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TOXICITY BIOASSAY OF CHLORPYRIFOS ON SOME LOCAL FISH SPECIES OF NORTHERN BANGLADESH

M.N. Zahan[1], M.J. Islam[1*], T. Mahajebin[2], M.S. Rahman[1] and A.K.M.M Hossain[3]

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Abstract

Four fish species namely, stinging catfish (*Heteropneustes fossilis),* spotted snakehead (*Channa punctatus*), climbing perch (*Anabas testudineus*) and tangra (*Batasio tengana*) were exposed to various concentrations of chlorpyrifos (Expert 20 EC) to investigate the mortality rate of fish species and the toxicity level of the pesticide. The LC_{50} values of chlorpyrifos on these fish species were 23.10, 20.32, 16.61 and 13.94 ppm, respectively at 96 hours of exposure. Among these results, it is clear that the lethal concentration is varying species to species because their strengths are different. During the experiments, some water quality parameters such as temperature, pH, dissolved oxygen, total dissolved solids and electrical conductivity were also analyzed. The temperature values remained within the ranges of 17-20°C and the pH was 6.64-7.30. Fish behaved irregular and erratic movements followed by hyper excitability, loss of balance and settling to the bottom of the test chamber. At higher concentration of chlorpyrifos, alterations in physiological and behavioural responses especially erratic swimming, gulping, mucus secretion, increased opercular movement and profuse emission of mucus all above the body were observed during the primary stages of contact after which it became occasional.

Keywords: Toxicity, Chlorpyrifos, Fish Species, Water Quality

[1]Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.
[2]Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.
[3]Department of Soil Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.

*Corresponding author's email: jahidul@hstu.ac.bd (M.J. Islam)

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Introduction

Application of organophosphorus insecticides in crop field has a great impact on aquatic system especially on fish population. In spite of terrible impact of pesticide on aquatic environment, it is vastly used in agricultural sector (Kadam and Patil, 2016). These pesticides are mixed with aquatic ecosystem by different ways such as rainfall, overflow of water bodies, drainage system *etc*. (Siddiqa *et al*., 2016). The pesticides enter the food chain and their subsequent bioaccumulation and biotransformation at different trophic levels have disastrous effect to the ecosystem (Grande *et al*., 1994).

Fish is very much important protein source and straightforwardly digestible food for human being. It plays a vital role to fulfill our nutritional demand and increase our total production (Pawarand Sonawane, 2014). However, now-a-days due to different natural and anthropogenic activities fishes are declined. Unexpected temperature variation, water reduction, unfavorable pH, dissolved oxygen, dissolved solids, insecticidal toxicity and biological agents like parasites, bacteria, virus, fungus *etc*. are the main causes of declining the fish population (Siddiqa *et al*., 2016). Among the different causes of fish decrease, use of pesticide is one of the important issues. Many authors studied the impact of organophosphorous insecticides on different fish species such as malathion on freshwater fish *Labeo rohita* (Thenmozhi *et al*., 2011), *Barbodes gonionotus* (Hoque *et al.,* 2000), *Cyprinus carpio* (Sharmin *et al*., 2014), chlorpyrifos and carbandazim on *Chanos chanos* (Palanikumar *et al*., 2014), and chlorpyrifos on *Channa gachua* (Kadam and Patil, 2016).

Chlorpyrifos, a widely used organophosphorus insecticide, enters into the aquatic ecosystem and affects aquatic organism (Chernyak *et al*., 1996; Livingstone, 2001). Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system as well as a skin and eye irritant of fish (Cox, 1994; 1995). It is most widely used in rice field in Bangladesh. In addition, the use of this insecticide can cause the decline of fish population. Freshwater fishes such as *H. fossilis* (stinging catfish), *C. punctatus* (spotted snakehead), *A. testudineus* (climbing perch), and *B. tengana* (tangra) are very much nutritional and popular in the study area. In order to assess the toxicity level of this insecticide, we have attempted to detect the behavioral patterns and mortality rate of the above-mentioned fish species as well as water quality parameters in laboratory experiment.

Materials and Methods

The experiments were conducted in the laboratory of the Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Freshwater fishes such as *H. fossilis*, *C. punctatus*, *A. testudineus*, and *B. tengana* were collected from the local market of Dinajpur, Bangladesh and brought to the laboratory. The average lengths of four fishes were 14.01±1.03cm, 12.88±0.98cm, 9.88±0.22cm, and 9.01±0.44cm, respectively. The freshwater healthy fishes were selected for the experiment. Fishes were screened for any pathogenic infections. Glass aquaria were washed with 1% $KMnO_4$ to avoid fungal contamination and then sun dried. Healthy fishes were then transferred to glass aquaria (35×20×20cm) containing dechlorinated tap water. Fishes were acclimated to laboratory conditions for 10 to 15 days prior to experimentation. They were regularly fed with commercial food *ad libitum* and the medium (tap water) was changed daily to remove faces and food remnants.

Organophosphate pesticide chlorpyrifos (Expert 20 EC) was collected from the agro chemist shop from Dinajpur, Bangladesh. The chemical was then taken into glass jar and requisite quantity of distilled water was added in order to prepare the desired concentration. Doses were 10, 20, 30, 40 and 50 ppm including a control with three replications. After 6, 12, 24, 48, and 96 hours, the external activities and the mortality rates of fishes were recorded and the dead fishes were removed.

Some water quality parameters viz. temperature, dissolved oxygen, electrical conductivity, total dissolved solids (TDS) and pH of the trial medium were recorded each day. A thermometer, DO meter (Model: DO-5509, TAIWAN) and pH meter (Model YSI 58, USA) were used for measurement of temperature, dissolved oxygen and pH, respectively. The TDS was determined using the method given by APHA (2005). The conductivity meter (Model 470 cond. meter, UK) was used for measurement of EC values of water samples.

In the present study, LC50 values for fish species were calculated for 96 hours of exposure time by probit analysis of the computer program SPSS. Probit analysis is a specialized regression model of binomial response variables. Regression is a method of fitting a line to the data to compare the relationship of the response variable or dependent variable (Y) to the independent variable (X).

$$Y = a + bX + e$$

Where,

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

Results and Discussion

Toxicity studies

Fish is very much sensitive to toxic substances (Misha and Verma, 2016). Acute toxicity studies are generally employed to compare the sensitivities of different species to different potencies of the chemicals using LC_{50} values. High concentrations of insecticide increase the mortality rates of fishes. Lethal concentrations estimated by probit analysis were shown in Table 1–4.

Table 1. Probit analysis on the effect of chlorpyrifos to stinging catfish at 96 hours of exposure.

Conc. ppm	Log conc.	No. of fishes	No. of dead fishes	% kill	Probit value	LC_{50} (ppm)	95% confidence limit	
							Lower (ppm)	Upper (ppm)
0	-	10	0	0	-	23.1	17.46	28.74
10	1	10	1	10	3.72			
20	1.3	10	3	30	4.48			
30	1.48	10	6	60	5.25			
40	1.6	10	9	90	6.28			
50	1.7	10	10	100	7.33			

Intercept (a) = -6.389, Regression co-efficient (b) = 4.685, Heterogenicity (χ^2) = 2.248 (Not significant), Probit = N.E.D. increased by 5, N.E.D = Normal equivalent deviate.

Table 2. Probit analysis on the effect of chlorpyrifos to spotted snakehead at 96 hours of exposure.

Conc. ppm	Log conc.	No. of fishes	No. of dead fishes	% kill	Probit value	LC_{50} (ppm)	95% confidence limit	
							Lower (ppm)	Upper (ppm)
0	-	10	0	0	-	20.3	14.7	25.5
10	1	10	1	10	3.72			
20	1.3	10	5	50	5.00			
30	1.48	10	7	70	5.52			
40	1.6	10	9	90	6.28			
50	1.7	10	10	100	7.33			

Intercept (a) = -5.850, Regression co-efficient (b) = 4.473, Heterogeneity (χ^2) = 0.787 (Not significant), Probit = N.E.D. increased by 5, N.E.D = Normal equivalent deviate.

Table 3. Probit analysis on the effect of chlorpyrifos to climbing perch at 96 hours of exposure.

Conc. ppm	Log conc.	No. of fishes	No. of dead fishes	% kill	Probit value	LC_{50} (ppm)	95% confidence limit	
							Lower (ppm)	Upper (ppm)
0	-	10	0	0	-	16.6	11.49	21.12
10	1	10	2	20	4.16			
20	1.3	10	6	60	5.25			
30	1.48	10	8	80	5.84			
40	1.6	10	10	100	7.33			
50	1.7	10	10	100	7.33			

Intercept (a) = -5.463, Regression co-efficient (b) = 4.476, Heterogenicity (χ^2) =1.310 (Not significant), Probit = N.E.D. increased by 5, N.E.D = Normal equivalent deviate.

Table 4.Probit analysis on the effect of chlorpyrifos to tengra at 96 hours of exposure.

Conc. ppm	Log conc.	No. of fishes	No. of dead fishes	% kill	Probit value	LC_{50} (ppm)	95% confidence limit	
							Lower (ppm)	Upper (ppm)
0	-	10	0	0	-	13.9	8.8	18.11
10	1	10	3	30	4.48			
20	1.3	10	7	70	5.52			
30	1.48	10	9	90	6.28			
40	1.6	10	10	100	7.33			
50	1.7	10	10	100	7.33			

Intercept (a) = -4.954, Regression co-efficient (b) = 4.329, Heterogenicity (χ^2) =0.616 (Not significant), Probit = N.E.D increased by 5, N.E.D = Normal equivalent deviate.

High probit values reflect the higher concentrations of insecticides applied in the water. The LC_{50} values at 96 hours of exposure were estimated to be 23.10 ppm, 20.32 ppm, 16.61 ppm and 13.90 ppm for stinging catfish (*H. fossilis*), spotted snakehead (*C. punctatus*), climbing perch (*A. testudineus*), and tangra (*B. tengana*), respectively. At 95% confidence level, the lower and upper limits were 17.46 ppm and 28.74 ppm for *H. fossili*, 11.49 ppm and 21.12 ppm for *A. testudineus*, 14.70 ppm and 25.50 ppm for *C. punctatus,* and 8.80 ppm and 18.11 ppm for *B. tengana,* respectively. The results were recorded as 10% to 90% mortality during the experiment; no mortality was found in control fishes at 24, 48, 72 and 96 hours, respectively. Variation in the LC_{50} depends on the number of biological and physicochemical factors which have been reported by many earlier workers (Srivastav *et al*., 2002; Jaroli and Sharma, 2005; Padmanabha *et al*., 2015). However, the same species may respond differently to same toxicant depending on size, age, sex and condition of test species along with experimental factors. The LC_{50} of chlorpyrifos to freshwater catfish (*M. seenghala*) at 96 hours of exposure was 1.776 ppmwith its lower and upper confidential limits (95%) of 1.583 ppm and 1.977 ppm, respectively (Khare, 2015). Ali *et al*. (2008) reported that the LC_{50} of chlorpyrifos for *C. punctatus* was estimated as 0.811 ppm at 96 hours of exposure.

Behavioral studies

The behavioral changes are linked to physiological responses capable of indicating the stress (Little and Finger, 1990). The behavioral patterns of the fish exposed to different insecticides are the changes in swimming behavior, feeding activities, predation, competition and species aggression (Cong *et al.*, 2008; 2009). Cholinesterase inhibition, altered brain neurotransmitter levels, sensory deprivation and impaired gonadal or thyroid hormone levels are the most commonly observed links of behavioral disruption (Scott and Sloman, 2004).

In this experiment, behavioral changes were observed in four species within 96 hours. The control group shows the normal behavior during the whole experiment and also normal responses was observed at the low concentration (10 ppm). After 24 hours of exposure to chlorpyrifos, a significant increase of hyperactivity was observed in terms of surfacing, scraping and schooling moments compared to control. At 48 hours of exposure, the surfacing as well as scraping moments decreased in fish species, other behaviors such as hyper secretion of mucus, opening mouth for gasping, losing scales, and hyperactivity were increased significantly. After 72 hours of exposure, decreased surfacing and jerky movements and increased grasping movements, and settled down to the bottom of the test chamber were observed. Normal shiny colour and behavior of test fishes were observed in the control groups, while the colour became light grayish-black in an increasing order towards the higher doses at the end of 96 hours exposure time.

Water quality during experiments

Some water quality factors are more likely involved in fish losses such as dissolved oxygen, temperature, and ammonia. Others, such as pH, alkalinity, hardness and clarity affect fish, but usually are not directly toxic. Each water quality parameter interacts with and influences other parameters, sometimes in a complex way. What may be toxic and cause mortalities in one situation can be harmless in another.The water quality parameters such as temperature, dissolved oxygen, pH, total dissolved solid (TDS) and electrical conductivity (EC) of the test media are presented in Table 5.

Table 5. Water quality parameters of the test media (chlorpyrifos) on tangra, stinging catfish, spotted snakehead and climbing perchduring the experimental period.

Fish	Conc.	Temperature	DO	pH	TDS	EC
species	ppm	°C	mg O_2/L		mg/L	µS/cm
Tengra	0	19.60±0.50	7.10±0.10	7.02±0.03	229	326
	10	19.72±0.50	6.90±0.10	7.07±0.03	234	343
	20	19.89±0.46	6.70±0.30	7.11±0.01	234	346
	30	20.10±0.30	6.40±0.40	7.10±0.01	235	350
	40	20.45±0.53	6.10±0.80	7.12±0.02	237	356
	50	21.25±0.26	6.45±0.50	7.14±0.01	238	358
Stinging catfish	0	19.40±0.40	7.40±0.40	6.94±0.13	220	284
	10	19.60±0.30	7.30±0.70	6.93±0.13	222	288
	20	19.70±0.30	6.90±0.80	7.05±0.05	224	290
	30	19.80± 0.20	6.85±0.15	7.16±0.14	226	293
	40	20.10±0.50	6.75±0.25	7.27± 0.23	228	298
	50	20.35±0.25	6.70±0.90	7.30 ±0.30	232	300
Spotted snakehead	0	17.50±0.50	7.40±0.20	6.74±0.24	200	246
	10	17.85±0.75	7.10±0.80	6.64±0.44	202	249
	20	18.20±0.90	7.00±0.60	6.68±0.38	205	252
	30	18.20± 0.98	7.00±0.40	6.73±0.33	209	254
	40	18.25±0.95	6.90±0.40	6.75±0.35	214	257
	50	18.40±0.60	6.65±0.60	6.80±0.30	217	260
Climbing perch	0	17.70±0.50	7.55±0.25	7.07±0.08	217	252
	10	17.80±0.60	7.51±0.15	7.05±0.05	222	251
	20	18.10±0.80	7.45±0.15	7.08±0.03	225	257
	30	18.15±0.35	7.30±0.40	7.08±0.05	228	260
	40	18.20±0.20	7.20±0.50	7.10±0.09	231	262
	50	18.30±0.10	7.17±0.50	7.18±0.03	236	265

The temperature levels ranged from 17–20 °C in these four experiments. The average dissolved oxygen was higher in the lower concentrated media in all experiments. The pH values of the tested media were neutral to slightly alkaline. However, the parameters varied little in different treatments and were in agreement with their requirements. There were relations among temperature, pH, dissolved solids, electrical conductivity and increasing concentration of pesticides. However, an inverse relation was there between dissolved oxygen and increasing concentration of pesticides.

Conclusion

At high concentration of pesticide the mortality rate as well as the toxicity level was high. The observations indicated that chlorpyrifos caused many behavioural and morphological alterations, which may result in severe physiological problems, ultimately leading to the death of fish. In spite of using lower doses than the recommended dose for controlling the insect in the field, all fish species used in these experiments were badly affected and their percentages of mortality were high. For sustainable development, we should try to use the minimal concentration of insecticide that can control the insects as well as can save the aquatic and terrestrial environment.

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