrations, yielding values of (21.3 ± 0.115 and 20.2 ± 0.145 mg/l) respectively, in contrast to the control group which recorded a protein level of 24.7 ± 0.033 mg/l following the 28-day exposure period (Table 1). Nonetheless, a marginal decrease in total protein content was observed in the CP group, registering at (20.2 ± 0.145 mg/l).

The lipid concentration experienced a substantial reduction at elevated concentrations of the toxicants CPS and CP, presenting values of (11.1 ± O.115 and 10.4 ± 0.115 mg/l) when compared to the control group (12.8 ± 0.040 mg/l), whereas a slight decrease in total lipid content was noted specifically within the CP concentrations (Table 4).

Table 4: Biochemical metrics in the musculature of zebrafish subjected to exposure of CPS and CP.

Pesticides	Protein content		Lipid content	
	Control	**Protein**	**Control**	**Lipid**
CPS	24.7 ± 0.033	21.3 ± 0.115	12.8 ± 0.040	11.1 ±0.115
CP	24.7 ± 0.033	20.2 ± 0.145	12.8 ± 0.040	10.4 ± 0.115

3.5 Histopathological alterations

Histopathological alterations have been employed as significant instruments in the realms of biomonitoring and ecotoxicological investigations owing to their interpretative simplicity, applicable in both acute and chronic exposure contexts. In this investigation, the sub-chronic toxicity of Chlorpyrifos (CPS) and Cypermethrin (CP) in zebrafish experiments was assessed to understand the toxicological capacity across the organs through the elucidation of histopathological observations. Zebrafish exposed to two distinct concentrations of CPS and CP for 28 days exhibited a diverse range of histopathological abnormalities within the Liver and Intestine of *Danio rerio* species. The findings have been meticulously documented and are visually represented in (Fig. 2. A - F)

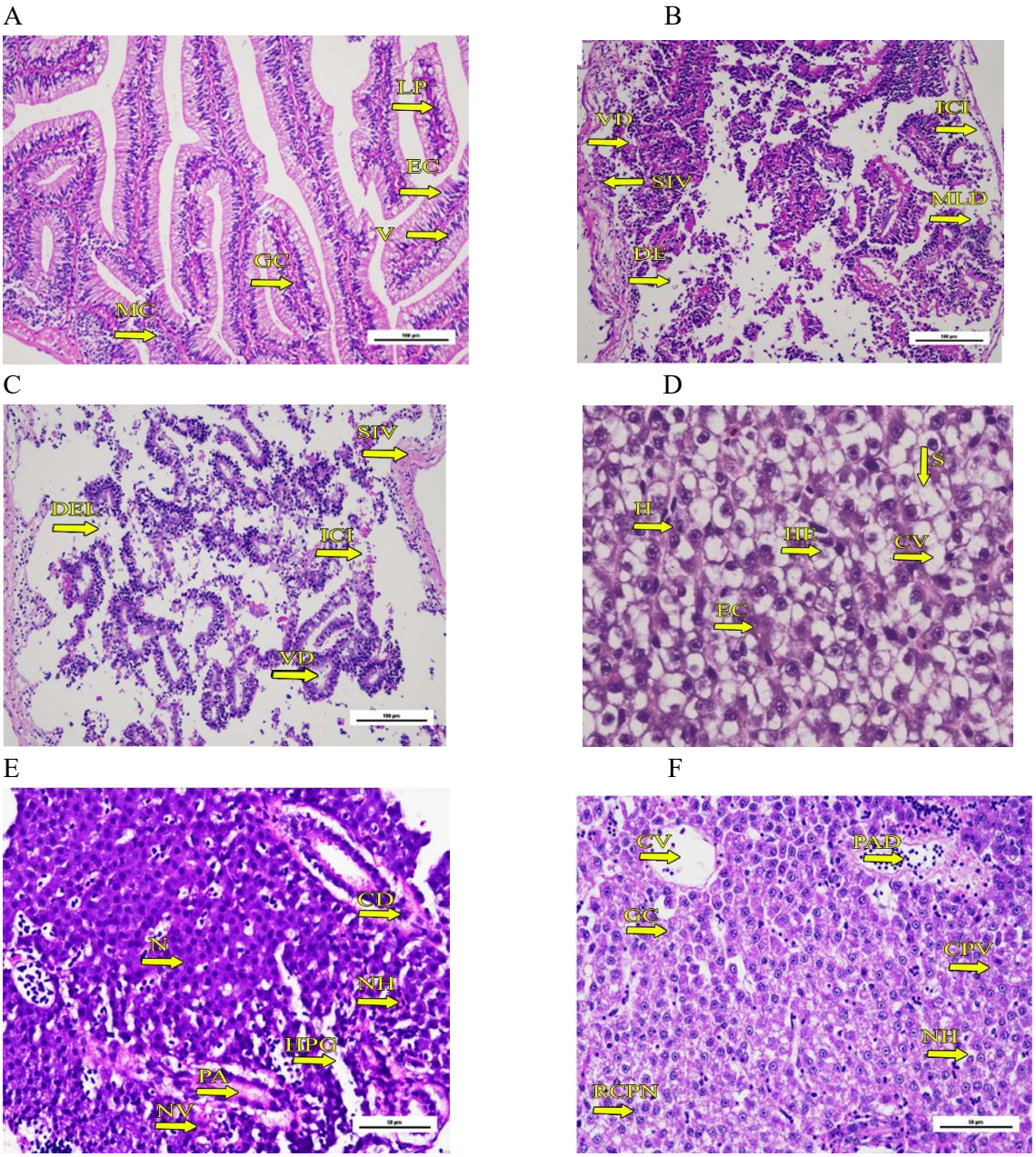

Figure: 2 Comparisons between control and changed intestine and liver tissues

(**A**) Control Intestine (CPS) LP, Lamina Propria; EC, Enterocytes; V, Villi; CC, Goblet cells; ML, Muscle layer (**B &C**) Intestine (CPS & CP) VD, Villi Degeneration; SIV, Shortened Intestinal Villi; DEL, Detachment of the Epithelium; MLD, Muscle Layer Degeneration; ICI, Inflammatory Cell Infiltration; (**D**) Control Liver (CP) H, Hepatocyte; EC, Endothelial Cell; CV, Central Vein; S, Sinusoid; (**E & F**) Liver (CPS & CP) CD, Cellular Degeneration; NH, Nuclear Hypertrophy; HPG, Hepatic Plate Gap, PA, Pyknotic Area, N, Necrosis, PAD, Portal Area Degenerate; CPV, Cytoplasmic vacuolation, NH, Nuclear Hypertrophy, RCPN, Round, centrally placed nucleus

CPS and CP exhibit significant histopathological effects on the intestinal morphology of zebrafish, including Villi Degeneration (VD) and Detachment of Epithelia (DEL). Muscle Layer Degeneration (MLD) and Inflammatory Cell Infiltration (ICI) were documented (refer to Fig B, C). Conversely, the control group exhibited no discernible alterations (see Fig. A). The control liver (illustrated in Fig D) displays a typical hepatocyte characterized by a polyhedral configuration and a prominent nucleus, with no pathological alterations detected. In contrast to the control fish, a variety of histological anomalies were identified in the livers of fish subjected to Chlorpyrifos exposure. (CPS) and Cypermethrin (CP) including Cytoplasmic Vacuolation (CV), Cellular Degeneration (CD), Pyknotic Nucleus(PN), Binucleated Hepatocytes(BH), Vacuolation(V), Portal Area Degeneration (PAD), Granular Cytoplasm (GC), and Necrosis (N), etc., The hepatocytes started degenerating leading to the loss of basophilia of liver tissue and the appearance of intracellular vacuoles due to necrosis (Fig E, F).

4. Discussion

The overreliance on pesticides presents a critical issue owing to their detrimental effects on non-target organisms, notably aquatic species such as fish. The occurrence of pesticides and chemical contaminants appears to exert a profound influence on the decline of fish populations, alongside ramifications for the aquaculture ecosystem and the evaluation of health hazards for human beings. As a result, this has instigated an intensified emphasis on investigating the acute toxicity and bioaccumulation of pesticides within living organisms Zhao [28]. The present investigation has revealed various effects of chlorpyrifos and cypermethrin on mature zebrafish. The current research demonstrates that both CPS and CP accumulated in fish after being exposed to high concentrations of CP (5.8 ± 0.014 mg/l), resulting in a higher level of bioaccumulation than CPS (1.63 ± 0.014 mg/l).

The investigation conducted by Zhao [28] elucidated that zebrafish subjected to exposure of the pyrimorph fungicide demonstrated a swift accumulation of the compound within their physiological systems shortly following their exposure to a sublethal concentration of pyrimorph. In an independent inquiry, Sun [29] probed the accumulation of HC Orange within the hepatic tissues of goldfish (*Carassius auratus*). The findings indicated that the accumulation within the fish's tissues transpired promptly after the commencement of exposure, achieving a peak concentration after a duration of 24 hours of exposure. The median lethal concentrations of CPS and CP in zebrafish were ascertained to be 0.25 and 0.15 mg/l, respectively, following a period of 96 hours. The outcomes of the present study revealed an elevated median lethal concentration (LC50) while exhibiting analogous effects on piscine behavior in comparison to the results presented by Pandey [30], wherein acute toxicity of profenofos to *Channa punctatus* was reported at 2.68 µg/l.

The effects of exposure to profenofos are evidenced by erratic swimming patterns, increased levels of excitability, changes in pigmentation, and the secretion of mucus in both the organism's body and gills, ultimately culminating in mortality. The empirical data we have amassed regarding the lethality of the present compound aligns with a scant number of antecedent

investigations. In the year 1980, Johnsson and Finley [30] documented a 96-hour LC50 value of 0.280 mg/l for chlorpyrifos in channel catfish (*Ictalurus punctatus*) and Lepomis microlophus. Ramesh and Munniswamy [31] reported an LC50 value of 0.16 mg/l for Cyprinus carpio subjected to chlorpyrifos in 2009. Additionally, Rao [32] ascertained that the toxicity of chlorpyrifos to *Oreochromis mossambicus* and *Gambusia affinis*, utilizing the semi-static methodology, was determined to be 0.0259 mg/l and 0.297 mg/l, respectively.

The compound CP exhibited significant pesticidal efficacy in the model organism zebrafish across all assessed exposure durations (24, 48, 72, and 96 hours) in a manner that was both time- and dose-dependent. Dongmei [33] proposed an LC50 value of 0.12 mg/l. Corroborating findings have similarly indicated that CP possesses a high level of toxicity towards aquatic organisms. The research conducted by Bradbury and Coats [34] has systematically reviewed the toxicological profiles of pyrethroids across various taxa, including mammals, avians, fish, amphibians, and invertebrates, and documented a 96-hour LC50 for CP toxicity of 2.2 mg/l for *Tilapia nilotica*, 0.9 to 1.1 mg/l for *Cyprinus carpio*, 1.2 mg/l for *Salmo trutta*, 0.5 mg/l for *Salmo gairdneri*, and 0.4 mg/l for *Scardinus erythrophthalmus*. Polat [35] determined that the LC50 value for beta-CP in male guppies over a 48-hour exposure period was 21.4 mg/l. Sarikaya [36] indicated that the range of the 96-hour LC50 value for alpha-CP in *Oreochromis niloticus* spans from 0.7 to 350 mg/l.

Alterations in biochemical parameters such as protein and lipid concentrations serve as crucial indicators of an organ's susceptibility to environmental pollutants. Proteins constitute vital organic compounds essential for tissue synthesis within the organism and are integral to energy metabolism. Kawade and Khillare [37] documented a statistically significant reduction ($p<0.05$) in muscle protein levels subsequent to exposure to sub-chronic concentrations of CPS (21.3 ± 0.115) and CP (20.2 ± 0.145). This study elucidated the extent of protein depletion observed in various fish species subjected to copper sulphate stress. Numerous prior investigations have indicated a progressive reduction in fish protein content when exposed to toxic substances, proposing that this phenomenon may stem from metabolic disturbances involving carbohydrates and proteins, leading to the impairment of protein synthesis mechanisms and inhibition of ATP production. The observed protein depletion may also be ascribed to the involuntary utilization of amino acids in diverse catabolic pathways within organisms as a response to stress conditions, as noted by Thenmozhi [38]. Sobha [39] reported a decrease in protein levels across different tissues of *Labeo rohita* and *Cirrhinus mrigala* subjected to sublethal and lethal concentrations of pyrethroid, suggesting that this decline may be attributable to the metabolic conversion of ketoacids for gluconeogenesis in glucose synthesis, or the direct utilization of free amino acids for the synthesis of requisite proteins. The research conducted by Kahtani [40] indicated a notable reduction in protein content within the liver of Tilapia Fish (*Oreochromis niloticus*) following exposure to insecticides, likely resulting from enhanced metabolic activity and proteolytic processes under toxic stress, alongside disrupted protein synthesis due to liver dysfunction.

Biological investigations have substantiated that lipids serve as a crucial energy source for metabolic processes in fish, particularly during periods of physiological stress, wherein there is

an elevation in overall energy expenditure, necessitating the curtailment of resource-intensive processes. Consequently, this situation triggers compensatory metabolic adaptations within the tissues aimed at modulating both the quantity and quality of numerous metabolites, including lipids, as articulated by Tulasi [41]. In the current research, a notable decline in total lipid content was observed following exposure to sub-chronic levels of CPS (11.1 ± 0.115) and CP (10.4 ± 0.115). This observation aligns with findings from Xiaoyu [42], which indicated that lipid accumulation exhibited a tendency to diminish post-CPS exposure, with the extent of reduction being contingent upon the concentration of CPS. The results obtained by Lixiao [43] demonstrated an increase in total lipid content within the tissues of zebrafish subjected to OP (MAL, ATR) and Cd exposure. Prior research has indicated that toxic pesticides possess the capacity to disrupt lipid metabolism in fish. Supporting this assertion, short-term exposure to propamocarb has been documented to induce lipid metabolic disturbances in zebrafish, as noted by Zang [44]. Hence, the interplay between pesticide toxicity and lipid metabolism warrants significant attention. Nandurkar and Zambare [45] reported an escalation in lipid content in both selected models*, Lamellidens corrianus* and *Parreysia* cylindrical, following both acute and chronic exposure to chloramphenicol. Concurrently, the augmented lipid content observed in fish may also result in adverse effects, as organisms with elevated lipid levels exhibit bio-concentration of such chemicals, a characteristic that leads to bioaccumulation and consequently poses a detrimental risk to the human food chain, as highlighted by Harald [46].

Furthermore, the histopathological examination of the intestinal architecture in adult zebrafish subjected to chronic exposure to CPS and CP was conducted following a 28-day treatment regimen. The investigation yielded the conclusion that exposure to CPS and CP instigated inflammatory cell infiltration within intestinal tissues, detachment of epithelial cells, degeneration of the muscular layer, and deterioration of the villi. The results presented by Mahmoud [47] indicated that the accumulation of fenvalerate significantly disrupts the structural integrity of the intestine and alters the specificity of protease and amylase regarding substrate interaction. In a similar vein, Bhattacharjee and Das [48] explored the histopathological implications of lindane on the intestinal tissue of *Channa punctata*, a species of teleost fish, and concluded that such exposure resulted in inflammatory cell infiltration. Carvalho [49] reported that exposure to highly toxic substances led to vacuolization within enterocytes, a condition that may be accompanied by edema. The latter phenomenon is frequently associated with degeneration of both the muscular layer and the villi. These pathological alterations impede the processes associated with nutrient absorption and often precede the onset of necrosis.

Das and Gupta [50] investigated the impact of mancozeb on *Esomus danricus* within the intestinal tissues, identifying pathological manifestations such as ulceration, vacuolization, and the infiltration of chronic inflammatory cells in the mucosal layer. In contrast to this investigation, our study did not reveal any instances of ulceration. Yön Ertug [51] explored the histopathological alterations induced by 2,4-dichlorophenoxyacetic acid in the intestinal tissue of zebrafish, documenting findings that included hyperplasia of goblet cells, degeneration and edema within villi structures, as well as necrosis and atrophy of epithelial cells. The outcomes of this study align with our observations. The intestinal morphology of zebrafish exhibits

significant homology to that of higher vertebrates, as noted by Brugman [52]. Consequently, the findings presented in this research will be instrumental in elucidating the detrimental effects of environmental pollutants on higher vertebrate species.

In the current investigation, the histopathological modifications within the hepatic tissue of zebrafish (*Danio rerio*) subjected to CPS and CP for a duration of 28 days were analyzed. The findings derived from this investigation revealed cytoplasmic vacuolation, cellular degeneration, pyknotic nuclei, binucleated hepatocytes, vacuolation, degeneration of portal areas, granular cytoplasm, and necrosis. Hamid [53] demonstrated that hepatocellular vacuolization (HV) and indicated congestion surrounding the central vein resulted from prolonged exposure to a mixture of PPCPs. Likewise, the vacuolation of hepatocytes and the presence of pyknotic nuclei were documented in the hepatic tissue of freshwater fish, specifically carp, subjected to the herbicide trifluralin, as reported by Poleksic [54]. Kaya [55] has documented comparable findings, including necrosis accompanied by condensed nuclear bodies and pyknotic nuclei in tilapia exposed to both small and large-sized zinc oxide nanoparticles. Abar [56] reported a range of histological alterations, including degeneration and the presence of vacuoles within the cytoplasm of hepatocytes in the liver of zebrafish following exposure to Poly (2-Ethyl-2-Oxazoline). Chen [57] noted analogous findings in the hepatic tissue of adult zebrafish (*Danio rerio*) subjected to mercuric chloride, as well as in *C. auratus* exposed to chromium. Agamy [58] asserted that necrotic lesions are regarded as a direct consequence of toxicants and represent one of the prevalent contributing factors, as they are typically irreversible and persistent, leading to a decline in organ functionality. Liu [59] has indicated that the ultraviolet filter benzophenone induces the formation of pyknotic nuclei in the hepatic tissue of freshwater fish, *Carassius auratus.*

5. Conclusion

The present investigation demonstrated that zebrafish exhibit a marked sensitivity to the toxicants examined, with mortality rates associated with chlorpyrifos (CPS) and cypermethrin (CP) showing a clear dependence on the concentration of these toxins. The capacity of zebrafish to endure dose-dependent bioaccumulation of toxicants serves as a robust indicator of their appropriateness as a model organism for biomonitoring initiatives. Furthermore, it is inferred from the current study that the indiscriminate application of pesticides leads not only to the eradication of target species but also adversely impacts numerous non-target organisms, thereby disrupting their normal physiological functions. Consequently, the application of these pesticides necessitates meticulous caution and sustainable practices to mitigate potential hazards to non-target biota. Additionally, the potential risks posed by the metabolites of chlorpyrifos and cypermethrin warrant thorough investigation to elucidate their toxicity profile more comprehensively. Moreover, our findings revealed intestinal and hepatic damage following sub-chronic exposure to these substances. The organ damage and metabolic disturbances induced by CPS and CP may impede the developmental processes of zebrafish. Furthermore, the detrimental effects on zebrafish merit careful consideration when these substances are utilized within the aquatic ecosystems of agricultural production zones.

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