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RESEARCH ARTICLE

IMPACT OF CHLORPYRIFOS ON CERTAIN HEAMATOLOGICAL PARAMETERS IN FRESH WATER FISH ANABAS TESTUDINEUS (BLOCH.)

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ABSTRACT

Fishes are very important food resources in developing countries like India. But due to indiscriminate use of the chemicals in the agriculture lands and other fouters the whole aquaculture ecosystem is adversely affect including fishes. The present study intended to assess the toxicity of Chlorpyrifos (20% EC), a popular organophosphate pesticide commonly used in agriculture fields, in a freshwater air-breathing fish *Anabas testudineus* in laboratory conditions. The fishes were exposed to different concentration of Chlorpyrifos and LC50 (15.5 ppm) was evaluated. Experimental fish have been exposed to several sublethal concentrations (1/10 and 1/50th of the LC50 value) for 15th, 30th, and 45th days, respectively, to assess certain haematological parameters (RBC and WBC count, Hb percentage, and PCV value). The impact was examined by comparing data between control and experimental groups of fish. The RBC and WBC count, Hb percentage and PCV value were found significantly decrease ($P < 0.05$) in compare to control group. Some behavioural changes, such as restlessness, irregular movement, hyperactivity, and so on, were observed in fish exposed to Chlorpyrifos. This was the result of stress produced by an abrupt shift in environment or the inactivation of a specific enzyme (acetylcholinesterase) activity. Hence it is recommended to use the pesticide at the lowest possible concentration to control and also to preserve the land and aquatic ecosystem for sustainable development.

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INTRODUCTION

The fishes are very important food resources in developing countries due to high protein content and its nutrition value. Fish diversity is one of the most important aquatic lives supporting system on the earth and also of much economic and scientific value (Dudgeon *et al*., 2006). Bihar is the state with diverse water resources supporting fish and fisheries as well (Raut *et al*., 2020). But due to serve anthropogenic activities, indiscriminate use of chemical in the agriculture field and climate change the aquatic organism are running under pitiable condition. Fish populations are diminishing due to a variety of factors, including unexpected temperature changes, water reduction, unfavourable pH, dissolved oxygen, dissolved solids, insecticidal toxicity, and biological agents including parasites, bacteria, viruses, fungi, etc (Siddiqa *et al*., 2016). Using pesticides is one of the major factors contributing to the decline in fish populations among the other causes. Some of the most frequently used insecticides in the world are organophosphates. They are utilized in veterinary clinics, homes, gardening, and agriculture. They generally don't last long in the environment because they degrade quickly. They have served as an effective substitute for the more persistent organochlorine because of their comparatively quick rate of degradation (Frederick, 2005). Numerous studies have demonstrated that fish are sensitive to low concentrations of chlorpyrifos. It has also been reported that fish exhibit behavioral (Levin *et al*., 2004), morphological (Sledge *et al*., 2011), biochemical (Kadam and Patil, 2016), and histopathological (Velmurugan *et al*., 2014) changes as a result of chlorpyrifos exposure. Chlorpyrifos (0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is a broad-spectrum organophosphate insecticide and it is the second largest selling OP (Organophosphate) and found to be more toxic to fish than organochlorine compounds (Tilak *et al*., 2001). It is used for the control of mosquitos, flies, various crop pests in soil and on foliage, household pests and aquatic larvae and may be used in some countries as an aquatic larvicide for the control of mosquito larvae (WHO, 2003). Some authors (Reddy and Bashamohideen, 1989; Chauhan *et al*., 1994; Agarwal and Chaturvedi, 1995) have reported a decrease in haematocrit, haemoglobin and red blood cells values of some fish after their exposure to insecticides. The present investigation was conducted to evaluate the toxicity of chlorpyrifos (20%EC) on a freshwater an air breathing fish *Anabas testudineus*. Blood parameters are intensively used as biological indicators to determine the health status of the fish (Lermen *et al*., 2004). So, haematological parameters were taken into consideration while doing this investigation.

MATERIALS AND METHODS

The experiment was carried out in the University Department of Zoology, T.M.B.U. Bhagalpur, Bihar. Fresh water fish *Anabas testudineus*were collected from local fish market of Nathnagar & Bhagalpur (Bihar) and bought to the Departmental laboratory without any physical injury. The average length of fish was 11.51 ± 1.298 cm. Fishes were acclimatized for about three weeks prior to experiments and screened for any pathogenic infections. The fishes were maintained in glass aquaria of volume (60×30×30) cm^3 (Choudhary and Naaz, 2025). For the purpose of preventing fungal contamination, glass aquaria were washed with 1% $KMNO_4$ solution (Anupama & Amit, 2023) to remove dermal infections if any. Healthy fishes were transferred to glass aquaria containing sufficient volume of water (20L). They were regularly fed with commercial fish food.

EXPERIMENTAL CHEMICAL: The commercial grade pesticide Chlorpyrifos (20% EC) manufactured by INDIA Pesticide ltd. was selected for this investigation to observe its effect on selected fishes. This chemical was taken into an experimental flask and required amount of distill water was added in order to prepare the desired concentration.

TOXICITY TEST-(96 hours of LC50): The toxicity range finding experiment was conducted to determine the Chlorpyrifos concentration that may kill 50% population of *Anabas testudineus* fish in 96 hours. The commercial grade Chlorpyrifos (20% EC, manufactured by India pesticide Ltd.) was diluted 1000 times with the distilled water to prepare the stock solution, which is equivalent to 1000ppm. After that, the stock solution was used accordingly to complete the experiment.

The experiment was performed in the glass aquaria, containing 20L of tap water with dual simultaneous replicates of 8 treatments containing 11, 12, 13, 14, 15, 16, 17, 16, 18 ppm of Chlorpyrifos. 10 healthy were fishes randomly removed from the glass aquarium and added to the experimental tank. Mortality was recorded after 24, 48, 72, 96 hours of exposure. Dead fishes were removed from the tank. Table-1 showing concentration of pesticide in ppm (parts per million) taken for the determination of LC50 and fishes (in number and in percentage) died at different interval of time at different concentration. LC50 is calculated as 15.5ppm. In the present study LC50 value of for fish *Anabas testudineus* were calculated for 96 hours of exposure time by Probit Analysis. Probit analysis is specialized regression model of binomial response variable. Regression is a technique for analyzing data by fitting line to it in order to compare variable, also known as the dependent variable (Y) and the independent variable (X).

$$Y = a + bx + e$$

Where,

a = y- intercept
b = the slope of the line
e = error term

ANIMAL & EXPERIMENTAL DESIGN: The fishes were separated into three groups Ⅰ, Ⅱ and Ⅲ, and was kept in different glass aquarium. To guarantee outcome, the current study was repeated three times. Ten fishes were used per replicates for each group. To act as a control Group Ⅰ was kept in the water without pesticides. Group Ⅱ & group Ⅲ were exposed to the sub lethal doses of Chlorpyrifos 1.55 and 0.31 ppm respectively ($1/10^{th}$ and $1/50^{th}$ of LC50 value).

METHODOLOGY FOR HAEMATOLOGICAL PARAMETERS

- Blood was collected directly by heart exposing by dissection and puncture it with syringe needle.
- RBC (Red blood count) and WBC (White blood count) count was determined by standard method using Neubauer haemocytometer. (Shrivastava and Das, 2010).
- Haemoglobin (Hb) concentration was evaluated by using Sahli’s haemometer. (Shrivastava and Das, 2010).
- PCV (Packed cell volume) value was evaluated by using standard method of Shrivastava and Das, 2010.
- Statical Analysis was done by Student’s T- test, ANOVA and POST- HOC TEST.

RESULTS

(TOXICITY STUDIES): Acute toxicity is typically used to examine how sensitive various species are to various chemical potencies using LC50 values. The mortality rate of fish increased by high pesticide concentration. The table displayed lethal concentrations determined by Probit Analysis.

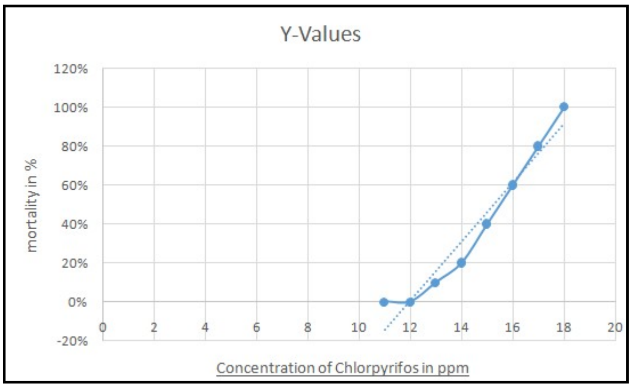

Figure 1. Showing Y- Value between pesticide (Chlorpyrifos) concentration and % mortality of fishes

BEHAVIORAL RESPOSNSE DURING TOXICITY ASSESEMENT: Over the course of 96 hours, the experimental fishes showed alterations in behaviour. Over the course of the trial, the control group exhibited normal behaviour, and at low concentrations (11 ppm), normal reactions were recorded. A considerable increase in hyperactivity was seen in terms of surfacing, scraping, and schooling moments following a 24-hour exposure to chlorpyrifos as compared to the control group. There was an increase in grasping and settling at the test chamber's bottom after 72 hours of exposure, along with a decrease in surfacing and jerking movements. Fishes in the control group showed typical pigment loss after 96 hours of exposure, whereas fish at higher dosages developed a light greyish-black colouring.

BEHAVIOURAL RESPONSES DURING SUB LETHAL DOSING: During a 45-day period of sub lethal dosages of 1/10 and 1/50 of LC50, the fish *Anabas testudineus* exhibits few behavioural changes. Normal behaviour was seen in the control group's fish. There had been no change in the behaviour of the fish in the experimental group at the start of the investigation (15th day). However, by the time the investigation was over (30 to 45 days), certain behavioural changes had started to show up in the experimental fishes, such as increased grasping, hyperactivity, and settling at the bottom of the tank. No fish death has been noted during the experiment. **Note**-Table-2,3 showing the increase or decrease in the level of behavioural parameters is shown by numbers of (+) sign. –, none (0%); +, mild (<10%); ++, moderate (10 to 50%); +++, severe (> 50%). The (-) sign indicates normal behavioural conditions.

IMPACT ON RBC (Red blood cell): Fish treated with Chlorpyrifos (20% EC) showed a significant decline in the number of RBC. It was observed that as the duration of treatment increased, the number of RBC showed a decline trend. At the 15th, 30th and 40th day of treatment with the dose 0.31ppm, the mean of the RBC count was 1.66±0.0527, 1.616±0.066, 1.51±0.07 ($10^6 \mu L^{-1}$) respectively ($p<0.05$) in compare to control of mean around 2.92±0.3002 at 45th day. Similarly with the dose 1.55ppm, at the 15th, 30th, 40th day of treatment, the mean was observed 1.4133±0.0185, 1.356±0.038, 1.2066±0.0033 ($10^6 \mu L^{-1}$) respectively ($p<0.05$) in contrast to control group. A significant decrease of number of RBC was observed in the fishes treated with chlorpyrifos for the duration of 45 day of treatment. It has been observed that RBC count decreases with increase in concentration of Chlorpyrifos on 15th, 30th, 45th days.

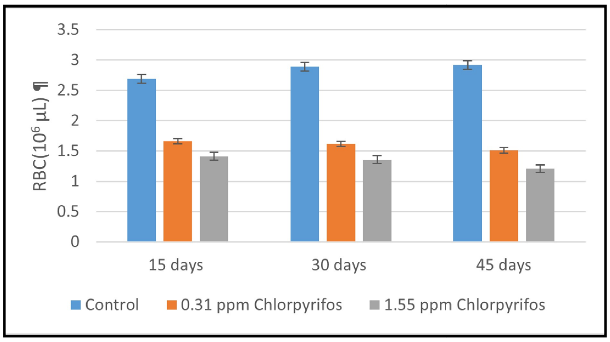

Figure 2. Showing the impact of Chlorpyrifos on RBC of *Anabas testudineus* after 15th, 30th and 45th days of exposure. Data is expressed as mean ± SEM (N=3), p < 0.05. (Based on T-test)

RESULTS

IMPACT ON WBC (White blood cell): Fishes treated with chlorpyrifos (20% EC) showed a significant decline in the number of WBC count. At the beginning of the experiment, it was observed that as the dosing days of experimental group fishes were increasing, the number of WBC was also increasing in compare to control group. But after 15thday it was notice that, as the dosing day increased, the WBC count started decreasing with respect to control group. At the 15th, 30th and 40th day of treatment with the dose 0.31ppm, the mean of the WBC count was 281± 6.35, 279±2.309, 282±2.309 ($10^3 \mu L^{-1}$) respectively ($p<0.05$) in compare to control of mean around 279.66±6.5773 at 45th day. Similarly with the dose 1.55ppm, at the 15th, 30th, 40th day of treatment, the mean was observed 282±1.154, 278±4.618, 277±2.962 ($10^3 \mu L^{-1}$) respectively ($p<0.05$) in compare to control group. The fish treated with chlorpyrifos showed a notable reduction in WBC over the course of the 45-days treatment period.

(White Blood Cell)

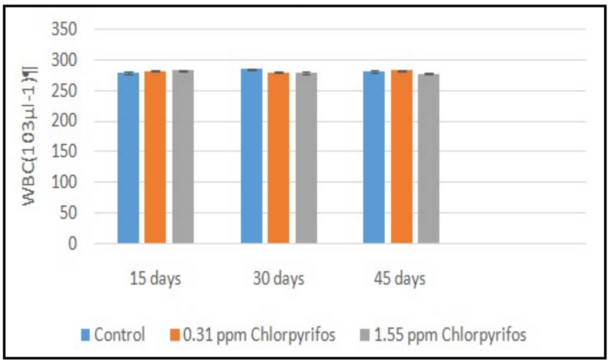

Figure 3. Showing the impact of Chlorpyrifos on WBC of *Anabas testudineus* after 15th, 30th and 45th days of exposure. Data is expressed as mean ± SEM (N=10), p < 0.05. (Based on T-test)

IMPACT ON HEAMOGLOBIN (HB%): Chlorpyrifos treatment showed some significant effect on the gram percentage hemoglobin of *Anabas testudineus* fish blood. In the experiment, it was found that as the number of days of Chlorpyrifos dosage was increased, the hemoglobin level decreased in the experimental fishes in compare to control group. At the 15th, 30th and 40th day of treatment with the dose 0.31ppm, the mean of the Hb% was 9.2±1.1732, 8.9±0.173, 8.8±0.2886 ($g\ dl^{-1}$) respectively ($p<0.05$) in compare to control of mean around 11.68±0.969 at 45th day. Similarly with the dose 1.55ppm, at the 15th, 30th, 40th day of treatment, the mean was observed 8.4±0.2886, 8.1±0.173, 7.9±0.173 ($g\ dl^{-1}$) respectively ($p<0.05$) in compare to control group. The Hb% of the fish treated with chlorpyrifos decreased significantly over the 45-day treatment period.

(Haemoglobin)

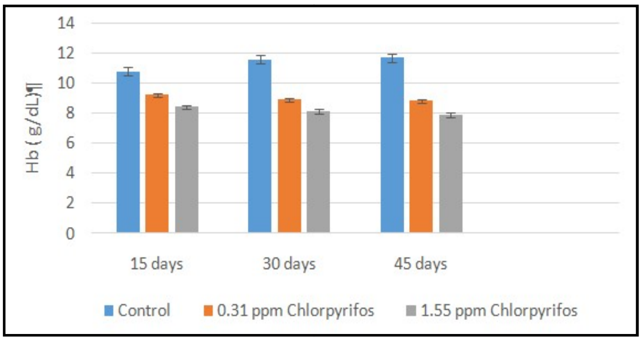

Figure 4. Showing the impact of Chlorpyrifos on HB level of *Anabas testudineus* after 15th, 30th and 45th days of exposure Data is expressed as mean ± SEM (N=10) p < 0.05 (Based on T-test)

IMPACT ON PACT CELL VOLUME (PCV): The effect of chlorpyrifos was also observed on the pact cell volume of fish *Anabas testudineus.* There was a very significant change in fish pact cell volume due to the dosage of chlorpyrifos. It was observed that as the dosage days of chlorpyrifos increased, the percentage of packed cell volume decreased in the experimental group fishes when compare to control group. At the 15th, 30th and 40th day of treatment with the dose 0.31ppm, the mean of the PCT percentage was 39±0.866, 37±1.732, 32±2.3094% respectively ($p<0.05$) in compare to control of mean around 47.74±0.0577 at 45th day. Similarly with the dose

Table 1. Probit analysis on the effect of Chlorpyrifos to *Anabas testudineus* at 96 hours of exposure

No. of fishes	Conc. In ppm	Log Conc.	Fish died (24 h)	Fish died (48h)	Fish died (72h)	Fish dead (96h)	Total fish dead	% Kill	Probit value	LC 50
10	11	1.041392685	0	0	0	0	0	0%	0	15.5ppm
10	12	1.079181246	0	0	0	0	0	0%	0	
10	13	1.113943352	0	0	0	1	1	10%	3.72	
10	14	1.146128036	0	0	1	1	2	20%	4.16	
10	15	1.176091259	1	2	0	2	5	40%	4.75	
10	16	1.204119983	3	2	0	1	6	60%	5.25	
10	17	1.230448921	2	1	3	2	8	80%	5.84	
10	18	1.255272505	3	2	1	4	10	100%	7.33	

Table 2. Impact of Chlorpyrifos on the behavioural pattern of *Anabas testudineus* (average length 18 ± 2 cm and weight 48 ± 2 g, n=10) at different concentration of pesticide after 96 hrs of exposure

Parameters	24hrs	48hrs	72hrs	96hrs
Skin color	+++	+++	+++	+++
Loss of Balance	+	++	++	+++
Surface activity	+	++	+	____
Rate of swimming	+++	++	+	____
Hyperactivity	+	++	+++	____
Convulsion	+++	++	++	____
Opercula activity rate	+	++	+++	____

Table 3. Impact of Chlorpyrifos on the behavioural pattern of *Anabas testudineus* (average length 18 ± 2 cm and weight 48 ± 2 g, n=10) at different sub lethal concentration of pesticide during 15, 30 & 45 days of exposure

Parameters	Control	0.31ppm	1.55ppm
Skin color		+++	+++
Loss of Balance	___	++	++
Surface activity	___	++	+
Rate of swimming	___	+++	+++
Hyperactivity	___	++	+
Convulsion	___	++	++
Opercula activity rate	___	+++	++

Table 3. Haematological values of *Anabas testudineus* exposed to sub lethal concentrations of chlorpyrifos. Data is expressed as Mean ± SEM, p<0.05 (*=significant), p<0.01 (= Highly significant), p<0.001 (***= Extremely significant)**

Hematological Parameters	Chlorpyrifos Concentration (in ppm)	15^{th} Day	30^{th} Day	45^{th} Day
RBC ($10^6\mu L^{-1}$)	**Control**	2.69±0.3505	2.89±0.222	2.92±0.3002
	0.31ppm	1.66±0.0527*	1.616±0.066*	1.51±0.07*
	1.55ppm	1.4133±0.0185*	1.356±0.038*	1.206±0.0033**
WBC ($10^3\mu L^{-1}$)	**Control**	278±1.527	284.33±0.769	279.66±6.5773
	0.31ppm	281± 6.351	279±2.309	282±2.309
	1.55ppm	282±1.154	278±4.618	277±2.962
HB (g dl^{-1})	**Control**	10.76±0.023	11.56±0.323	11.68±0.969
	0.31ppm	9.2±1.1732***	8.9±0.173**	8.8±0.2886*
	1.55ppm	8.4±0.2886***	8.1±0.173***	7.9±0.173**
PCV (%)	**Control**	44.81±0.1732	46.83±0.115	47.74±0.0577
	0.31ppm	39±0.866**	37±1.732**	32±2.3094***
	1.55ppm	29±0.2886***	28±0.4409***	24±1.1547***

1.55ppm, at the 15^{th}, 30^{th}, 40^{th} day of treatment, the mean was observed 29±0.2886, 28±0.4409, 24±1.1547 % respectively (p<0.05) in compare to control group. The percentage PCV of the fish treated with chlorpyrifos decreased significantly over the 45-day treatment period.

(Packed cell volume)

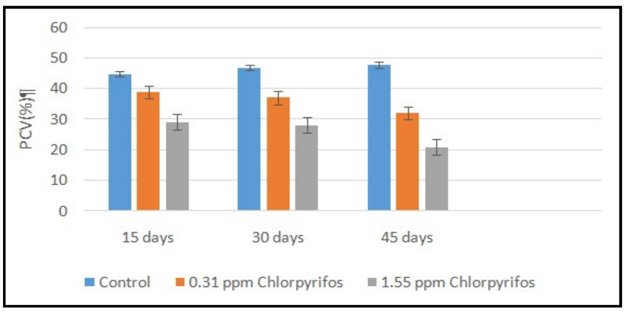

Figure 5. Showing the impact of Chlorpyrifos on PCV% of *Anabas testudineus* after 15th, 30th and 45th days of exposure Data is expressed as mean ± SEM (N=10) p < 0.05 (Based on T-test)

DISCUSSION

Haematological change: The haematological parameters of *Anabas testudines* treated with sublethal doses of Chlorpyrifos on days 15, 30, and 45 showed a significant change in blood parameters as compared to the control group. Haematological factors usually make an impact on the health of any organism (Baker *et al*., 2001; Satheeshkumar *et al.* 2011). Some kind of behavioural change was observed in the fishes treated with sublethal dosages. Fishes exhibited more behavioural changes throughout the toxicity assessment test. The result found in the present investigation showed decrease in RBC count, HB%, PCV levels. These results align with the findings of earlier research examining the haematological responses of several fish species subjected to different organophosphate insecticides. Reports of comparable declines in Hb levels and RBC counts following diazinon exposure have also been made in *Cyprinus carpio, Silurusglanis*, *Clarias gariepinus* and *Cirrhinusmrigala* (Svobodam*et al.,* 2001; Koprucu*et al.*, 2016;

Adedeji *et al*., 2009; Haider and Rauf, 2014) methyl parathion in *Mystuskeletius* (Sampath *et al*., 2003) monocrotophos in *Clarias gariepinus* (Yagi and Auta, 2007), *Cyprinus carpio* exposed to Chlorpyrifos (Ramesh and Saravanan, 2008). Lower Hb level and decrease PCV in fishes was also reported in cypermethrin exposure that may be due to decrease RBC count (Paul, 2004; Chaudhary & Naaz, 2025). Due to a shortage of Hb in the cellular medium, the development of hypoxic conditions during treatment caused a reduction in RBC production, which in turn caused decrease in the rate of RBC synthesis (Chen *et al*., 2004). Changes in the haematological parameters might have been brought about by chlorpyrifos as an anaemic condition due to decrease synthesis of haemoglobin and RBC numbers in hemopoietic organs and increase in the rate at which haemoglobin is destroyed. (Masud & Singh, 2013; Raibeemol & Chitra, 2018).

Fishes treated with chlorpyrifos (20% EC) showed the significant decline in the number of WBC count. At the beginning of the exposure, it was noticed that the WBC count was slightly increased but after 15 days the WBC count suddenly dropped in comparison to the fishes of control group. Increase in WBC count during early period of treatment may be due to some pathological response because WBC play a great role during infection by stimulating the hematopoietic tissue and the immune system by producing antibodies working as defence against any infection (Lebelo *et al*., 2001; Hassen, 2002; Masud & Singh, 2013; Haque *et al.,*2021).

Behavioural change: The behavioural changes are associated with the physiological reactions that can indicate stress (Little & Finger, 1990). Fish that are exposed to a variety of pesticides demonstrate behavioural modifications, including predation, competition, swimming, feeding, and species aggressiveness (Cong *et al.,* 2008 & 2009). Behavioural aberrations resulting from stress are increasingly recognized as the most sensitive indicators of deleterious effects in aquatic environments (Nwani *et al*., 2010).

Hassanein & Okail (2008) had also found loss of buoyancy and balance with an initial increase in the opercular ventilation rate which then decreased significantly in *Ctenopharyngodon idella* (grass carp) after the exposure of pesticide. According to Fulton & Key (2001), the restlessness and hyperactivity in fish may occur due to the stress or inactivation of acetylcholinesterase, enzyme leading to accumulation of acetylcholine at synaptic junctions. Eventually, fishes were almost paralyzed and settled on the bottom of the tank and died (Choudhary & Azad, 2024).

CONCLUSION

The death rate and toxicity level were both high at high pesticide concentrations. The findings showed that chlorpyrifos produced a wide range of behavioural and morphological changes in fishes, which might lead to serious physiological issues and eventually fish mortality. Despite employing dosages that were lower than those advised for managing the bug in the field, all fish species utilized in these studies suffered greatly, and their fatality rates were elevated. We should aim to apply the lowest concentration of pesticide possible to control insects and preserve the land and aquatic environments for sustainable development.

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Authors contribution: This work was carried out in collaboration between both authors. First Author conducted the whole experiment and analysed the data. Second Author prepared the manuscript.

Ethical approval: Animal ethic committee approval has been collected and preserved by author(s).

Disclaimer (Artificial Intelligence): Author(s) here by declare that NO generative AI technologies such as large language models (Chat GPT, COPILOT etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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