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Acute Toxicity and Behaviour of Fresh Water Murrel (*Channa punctata*, Bloch 1793), Exposed to Agricultural Pesticides

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ABSTRACT
Pesticides are employed in agricultural practices to manage insect infestations. However, they can significantly impact all levels of biological organization, particularly affecting fish, populations and contaminating surface waters through mechanisms such as irrigation or precipitation-driven runoff. This investigation examined the acute toxicity of several commonly used pesticides in croplands, including Chlorpyrifos (20%), Cypermethrin (10%), and Hamla550 (combination of Chlorpyrifos 50% and Cypermethrin 5%), utilizing static renewal bioassay experiments. Fingerlings of both edible and ornamental fish, specifically *Channa punctata*, with an average size of 8.16±0.86 cm and a body weight of 7.53±0.65 g, were selected for the study. The 96 hr LC_{50} (Probit) values Chlorpyrifos, Cypermethrin, and Hamla550, were determined to be 1.3836, 0.2841, and 0.0010 mg L^{-1}, respectively. The physicochemical parameters of the test water were analyzed following standard protocols. The behavioural responses of the exposed fish were monitored throughout the exposure period. As the concentrations of the toxic pesticides increased, the fish exhibited various abnormal behaviours, including hyperactivity, erratic swimming, patterns, heightened surface activity, rapid and erratic movements, body imbalance, and loss of equilibrium. This study highlights the importance of assessing appropriate pesticide dosages prior to field application to help preserve fish populations.

Key words: *Channa punctata*, Chlorpyrifos, Cypermethrin, LC_{50}, Organophosphate, Pesticide

INTRODUCTION

Pesticides are hazardous materials used extensively in India to manage agricultural pests, particularly insects. They can contaminate surface waters, through various means, primarily via irrigation or runoff caused by precipitation. Exposure to pesticides in aquatic environments can affect all levels of biological organization, with fish being particularly vulnerable. The chemical composition of pesticides significantly influences their mode of action leading to various physiological and biochemical changes in affected organisms due to prolonged exposure. While pesticides are commonly employed to control agricultural pests, synthetic pesticides have posed serious health risks to workers and caused significant ecological damage. *Channa punctata* is commonly used as a model in pollution studies due to its availability and suitability for research. Chlorpyrifos also known as Chlorpyrifos ethyl in organic chemistry is considered one of the most toxic pesticides in India (Jaffrey et al. 1989). As an organophosphate pesticide it targets insects by inhibiting the action of the acetylcholinesterase enzyme in their nervous systems (Sparks et al. 2020). Chlorpyrifos can enter the food chain through bioaccumulation and biomagnification, posing ecosystem threats. Chlorpyrifos is one of the most widely used insecticides in agricultural practices globally (Hasanuzzaman et al. 2018) and is the second most utilized synthetic pesticide in India (Stalin et al. 2008). Various studies have documented the LC_{50} values of Chlorpyrifos in different fish species. According to De Silva and Samayawardhena (2002), the LC_{50} of Chlorpyrifos for Guppies was 7.17 µg L^{-1} and in *Gambusia yucatana*, it was 0.085 mg L^{-1} (Rendón-von Osten et al. 2005). Halappa and David (2009) reported an acute toxicity LC_{50} for common carp of 0.160 mg L^{-1}. Thaimuangphol and Kasamesiri (2015) investigated the LC_{50} value for 24 hr in the fairy shrimp, *Branchinella thailandensis*, and reported it to be 210 µg L^{-1}. The 96 hr LC_{50} values of Chlorpyrifos have also been studied in fish (*Cnesterodon decemmaculatus*) and snails (*Lanistes carinatus*) by Paracampo et al. (2015) and Khalil (2015). Suja and Sherly (2017) reported that the acute toxicity dose (96 hr LC_{50}) of Chlorpyrifos on *Channa striata* was 4.521 ppm.

Cypermethrin is a widely utilized pesticide recognized as one of the most potent formulations of pyrethroids (Bradbury and Coats 1989a, b). Its mode of action in fish is closely related to that of other pyrethroids featuring cyano-3-phenoxybenzyl groups. Specifically, Cypermethrin extends the depolarization phase of GABA receptors in nerve fibers by obstructing sodium channels (Stephanson 1982). Numerous studies have assessed the LC_{50} of Cypermethrin across various fish species. According to research by Stephanson (1983), the 96 hr LC_{50} values for several species are as follows: 2.8 µg L^{-1} for rainbow trout (*Oncorhynchus mykiss*), 1.2 µg L^{-1} for fathead minnows, and 0.93 µg L^{-1} for juvenile *Pimephales promelas*. Baser et al. (2003) investigated the acute toxic effects of Cypermethrin on guppies 48 hr LC_{50} value of 245.7 µg L^{-1}. In their study, Das and Mukherjee (2003) reported the LC_{50} for *Labeo rohita*, 0.139 mg L^{-1}., while Sarkar et al. (2005) reported an LC_{50} of 5.13 µg L^{-1} in *Cirrhinus mrigala*. Additionally, Ayoola and Ajani (2008) established an LC_{50} of 0.063 mg L^{-1} in *Clarias gariepinus*, and Küçükbay et al. (2009) found the value to be 41.786 µg L^{-1} in *Oncorhynchus mykiss*. Bhoi et al. (2021) have examined the long-term toxicity of Cypermethrin on *Channa marulius* (Ham Buch).

Kumar et al. (2020) researched the morphological and behavioural effects of a mixture containing 50% Chlorpyrifos and 5% Cypermethrin on the freshwater fish *Clarias batrachus*. However, no studies have yet reported the impacts of the hybrid pesticide Hamla550, which comprises both Chlorpyrifos (50%) and Cypermethrin (5%), on *C. punctata*.

MATERIALS AND METHODS

Test animal

The fingerling stage of the freshwater murrel, *Channa punctata* (Bloch 1793), with an average weight of 7.53±0.65 g and a length of approximately 8.16±0.86 cm, was sourced from a local fish market for use as test subjects. The fish were placed in three separate holding tanks, each containing 100 L of water, and equipped with aeration, and were allowed to acclimate for 30 days. During this acclimatization phase, the fish were fed pelleted feed twice daily at a rate of 5% of their body weight. Throughout the acclimatization period, no mortality was observed among the test fish.

Test chemical

Three conventional pesticides, "Dhanvan-20" (Chlorpyrifos 20% EC), "Superkiller-10" (Cypermethrin 10% EC), and Hamla550 (50% Chlorpyrifos + 5% Cypermethrin), were used as test chemicals. The physicochemical properties of the above-mentioned pesticides are summarised in Table 1.

Test water

In the present experiment, each tank was filled with 20 L of tap water and maintained under static conditions without aeration throughout the experimental period. Various physiochemical parameters were assessed daily in each tank. Water temperature was measured using a digital thermometer (Zacro), while a digital pH meter (Hanna HI98107) was utilized to determine pH levels. Standard techniques (Anonymous 2017) were employed to evaluate parameters such as dissolved oxygen (DO), total alkalinity (TA), total hardness (TH), and chlorides.

Methodology

Fish of similar sizes, averaging a diameter of approximately 8.16±0.86 cm, were selected and divided into five groups: four containing twelve fish each for Chlorpyrifos and Cypermethrin and one with ten fish for Hamla550. These groups were placed in a 20 L glass tank with dimensions of 18×10×10 inches. Each group was exposed to varying concentrations of pesticides, including one control group and four with different concentrations. To determine the 96 hr LC_{50} value, three replicates and a control were simultaneously assessed from each group. Throughout this period, the fish were not fed. The mortality rate for each group was meticulously recorded, with any deceased fish being promptly removed. Probit analysis was employed to evaluate fish mortality. Additionally, the fish behaviour was monitored during the first hour of chemical exposure over the four-day experimental period.

Table 1. Physicochemical properties of Chlorpyrifos, Cypermethrin and Hamla550

Brand Name	**Chemical composition**	**Chemical formula**	**Target pest**	**Physical property**
"DHANVAN-20" Dhanuka Agritech Ltd, Gurugram, 122002, Haryana, India	Chlorpyrifos (20%)	$C_9H_{11}C_{13}NO_3PS$	Aphids, whiteflies, jassids, leaf-eating caterpillars, spider mites, bollworms	Liquid, colouress, water soluble
"SUPERKILLER-10" Dhanuka Agritech Ltd, Gurugram, 122002, Haryana, India	Cypermethrin (10%)	$C_{22}H_{19}C_{12}NO_3$	Aphids, whiteflies, jassids, leaf-eating caterpillars, spider mites, bollworms	Liquid, colouress, water soluble
"HAMLA 550" Gharda Chemicals Limited, Howrah, West Bengal	50% Chlorpyrifos + 5% Cypermethrin	Chlorpyrifos - $C_9H_{11}C_{13}NO_3PS$ + Cypermethrin - $C_{22}H_{19}C_{12}NO_3$	Aphids, jassids, thrips, whitefly, thrips, caterpillars, and beetles	Liquid, colouress, water soluble

RESULTS

Acute toxicity study

The toxicity study results are given in Table 2. The percentage of mortality in *C. punctata* was recorded and calculated using the probit analysis method. In the acute toxicity study of Chlorpyrifos on *C. punctata*, four different pesticide concentrations (1, 2, 3, and 4 mg L^{-1}) were administered over 24, 48, 72, and 96 hrs. The highest mortality rate was observed at a concentration of 4 mg L^{-1}, while the lowest at 1 mg L^{-1}. After 96 hr of pesticide exposure, the 2 mg L^{-1} concentration resulted in over 50% mortality.

In a separate exploratory test with Cypermethrin over 96 hr 80% mortality rate was recorded at a concentration of 0.60 mg L^{-1}, while 100% mortality occurred at 0.80 mg L^{-1}. Additionally, at 0.40 mg L^{-1} concentration, more than half of the exposed fish succumbed.

Calculation of LC_{50} using the direct interpolation method

The concentrations from the definitive test were converted into log concentrations, allowing for calculating a corrected percentage, which was subsequently utilized to determine the LC_{50} through probit analysis. A curve was then plotted between the log concentrations and the probit values, drawing a perpendicular line at five probits, corresponding to 50% mortality, the LC_{50} values for various time intervals were ascertained from these curves. The true LC_{50} was obtained by applying the inverse log to the associated concentrations. Additionally, the toxicants' percentage mortality and log concentrations were graphed to establish the LC_{50} values for different pesticides (Fig. 1).

Behavioural study

Based on the findings of this investigation, *C. punctata* exposed to lethal concentrations of Chlorpyrifos, Cypermethrin, and Hamla550 exhibited noticeable morphological and behavioural abnormalities. While the fish demonstrated independent movements, they tended to remain near the bottom of the test tank when exposed to lower pesticide concentrations. Elevated levels were linked to an imbalance and irregular, darting, and unpredictable swimming patterns. The fish displayed

Table 2. 96 hr LC_{50} of Chlorpyrifos, Cypermethrin and Hamla550 on *Channa punctata*

	Chlorpyrifos concentration (mg L^{-1})	% of mortality per exposure time 24 hr	48 hr	72 hr	96 hr	Total % of dead	Cypermethrin concentration (mg L^{-1})	% of mortality per exposure time 24 hr	48 hr	72 hr	96 hr	Total % of dead	Hamla550 concentration (mg L^{-1})	% of mortality per exposure time 24 hr	48 hr	72 hr	96 hr	Total % of dead
Control	**0.0**	00	00	00	00	00	**0.00**	00	00	00	00	00	**0.0**	00	00	00	00	00
Tank 1	**1.0**	8.33	8.33	00	16.67	33.33	**0.20**	8.33	8.33	00	16.67	33.33	**0.000687**	10.00	00	00	00	10.00
Tank 2	**2.0**	25.00	16.66	8.33	8.33	58.33	**0.40**	16.67	8.33	16.67	16.67	58.33	**0.001375**	20.00	10.00	00	20.00	50.00
Tank 3	**3.0**	33.33	16.67	16.67	16.67	83.33	**0.60**	33.33	8.33	8.33	33.33	83.33	**0.00200**	50.00	30.00	20.00	00	100.00
Tank 4	**4.0**	50.00	50.00	00	00	100.00	**0.80**	50.00	25.00	25.00	00	100.00	**0.004125**	60.00	40.00	00	00	100.00
LC_{50} probit (96 hr)		**1.3836 mg L^{-1}**						**0.2841 mg L^{-1}**						**0.0010 mg L^{-1}**				

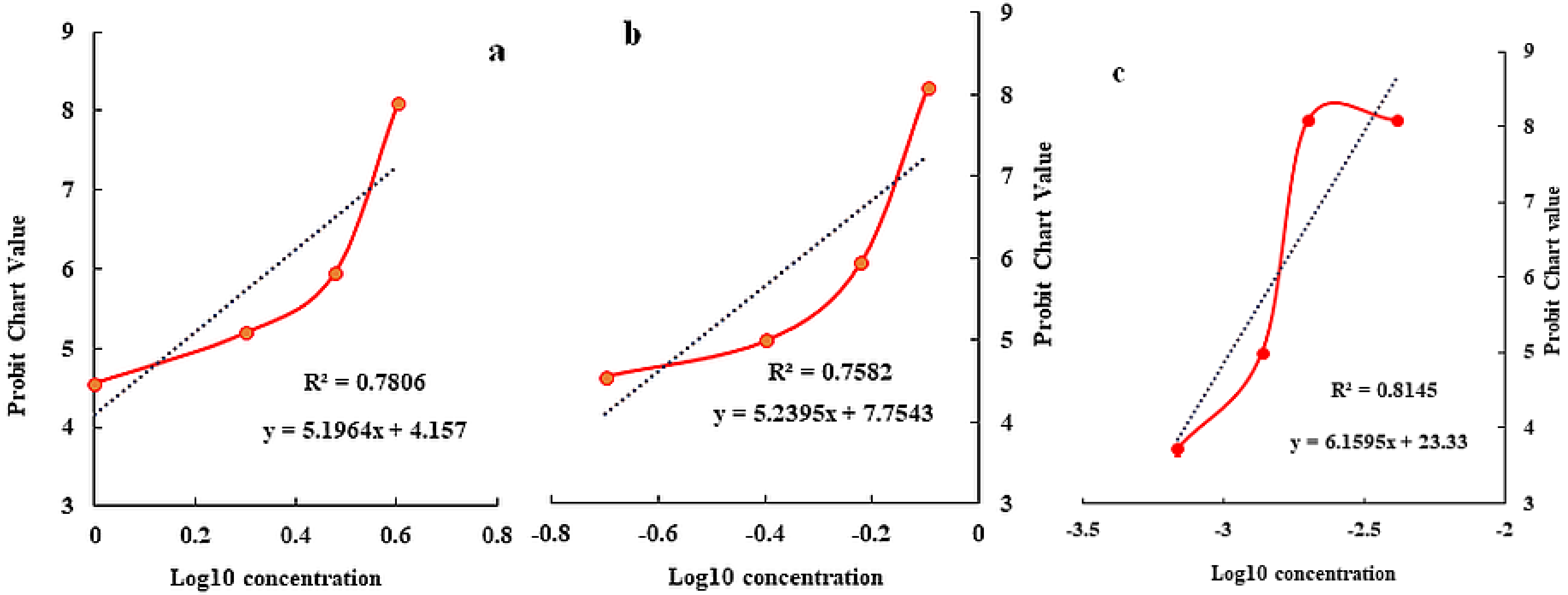

Figure 1. LC_{50} Probit graph for the 96-hrs acute toxicity of different pesticides. a) Chlorpyrifos, b) Cypermethrin, and c) Hamla550

Table 3. Behaviour of fish observed in different concentration of Chlorpyrifos, Cypermethrin and Hamla550 in respect of exposure time

Behaviour	24 hr				48 hr				72 hr				96 hr				
	T 1	T 2	T 3	T 4	T 1	T 2	T 3	T 4	T 1	T 2	T 3	T 4	T 1	T 2	T 3	T 4	
Chlorpyrifos																	
Migration to the base	++	++	+++	++	++	++	+++	++	++	++	++	-	++	++	-	-	
Swimming frequently	+++	+++	+++	++++	+++	+++	+++	++++	+++	+++	+++	-	++	++	-	-	
Loss of balance	++	++	++	+++	++	++	+++	+++	+++	+++	++++		-	+++	+++	-	-
Sudden rapid movement	+++	+++	+++	++++	+++	+++	+++	++++	++	++	+++	-	++	++	-	-	
Engulfment of air	++	++	+++	++++	++	+++	+++	++++	++	++	+++	-	+++	+++	-	-	
Mouth, operculum opening	++	++	++	++++	++	++	++	++++	++	+++	+++	-	+++	+++	-	-	
Surface activity	+++	+++	+++	++++	+++	+++	+++	++++	++	+++	+++	-	++	++	-	-	
Migration to the base	++	++	+++	++	++	++	+++	++	++	++	++	-	++	++	-	-	
Cypermethrin																	
Migration to the base	++	++	+++	+++	++	++	+++	++	++	++	++	++	++	++	++	-	
Swimming frequently	+++	+++	+++	++++	+++	+++	+++	++++	+++	+++	+++	++++	++	++	++	-	
Loss of balance	++	++	++	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	
Sudden rapid movement	+++	+++	+++	++++	+++	+++	+++	++++	++	++	+++	+++	++	++	++	-	
Engulfment of air	++	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++	-	
Mouth, operculum opening	++	++	++	+++	++	++	+++	+++	+++	+++	+++	++++	+++	+++	+++	-	
Surface activity	+++	+++	+++	++++	+++	+++	+++	++++	++	+++	+++	++++	++	++	+++	-	
Hamla550																	
Migration to the base	+++	+++	+++	+++	++	++	+++	+	+++	++	+	-	+++	-	-	-	
Swimming frequently	+++	+++	+++	++++	++++		+++	+++	++	+++	+++	++	-	++	-	-	-
Loss of balance	++	++	++	+++	++	++	+++	+++	+++	+++	+++	-	++++		-	-	-
Sudden rapid movement	+++	+++	+++	++++	+++	+++	+++	++++	+++	++	++	-	++	-	-	-	
Engulfment of air	++	+++	++	++++	++	++	+++	+++	++	++	+++	-	+++	-	-	-	
Mouth, operculum opening	++	++	++	++	++	++	++	+	+++	+++	+++	-	+++	-	-	-	
Surface activity	+++	+++	+++	++++	++	+++	+++	+++	++	+++	++++		-	++	-	-	-

Refer to Table 2 for the details of concentration of respective pesticide in tanks T1, T2, T3, T4; + = very low, ++ = low, +++= moderate, ++++= high, - = Not applicable restlessness, lethargy, and mucous discharge covering their bodies. Those exposed to Chlorpyrifos and Cypermethrin exhibited aberrant behaviour, including hyperactivity, erratic swimming with increased surface activity, and mucus secretion. Peritoneal movements initially increased but then gradually decreased throughout the exposure periods. The fish exposed to Hamla550 (a mixture of 50% Chlorpyrifos and 5% Cypermethrin) displayed the most severe behavioural issues, such as hyperactivity, erratic swimming, loss of balance, excessive mucus production, and heightened surface activity. Similar to earlier observations, peritoneal movements rose initially and diminished slowly. Throughout the exposure, the affected fish secreted large amounts of mucus and experienced disorientation and lack of equilibrium. At lower concentrations, of the toxicants, the behavioural changes were less pronounced. Fish produced mucus to mitigate the adverse effects of elevated toxicant levels.

The various behavioural abnormalities of *C. punctata* observed during different exposure periods to various pesticides are summarized in Table 3. At a concentration of 4 mg L^{-1} for Chlorpyrifos, the exposed fish exhibited maximum surface activity, rapid movements, and frequent swimming throughout the 24 and 48 hrs period. This indicates that the pesticide concentration significantly affects the fish's behaviour. Conversely, moderate to mild behavioural changes were noted at a concentration of 1 mg L^{-1}.

Fish exposed to Cypermethrin at a concentration of 0.80 mg L^{-1} demonstrated heightened surface activity and a tendency to migrate to the bottom within 24 and 48 hrs. In contrast, the lowest to moderate behavioural abnormalities were observed at 0.20 mg L^{-1} over 24, 48, 72, and 96 hrs exposures. Fish subjected to Hamla550 (a mixture containing 50% Chlorpyrifos and 5% Cypermethrin), displayed the most severe behavioural abnormalities. These included hyperactivity, erratic swimming, loss of equilibrium, excessive mucus secretion, and increased surface activity. Initially, peritoneal movements increased but gradually declined over the entire exposure period. The affected fish secreted a significant amount of mucus, experiencing disorientation and a loss of balance. The behavioural changes were less pronounced at lower toxicant concentrations of 0.000687 mg L^{-1}, where the mucus secretion served as a coping mechanism to alleviate the adverse effects of higher toxicant levels.

Physicochemical parameters of test water

The selected water quality parameters for Chlorpyrifos, Cypermethrin, and Hamla550 acute toxicity tests are summarized in Table 4. In the current study of Chlorpyrifos on *C. punctata*, the recorded water temperature varied from 21.54±0.41 to 22.87±0.15 °C. The average pH values, which showed progressive fluctuations during the pesticide application, ranged from 6.82±0.12 to 7.10±0.10 throughout the experimental period, indicating that the pH of the test water remained slightly basic. During the study, the dissolved oxygen (DO) content gradually decreased as the pesticide concentration increased. Specifically, for Chlorpyrifos, the mean DO level dropped after 72 hr of exposure to the pesticide. The total alkalinity of the test water ranged between 159.65±0.41 to 161.18±0.61 mg L^{-1}. In the investigation of Cypermethrin on *C. punctata*, the DO content also decreased as the pesticide concentration rose. The lowest DO levels were recorded after a 96 hr exposure to both Cypermethrin and Hamla550. The average pH values for Cypermethrin ranged from 6.79±0.10 to 7.10±0.10, while for Hamla550, they ranged from 6.88±0.14 to 7.05±0.13, showing gradual variations depending on the insecticide used. The pH of the test water consistently remained in a slightly acidic to neutral range in both cases. Regarding alkalinity, Hamla550, had the lowest recorded levels, whereas Cypermethrin, had the highest. The average water hardness levels in this investigation ranged from 104.65±0.10 to 106.10±0.13 mg L^{-1} for Cypermethrin, and from 100.36±0.18 to 102.07±0.20 mg L^{-1} for Hamla550, which are considered quite challenging. Water hardness values typically range from 0- to 60 mg L^{-1} and fall into the soft category (Kannan 1991). The mean water hardness values across all cases in this study varied between 107.90±0.27 and 108.70±0.59 mg L^{-1}, placing them within the hard water range. Lower chloride concentrations were recorded overall during this investigation, although slightly higher levels were detected at higher concentrations of Chlorpyrifos. In the Chlorpyrifos-treated tanks, chloride

Table 4. Physicochemical study of Test water for LC_{50} of Chlorpyrifos, Cypermethrin and Hamla550

	Chlorpyrifos						Cypermethrin						Hamla550					
	T (°C)	pH	DO (mg L^{-1})	TA (mg L^{-1})	TH (mg L^{-1})	TC (mg L^{-1})	T (°C)	pH	DO (mg L^{-1})	TA (mg L^{-1})	TH (mg L^{-1})	TC (mg L^{-1})	T (°C)	pH	DO (mg L^{-1})	TA (mg L^{-1})	TH (mg L^{-1})	TC (mg L^{-1})
T1																		
24 hr	22.87 ± 0.15	7.10 ± 0.10	5.51 ± 0.07	161.12 ± 0.60	108.60 ± 0.66	42.99 ± 0.22	25.87 ± 0.11	7.10 ± 0.10	5.22 ± 0.07	168.50 ± 0.60	106.05 ± 0.14	41.10 ± 0.11	24.95 ± 0.33	7.01 ± 0.10	5.55 ± 0.14	145.59 ± 0.33	102.07 ± 0.20	42.09 ± 0.16
48 hr	22.06 ± 0.27	7.09 ± 0.10	5.50 ± 0.06	161.18 ± 0.61	108.70 ± 0.59	43.00 ± 0.22	24.90 ± 0.23	6.94 ± 0.11	5.25 ± 0.07	168.28 ± 0.42	105.94 ± 0.16	41.00 ± 0.09	25.00 ± 0.31	6.95 ± 0.11	5.42 ± 0.14	145.55 ± 0.29	101.87 ± 0.18	41.90 ± 0.16
72 hr	21.54 ± 0.41	7.02 ± 0.12	5.49 ± 0.05	160.91 ± 0.61	108.52 ± 0.41	43.04 ± 0.23	25.07 ± 0.20	6.84 ± 0.10	5.20 ± 0.09	168.40 ± 0.46	105.90 ± 0.16	41.15 ± 0.11	25.04 ± 0.28	6.90 ± 0.11	5.47 ± 0.12	145.25 ± 0.29	101.84 ± 0.18	42.10 ± 0.12
96 hr	22.44 ± 0.32	6.92 ± 0.11	5.47 ± 0.08	160.85 ± 0.54	108.63 ± 0.36	43.08 ± 0.20	26.00 ± 0.16	6.86 ± 0.10	5.15 ± 0.10	168.09 ± 0.25	106.00 ± 0.12	41.10 ± 0.09	24.84 ± 0.13	6.89 ± 0.10	5.36 ± 0.14	144.93 ± 0.26	100.84 ± 0.24	41.75 ± 0.12
T2																		
24 hr	21.82 ± 0.26	7.10 ± 0.10	5.52 ± 0.11	160.91 ± 0.48	108.65 ± 0.35	43.00 ± 0.18	25.92 ± 0.10	7.01 ±0.12	5.17 ± 0.07	168.22 ± 0.37	105.90 ± 0.15	41.19 ± 0.11	25.00 ± 0.19	7.05 ± 0.13	5.48 ± 0.12	145.00 ± 0.26	101.02 ± 0.30	42.09 ± 0.16
48 hr	21.62 ± 0.19	6.98 ± 0.11	5.50 ± 0.08	160.64 ± 0.55	108.67 ± 0.37	42.96 ± 0.19	25.10 ± 0.19	6.96 ± 0.11	5.20 ± 0.08	167.96 ± 0.11	105.81 ± 0.16	41.26 ± 0.10	25.37 ± 0.21	7.01 ± 0.10	5.45 ± 0.11	145.05 ± 0.26	100.67 ± 0.23	41.68 ± 0.17
72 hr	22.00 ± 0.21	6.95 ± 0.10	5.45 ± 0.08	160.90 ± 0.46	108.48 ± 0.41	43.02 ± 0.17	25.95 ± 0.13	6.80 ±0.11	5.12 ± 0.09	167.84 ± 0.22	104.93 ± 0.28	41.50 ± 0.21	25.56 ± 0.29	7.00 ± 0.10	5.37 ± 0. 14	144.85 ± 0.19	100.50 ± 0.17	41.52 ± 0.12
96 hr	22.60 ± 0.28	6.90 ± 0.11	5.41 ± 0.06	160.56 ± 0.47	107.93 ± 0.45	43.04 ± 0.17	25.87 ± 0.14	6.86 ± 0.10	5.11 ± 0.09	167.08 ± 0.19	104.85 ± 0.17	41.46 ± 0.16	25.65 ± 0.25	6.95 ± 0.12	5.33 ± 0. 14	143.80 ± 0.36	100.36 ± 0.18	41.61 ± 0.13
T3																		
24hr	22.82 ± 0.23	7.00 ± 0.13	5.52 ± 0.10	160.90 ± 0.33	108.10 ± 0.37	42.98 ± 0.15	25.90 ± 0.14	6.93 ±0.10	5.10 ± 0.10	167.10 ± 0.17	106.07 ± 0.12	42.00 ± 0.20	25.44 ± 0.15	7.05 ± 0.12	5.21 ±0.15	145.55 ± 0.29	101.00 ± 0.09	42.10 ± 0.12
48hr	22.19 ± 0.24	6.98 ± 0.10	5.48 ± 0.06	160.45 ± 0.45	108.05 ± 0.33	43.00 ± 0.22	24.98 ± 0.17	6.89 ± 0.11	5.07 ± 0.10	167.20 ± 0.16	105.90 ± 0.14	41.92 ± 0.17	25.70 ± 0.17	6.95 ± 0.13	5.25 ±0.14	143.90 ± 0.29	100.50 ± 0.17	42.00 ± 0.23
72hr	22.07 ± 0.25	6.89 ± 0.13	5.44 ± 0.08	159.92 ± 0.50	107.90 ± 0.27	43.05 ± 0.18	25.07 ± 0.20	6.85 ± 0.12	5.05 ± 0.10	167.25 ± 0.16	104.78 ± 0.17	42.02 ± 0.19	-	-	-	-	-	-
96hr	21.60 ± 0.27	6.88 ± 0.11	5.44 ± 0.06	159.83 ± 0.40	107.95 ± 0.19	43.08 ± 0.22	25.65 ± 0.28	6.86 ± 0.11	5.05 ± 0.10	166.92 ± 0.25	104.65 ± 0.10	41.91 ± 0.17	-	-	-	-	-	-
T4																		
24 hr	21.80 ± 0.23	6.96 ± 0.12	5.48 ± 0.10	159.65 ± 0.41	108.13 ± 0.22	42.99 ± 0.17	25.80 ± 0.12	6.91 ± 0.12	5.12 ± 0.09	167.00 ± 0.21	106.10 ± 0.13	40.75 ± 0.28	25.55 ± 0.13	6.94 ± 0.14	5.20 ± 0.11	145.05 ± 0.26	101.00 ± 0.08	41.58 ± 0.23
48 hr	22.21 ± 0.23	6.85 ± 0.12	5.45 ± 0.08	159.71 ± 0.35	108.06 ± 0.18	43.01 ± 0.18	24.95 ± 0.14	6.85 ± 0.12	5.11 ± 0.07	166.60 ± 0.21	105.92 ± 0.13	40.82 ± 0.28	25.37 ± 0.21	6.88 ± 0.14	5.17 ± 0.20	143.50 ± 0.32	100.94 ± 0.12	41.52 ± 0.12
72 hr	22.01 ± 0.18	6.82 ± 0.12	5.42 ± 0.09	159.61 ± 0.36	107.98 ± 0.19	42.92 ± 0.19	25.92 ± 0.15	6.79 ± 0.10	5.09 ± 0.11	166.84 ± 0.16	105.90 ± 0.16	40.75 ± 0.17	-	-	-	-	-	-
96 hr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: Dissolved oxygen = DO, Temperature = T, Total alkalinity = TA, Total Hardness = TH, Total Chloride = TC, - = Not applicable; Refer to Table 2 for the details of concentration of respective pesticide in tanks T1, T2, T3, T4 concentrations in the test water ranged from 42.92±0.19 to 43.08±0.20 mg L^{-1}. For the Cypermethrin and Hamla550 treated tanks, the chloride concentrations ranged from 40.75±0.17 to 42.02±0.19 mg L^{-1} and from 41.52±0.12 to 42.10±0.12 mg L^{-1}, respectively. Overall, lower chloride concentrations were observed across all cases, though slightly elevated levels were noted with higher pesticide concentrations.

DISCUSSION

The present study assessed evaluated the acute toxicity of Chlorpyrifos on *Channa punctata*, determining a 96 hr LC_{50} value of 1.3836 mg L^{-1}. This finding highlights the species' sensitivity to Chlorpyrifos exposure *C. punctata* exhibits moderate susceptibility to this organophosphate insecticide compared to previous investigations on other fish species. For instance, *Oncorhynchus mykiss* (rainbow trout) and *Oreochromis mossambicus* demonstrated significantly higher sensitivity, with LC_{50} values of 24 µg L^{-1} (Mayer and Ellersieck 1986) and 25.7 µg L^{-1} (Rao et al. 2003), respectively. Similarly, *Cyprinus carpio* exhibited relatively low sensitivity, with a 96 hr LC_{50} of 58 µg L^{-1} (Xing et al. 2012). These values are considerably lower than that observed for *C. punctata*, indicating that these species are more vulnerable to Chlorpyrifos exposure. This increased sensitivity may be attributed to physiological differences, metabolic detoxification capacity, or behavioural responses. In contrast, the LC_{50} value for *Nile tilapia* (*Oreochromis niloticus*) was reported to be 1.57 mg L^{-1} (Gul 2005), comparable to that of *C. punctata*, suggesting a similar tolerance level. This highlights the interspecific variation in pesticide susceptibility, which genetic, ecological, and physiological factors can influence.

The present study established the 96 hr LC_{50} value of Cypermethrin for *C. punctata* at 0.2841 mg L^{-1}, indicative of a moderate acute toxicity level. When this figure is compared to those reported for other fish species, it becomes clear that *C. punctata* demonstrates a relatively higher tolerance to Cypermethrin than many species previously studied. Bradbury and Coats (1989a, b) provided a thorough review of pyrethroid toxicity, noting significantly lower 96 hr LC_{50} values for several species: 2.2 µg L^{-1} for *Tilapia nilotica*, 0.9-1.1 µg L^{-1} for *Cyprinus carpio*, 1.2 µg L^{-1} for *Salmo trutta* (brown trout), 0.5 µg L^{-1} for *Salmo gairdneri* (rainbow trout), and 0.4 µg L^{-1} for *Scardinius erythropthalmus*. Compared to *C. punctata*, these values are significantly lower, signifying that these species are much more vulnerable to Cypermethrin. This increased susceptibility may be attributed to variations in physiological or metabolic responses, such as reduced detoxifying enzyme activity or more permeable gill structures.

Additional studies further corroborate this interspecific variation. Das et al. (2003) reported a 96 hr LC_{50} of 0.139 mg L^{-1} for *Labeo rohita*, while Ayoola and Ajani (2008) discovered an even lower LC_{50} of 0.063 mg L^{-1} for *Clarias gariepinus*. These species exhibited greater sensitivity than *C. punctata*, underscoring the variability in susceptibility among different species. Conversely, Küçükbay et al. (2009) recorded a significantly higher LC_{50} of 41.786 µg L^{-1} for *Oncorhynchus mykiss*, and Sarkar et al. (2005) found an LC_{50} of 5.13 µg L^{-1} for *Cirrhinus mrigala*, suggesting that particular species may demonstrate higher resistance under specific conditions. In addition, Polat et al. (2002) reported a 48 hr LC_{50} of 21.4 µg L^{-1} for beta-Cypermethrin in male guppies, highlighting the rapid toxic effects of pyrethroids even during short-term exposures.

The variation observed in LC_{50} values across different species highlights the complexity of pesticide toxicodynamics within aquatic ecosystems. Various factors - such as metabolic detoxification capacity, behaviour, life stage, habitat preferences, and environmental conditions (including temperature, pH, and dissolved oxygen) can all influence toxic responses. The greater resistance of *C. punctata* to Cypermethrin might be attributed to reduced absorption rates through the gill epithelium or more efficient enzymatic degradation. Overall, the findings of this study enhance our understanding of species-specific responses to pyrethroid insecticides. While *C. punctata* may exhibit more resilience than several other freshwater fish species, the observed LC_{50} still indicates a level of toxicity that could be harmful in natural environments, especially with prolonged or repeated exposure. These results underscore the necessity for species-specific risk assessments and highlight the importance of regulating pesticide use to minimize adverse ecological impacts.

This study evaluated the acute toxicity of the hybrid pesticide Hamla550 compared to its active components, Cypermethrin and Chlorpyrifos, on the freshwater fish *C. punctata*. The results showed that Hamla550 exhibited significantly higher toxicity, evidenced by its 96 hr LC_{50} value of 0.0010 mg L^{-1}, which is substantially lower than the LC_{50} values for Cypermethrin (0.2841 mg L^{-1}) and Chlorpyrifos (1.3836 mg L^{-1}). These findings suggest that Hamla550 is more toxic to *C. punctata*, even at much lower concentrations.

This pronounced toxicity may be due to the synergistic interaction between Cypermethrin and Chlorpyrifos within the formulation of Hamla550. Cypermethrin, a synthetic pyrethroid, primarily affects the nervous system by delaying the closure of voltage-gated sodium channels. At the same time, Chlorpyrifos, an organophosphate, inhibits acetylcholinesterase activity, accumulating acetylcholine at synapses. The combination of these mechanisms could enhance neurotoxicity.

Furthermore, the formulation components of Hamla550 such as surfactants or emulsifiers may improve the bioavailability of the active ingredients, thereby increasing their toxic effects. The study also indicates that the toxicity of Hamla550 is both concentration and time dependent, consistent with typical patterns of pesticide toxicity observed in aquatic organisms. Even at low concentrations, prolonged exposure could worsen physiological stress and mortality in *C. punctata*, emphasizing the risks of such hybrid formulations in aquatic ecosystems.

The physicochemical parameters of the test water play a crucial role in evaluating the environmental impact and toxicological effects of pesticide exposure on *C. punctata*. To simulate various environmental conditions, the physicochemical properties of the water were systematically altered across multiple experimental tanks during this study. Key parameters were monitored throughout the experiment, including temperature, pH, dissolved oxygen, total hardness, alkalinity, and chloride levels. These modifications aimed to assess their potential effects on the biological and ecological responses under investigation. The observed changes in water quality parameters were consistent with findings reported by Hermann et al. (2023), who documented similar variations in physicochemical properties within controlled aquatic environments. This congruence with previous research enhances the reproducibility and relevance of the experimental design for evaluating the impact of water quality on aquatic systems.

In the present study, water temperature remained relatively stable, ranging from 21.54±0.41 to 22.87±0.15 °C. This narrow temperature variation falls within the optimal thermal range for *C. punctata*, suggesting that temperature did not serve as a confounding factor when interpreting the observed toxicological effects of the insecticides.

The pH levels across the different pesticide treatments exhibited minor yet consistent fluctuations. Regarding Chlorpyrifos exposure, pH values ranged from 6.82±0.12 to 7.10±0.10, indicating slightly basic conditions. In contrast, exposure to Cypermethrin and Hamla550 resulted in pH ranges from slightly acidic to neutral (specifically 6.79±0.10 to 7.10±0.10 and 6.88±0.14 to 7.05±0.13, respectively). Although these variations were subtle, they suggest a potential influence of the chemical composition of the insecticides on water chemistry. A slightly acidic to neutral pH may enhance the bioavailability of specific toxicants, thereby increasing their physiological impact on aquatic organisms.

Dissolved oxygen (DO) levels exhibited a significant decline in correlation with increasing concentrations of pesticides, suggesting either heightened microbial activity due to organic load or a reduction in photosynthetic processes. In tanks exposed to Chlorpyrifos, a noticeable decrease in DO was recorded after 72 hr, while the most substantial reduction was noted following a 96 hr exposure to Cypermethrin and Hamla550. Lower DO concentrations can hinder respiratory efficiency in fish, and chronic exposure to sub-optimal DO levels may lead to physiological stress and behavioural changes in aquatic species.

Total alkalinity remained relatively stable, with minor variations observed across the treatments. In Chlorpyrifos-treated tanks, alkalinity levels ranged from 159.65±0.41 to 161.18±0.61 mg L^{-1}, indicating a buffering capacity sufficient to moderate pH fluctuations. The lowest alkalinity values were found in the tanks treated with Hamla550, suggesting a depletion of buffering agents or chemical interactions that consumed bicarbonate ions. In contrast, tanks exposed to Cypermethrin displayed the highest alkalinity values, which may reflect the presence of basic compounds or reduced utilization of carbonates.

Water hardness, which indicates the concentration of calcium and magnesium, remained consistently within the "hard" range across all treatments, ranging from 100.36±0.18 to 108.70±0.59 mg L^{-1}. According to Kannan (1991), water hardness values between 0 and 60 mg L^{-1} are classified as "soft." Such conditions are typically less sensitive to fluctuations in pH and may decrease the bioavailability of certain heavy metals and pesticides potentially modulating their toxicity. However, the stable hardness values observed across the tanks suggest that this parameter was relatively constant and unlikely to affect pesticide efficacy or toxicity.

Significantly, chloride concentrations were generally low, varying narrowly from 42.92±0.19 to 43.08±0.20 mg L^{-1} for Chlorpyrifos, from 40.75±0.17 to 42.02±0.19 mg L^{-1} for Cypermethrin, and at 42.10±0.12 mg L^{-1} for Hamla550. Notably, slightly elevated levels were observed at higher pesticide concentrations, particularly Chlorpyrifos. This marginal increase in chloride content could be attributed to ionic release during pesticide degradation or slight shifts in ionic balance due to pesticide interactions. Even elevated chloride concentrations, within the sublethal range, can affect osmoregulatory functions in fish, contributing to cumulative stress.

Overall, although the physicochemical parameters of the test water remained within acceptable limits for aquatic life, minor yet consistent changes linked to pesticide exposure indicate potential stress within the aquatic ecosystem. These alterations, particularly in dissolved oxygen (DO), pH, and alkalinity, emphasize the necessity for ongoing monitoring when evaluating pesticide toxicity and underscore the importance of interpreting the biological effects environmental variables.

Aquatic ecosystems are negatively affected by the persistently hazardous conditions created by these pesticides, which contaminate aquatic environments. Due to their hybrid chemical nature, these pesticides are highly toxic to aquatic biota, especially fish, posing a serious threat to food webs in these ecosystems. Therefore, evaluating the potentially dangerous effects of pesticide residues in aquatic environments is crucial. Additionally, the pesticides used on farms may harm human health while eliminating target organisms and non-target species. This approach could help assess the potential health risks pesticide exposure poses to non-target animals. From an ecological perspective, the elevated toxicity at minimal concentrations is particularly concerning. *C. punctata* is a common freshwater species that plays vital role in maintaining the balance of aquatic ecosystems and holds economic value in many regions. Using such potent formulations may lead to the mortality of non-target species, reduced biodiversity, and disruption of aquatic food webs. Furthermore, persistent exposure could impair fish behaviour, reproduction, and overall population health. Even at sub-lethal levels. These results emphasize the urgent need for stringent regulatory oversight and environmental risk assessments of mixed or hybrid pesticide formulations. While these compounds may provide agricultural benefits in pest control efficiency, their ecotoxicological implications warrant caution, especially in areas adjacent to freshwater bodies. Adopting safer pest management strategies and routinely monitoring pesticide residues in aquatic habitats, is crucial for mitigating long-term ecological damage.

From an ecotoxicological standpoint, the high toxicity of Hamla550, compared to its constituent chemicals, raises serious concerns regarding its use, particularly in regions where *C. punctata* is ecologically or economically important. The findings highlight the need for comprehensive risk assessments of combined pesticide formulations, as their toxicological profiles can vary significantly from those of their components. These findings underscore the importance of species-specific toxicological assessments in environmental risk evaluation.

CONCLUSIONS

The results of this study indicated that Hamla550 is significantly more toxic to *Channa punctata* fingerlings than Cypermethrin or Chlorpyrifos. Unlike Cypermethrin and Chlorpyrifos, Hamla550 appears to have greater absorption and toxicity in aquatic creatures. The increased toxicity indicates a synergistic interaction between the two active ingredients, which produces amplified neurotoxic effects on aquatic organisms when combined in a single formulation. Given the widespread use of these pesticides in agricultural practices and their potential runoff into aquatic ecosystems, understanding the differential toxic responses among fish species is essential for developing protective regulatory standards and effective mitigation strategies. Furthermore, *C. punctata* may serve as a suitable bio-indicator species for monitoring organophosphate pesticide contamination in freshwater environments, particularly in where it is ecologically or economically significant.

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