Volume: 2, Issue: 3
Page: 432-442
2018

ISSN 2520-4750 (Online) & ISSN 2521-3040 (Print)

International Journal of Science and Business

Response of Four Local Freshwater Fish Species to The Toxicity of Thiocarbamate Insecticide Cartap (Suntap 50 SP)

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Abstract:

An investigation was conducted at Agricultural Chemistry Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur during November 2016 to April 2017 to study the response of four local freshwater fish species (Stinging catfish, Spotted snakehead, Climbing perch and Tangra) to the toxicity of a neurotoxic insecticide thiocarbamate Cartap (Suntap 50 SP). Results indicated that the limit of probit was increased with the increment of Cartap concentration and time of exposure for all the fish species. The lethal concentration (LC50) of Cartap was 2.94 ppm in Stinging catfish, 2.26 ppm in Spotted snakehead, 3.48 ppm in Climbing perch and 2.69 ppm in Tangra, respectively with the 95% confidence limit. The results reveal that the Cartap was more toxic to Spotted snakehead and less toxic to climbing perch as compared to the other two species. Water quality parameter such as pH, temperature, electrical conductivity and total dissolved solid of the experimental aquatic conditions were increased but the dissolved oxygen of that was reduced with the increment of Cartap concentration and exposure of time which also affected the fish behaviors negatively. Therefore, in field condition, if water level is 3 cm then the total amount of water 312500 litres is needed for 1 hector of land approximately and 800 ppm insecticide is dissolved in this field. Although its concentration is lower than exact dose it will seriously hamper the aquatic organism.

IJSB
Accepted 27 July 2018
Published 30 July 2018
DOI: 10.5281/zenodo.1324445

Keywords: Cartap, LC_{50}, Local fish, Neurotoxic, Toxicity, Water quality.

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Introduction

Nowadays the agricultural sector all over the world faces many challenges owing to ensuring global food security for rapid population growth. Bangladesh is also predominantly an agricultural country with over population and agriculture plays an important role in the lives of its people. Due to land scarcity and integrated pest management practice the use of pesticides and fertilizers has increased swiftly during the past two decades for increasing the productivity in the agricultural sector. The excessive use of these pesticides causes contamination or degradation of the aquatic environment and surrounding ecosystems. It also responsible to human's health hazard and their economic interests (Panigrahi *et al.*, 2014). Aquaculture is one of the fastest growing food producing sectors, supplying approximately 40% of the world's fish food. Since fishes are important sources of proteins and lipids for humans and domestic animals, and 80 percent of the animal protein in the Bangladeshi diet comes from fish so the health of fishes is very important for human beings. In Bangladesh, more than 300 types of pesticides and insecticides are used for crop protection in agriculture (Uddin *et al.*, 2016). The use of toxic pesticides by Bangladeshi farmers increased by 328 percent during the past 10 years, posing a serious health hazards on human health due to its long-term residual effect. By surface runoff and leaching these pesticides reaches in the unrestricted agricultural areas like ponds and rivers which alters the physicochemical properties of water and is toxic to aquatic organism and cause deleterious effect or even death to the aquatic animal (Nikam *et al.*, 2011; Neelima *et al.*, 2016). Fish are being used as useful genetic models for evaluation of pollution in aquatic ecosystems. Fish as bio-indicators of pollutant effects are very sensitive to the changes in their environment and play significant roles in assessing potential risk associated with contaminations of new chemicals in aquatic environment (Lakra *et al.*, 2009). The contamination of surface waters by insecticides effects on the growth, reproduction, spinal deformities, histopathological changes in gills, liver, renal tubules, brain, behavioral disorder, genetic defect and survival of the fish (Sabra *et al.*, 2015). Pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (John, 2007; Banaee *et al.*, 2011). Cartap is one of the most popular and widely used thiocarbamates group insecticides which contain of organosulfur compounds in Bangladesh. It is essentially contact and stomach poison. It effects on indigenous local fish species like *Anabas testudineus*, *Channa punctatus* and other small fish species. However, very little works have been done on the effects on Cartap on indigenous fishes of Bangladesh particularly on the residual effects and rate of mortality. The experiment was conducted to estimate the LC_{50} value of Cartap against four local freshwater fish species and also to predict the applied doses of Cartap for the safety of aquatic organisms especially fish population.

Materials and methods

Location

The experiment was conducted in the laboratory of the Department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Experimental fish species

Four local fish species were selected for the study. The local fish viz. Climbing perch (*Anabas testudineus*), averaging size 11.68 ± 0.30 cm, Spotted snakehead (*Channa punctatus*), averaging size 13.25 ± 0.75 cm, Stinging catfish (*Heteropneustes fossilis*), avereging size 13.65 ± 0.44 cm and *Tangra (Batasio tengana*), averaging size 10.85 ± 0.55 were collected from unpolluted water body without any injury.

Collection of insecticides and preparation of test solution
Cartap (Suntap 50 SP) was collected from the authorized dealer of the insecticide in original sealed container from Dinajpur, Bangladesh and requisite quantity of distilled water was added in order to prepare the desired concentration.

Fish bio-assay
Eighteen plastic dishes (6°C m x 3°C m x 3°C m) with 50 liters of tap water were used in the experiments. Firstly, range finding tests were performed. It was observed that climbing perch, spotted snakehead, stinging catfish and tangra were 11.12, 12.31, 14.29 and 10.88 cm, respectively and all of them died at concentration of 01, 02, 04, 06 and 10 ppm, respectively. Accordingly, for each case six concentration of a particular order were chalked out for definitive tests within these highest and lowest concentrations of range was found. Concentration of Cartap (Suntap 50 SP) for climbing perch, spotted snakehead, stinging catfish and tangra were 01, 02, 04, 06 and 10 ppm, respectively with three replications including a control. In the test dishes desired concentration of insecticides were poured carefully and mixed gently with a glass rod. Ten fishes each were released after proper acclimatization in dishes containing different concentrations of insecticides as well as in the control. All tests were done at ambient temperature. The behavior and other external changes in the body of fishes were observed. Dead fishes were removed and mortality was recorded at 6, 12, 24, 48, 72 and 96 hours of exposure time. During the experimental period, the fishes were in starved condition.

pH (Potential of Hydrogen)
pH of pond water was measured by a digital pH meter.

Temperature
The temperature of water was measured at the time of sample collection by an alcoholic centigrade thermometer with the range 0°C to 110°C.

Dissolved oxygen (DO)
The dissolved oxygen (DO) concentrations of the collected samples were determined immediately by titration method following the Winkler's method (APHA 1976). DO was measured weekly.

Electrical conductivity
The EC values of collected samples were measured by the conductivity bridge (Model 470 Cond meter, UK).

Total dissolved solids (TDS)
To convert the electric conductivity of water sample (mS/cm^{-1}) into the approximate concentration of total dissolved solids (ppm), the mS/cm^{-1} was multiplied by a conversion factor by using the formula, [TDS (ppm) = Conductivity $\mu S/cm^{-1}$ x 0.67].

Probit analysis
The LC_{50} value for different fish species were calculated for 96 hours of exposure time by probit analysis using the computer program SPSS.

Results and discussion

In control condition, there were no changes founded in the tested fish species but increasing the concentration of insecticide significantly damaged certain physiological and biochemical processes because by increasing time of exposure insecticide enter into the vital organs of fishes. No marked acute response was observed. At the higher levels, slight increase in opercular movement and restlessness were found. By the increase of exposure time the fishes became sluggish, settled at the bottom, opercular activities became slower, ultimately the fish became paralyzed and died in an increasing order at the higher doses.

The behavioral changes were also seen in the present investigation, over the duration of 96 hours of exposure to, overall statistically significant changed in almost all the behavioral patterns were observed in four fish species. The surfacing phenomenon of fish observed under Cartap (Suntap 50 SP), overall exposure. The control group showed the normal behavior during the whole experiment and also normal responses was observed at the low concentration (10 mg/L). After 24 hours of exposure to Cartap (Suntap 50 SP), significantly increased hyperactivity in terms of surfacing and scraping moments and schooling were observed in comparison to control. At 48h of exposure, the surfacing as well as scraping moments decreased in fish species, other behaviors increased significantly hyper secretion of mucus, opening mouth for gasping, losing scales, hyper activity were observed. After 72 hours of exposure, all the fishes showed decreased surfacing and jerky movements and increased grasping movements, sank to bottom of the test chamber and independency in swimming. Normal shiny color 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.

The water quality parameters viz., pH, dissolved oxygen, temperature, electrical conductivity (EC) and total dissolved solid (TDS) of the test media are presented in the table 5, 6, 7 and 8. The average dissolved oxygen was higher in the lower concentrated media. However, the parameters varied little in different treatments and agreed with their requirements.

In Table 1, the probit of stinging catfish was different in different concentration i.e. limit of probit was increased with the increasing rate of concentration. The lethal concentration (LC_{50}) was 2.94 ppm at 95% confidence, where lower limit was 2.04 ppm and upper limit was 4.05 ppm.

Table 2 shows that the probit of spotted snakehead was different in different concentration i.e. limit of probit was increased with the increasing rate of concentration. The lethal concentration (LC_{50}) was 2.26 ppm at 95% confidence, where lower limit was 1.41 ppm and upper limit was 3.19 ppm.

Table 3 shows that the probit of climbing pearch was different in different concentration i.e. limit of probit was increased with the increasing rate of concentration. The lethal concentration (LC_{50}) was 3.48 ppm at 95% confidence, where lower limit was 2.45 ppm and upper limit was 4.89 ppm.

In Table 4, the probit of tangra was different in different concentration i.e. limit of probit was increased with the increasing rate of concentration. The lethal concentration (LC_{50}) was 2.69 ppm at 95% confidence where lower limit was 1.70 ppm and upper limit was 3.90 ppm.

In Table 5, water quality in case of stinging catfish in concentration of 01 ppm water pH was 6.81 ± 0.24, and in concentration of 10 ppm pH was 6.96 ± 30. With the increasing of concentration of insecticide, pH was increased. In case of dissolved oxygen with the increase of concentration of

insecticide dissolved oxygen was reduced. In 01 ppm dissolved oxygen was 3.15 ± 0.80 ppm, and in 10 ppm dissolved oxygen was 2.94 ± 0.60 ppm, respectively. Temperature of water in 01 ppm was 22.09 ± 0.35°C, with the increasing of concentration of insecticide, temperature was increased. In concentration of 10 ppm temperature was 22.32 ± 0.60°C. Electrical conductivity of water in 01 ppm was 255 µS cm^{-1} and in 10 ppm EC was 282 µS cm^{-1}. Electrical conductivity (EC) of control (Tap water) was 322 µS cm^{-1}. Total dissolved solid of water in 01 ppm was 197 mgL^{-1} and in 10 ppm total dissolved solids was 212 mgL^{-1}. Total dissolved solid (TDS) of control (Tap water) was 192 mgL^{-1}.

Table 6 shows that water quality in case of spotted snakehead. In concentration of 01 ppm water pH was 6.37 ± 0.25, and in concentration of 10 ppm pH was 6.66 ± 30. When the concentration of insecticide increased pH of water was also increased. In 01 ppm dissolved oxygen was 3.90 ± 0.10 ppm, and in 10 ppm dissolved oxygen was 2.45 ± 0.50 ppm, respectively. In case of temperature, in 01 ppm it was 23.92 ± 0.55 °C, with the increasing of concentration of insecticide, temperature was also increased. In concentration of 10 ppm temperature was 23.99 ± 0.25°C. Electrical conductivity of water in 01 ppm was 256 µS cm^{-1} and in 10 ppm EC was 279 µS cm^{-1}. Electrical conductivity (EC) of control (Tap water) was 251 µS cm^{-1}. Total dissolved solid in water was 197 mg L^{-1} in 01 ppm and 213 mg L^{-1} in 10 ppm. Total dissolved solid (TDS) of control (Tap water) was 195 $mgL^{-1.}$

In Table 7, water quality in case of climbing pearch indicates that in concentration of 01 ppm water pH was 6.46 ± 0.02, and in concentration of 10 ppm it was 6.82 ± 0.05. With the increasing of concentration of insecticide, pH was also increased. In case of dissolved oxygen with the increase of concentration of insecticide dissolved oxygen was reduced. In 01 ppm dissolved oxygen was 3.48 ± 0.60 ppm, and in 10 ppm dissolved oxygen was 3.35 ± 0.70 ppm, respectively. Temperature of water in 01 ppm was 23.89 ± 0.60°C and with the increasing of concentration of insecticide, temperature was increased. In concentration of 10 ppm temperature was 23.99 ± 0.10°C. Electrical conductivity of water in 01 ppm was 258 µS cm^{-1} and in 10 ppm it was 287 µS cm^{-1}. Electrical conductivity (EC) of control (Tap water) was 255 µS cm^{-1}. Total dissolved solid of water in 01 ppm was 194 mgL^{-1} and 10 ppm total dissolved solids was 210 mgL^{-1}. Total dissolved solid (TDS) of control (Tap water) was 190 mgL^{-1}.

Table 8 reveals water quality in case of tangra. In case of pH, with the increasing of concentration of insecticide pH of water was increased. In concentration of 01 ppm water pH was 6.93 ± 0.13, and in concentration of 10 ppm pH was 6.96 ± 0.30. In case of dissolved oxygen with the increase of concentration of insecticide dissolved oxygen was reduced. In 01 ppm dissolved oxygen was 3.27 ± 0.60 ppm, and in 10 ppm dissolved oxygen was 2.79 ± 0.60 ppm, respectively. Temperature of water in 01 ppm was 22.66 ± 0.30°C, with the increasing of concentration of insecticide, temperature was increase. In concentration of 10 ppm temperature was 22.93 ± 0.25°C. Electrical conductivity of water in 01 ppm was 261 µS cm^{-1} and in 10 ppm EC was 278 µS cm^{-1}. Electrical conductivity (EC) of control (Tap water) was 253µS cm^{-1}. Total dissolved solid of water in 01 ppm was 189 mgL^{-1} and 10 ppm total dissolved solids was 207 mgL^{-1}. Total dissolved solid (TDS) of control (Tap water) was 185 mgL^{-1}.

Behavioral and biological responses included loss of reflex, air gulping, erratic swimming, discoloration, hemorrhage and molting were also observed by Ada *et al.*, (2012). Metabolites and pesticidal effects might be the probable reasons of declining oxygen concentration in the lower to higher concentrated test media during the present study. Fish showed uncontrolled behavior like mucus secretion, decreased agility and inability to maintain normal position and erradic swimming were seen in exposure time of the toxicant also observed by Anitha *et al.*, (2016).

The LC_{50} values for 96 hours of exposure were estimated 2.94, 2.26, and 3.48 and 2.69 in Stinging catfish (*Heteropneustes fossilis*) Spotted snakehead *(Channa punctatus)* Climbing perch (*Anabas testudineus)* and Tangra *(Batasio tengana*), respectively. Results indicate that, the LC_{50} value gradually decreased with the increase of exposure period and the mortality rate increased with increase in concentration of pesticide. The results of the present study are in a line with the results Nikam *et al.*, (2011) and Karra *et al.*, (2015). They also observed that the LC_{50} values decreased with increase in exposure period and concentration. The dissolved oxygen was reduced with the increase of Cartap concentration. It might be due to excessive used of dissolved oxygen by the fish species. Gavit and Patil, (2016) also concluded similar observation. Ada *et al.*, (2012) also concluded that dissolved oxygen content was reducing with increasing insecticide concentration, while temperature and pH increased with increasing insecticide concentration. Findings of Nwani *et al.*, (2010) also support the findings on the water quality of the present study. However, the water quality parameters of the test media varied little and were within the desirable range for different four fish species.

Conclusion

From the overall results it may be concluded that the Cartap was more toxic to Spotted snakehead and less toxic to climbing perch as compared to the other two species. The recommended dose for insecticide Cartap (Suntap 50 SP) is 800 ppm (800 mg L^{-1}) for 1 hector of land in field. In this experiment six treatment was used including a control dose, where the doses were lower than the recommended does use in the field by farmers. So, it is clear, that though in this lower dose fish species were seriously injured they can be more affected by the recommended dose applied in the field. Thus, as in field condition if water level is 3 cm than the total amount of water 312500 litre is needed for 1 hectare of land approximately and 800 ppm insecticide is dissolved in this amount although its concentration is lower than exact dose, it will seriously hamper the aquatic organism.

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Table 1. Probit analysis on the effect of Cartap (Suntap 50 SP) to Stinging Catfish at 96 hours of exposure

Concentration (ppm)	Log concentration	No. of Organism	No. of Organism dead	Percent kill	Probit	LC50 (ppm)	95% confidence Limit	
							Lower (ppm)	Upper (ppm)
00	-	10	0	00	-			
01	0	10	1	10	2.67	2.94	2.04	4.05
02	0.30	10	3	30	2.95			
04	0.60	10	5	50	3.25			

06	0.78	10	9	90	3.45
10	01	10	10	100	3.72

Intercept (a) = -1.540 Regression co-efficient (b) = 3.294, Heterogenicity (X^2) = 2.214 (Not significant), Probit = N.E.D. increased by, N.E.D. Normal equivalent deviate.

Table 2. Probit analysis on the effect of Cartap (Suntap 50 SP) to Spotted snakehead at 96 hours of exposure

Concentration (ppm)	Log concentration	No. of Organism	No. of Organism dead	Percent kill	Probit	LC50 (ppm)	95% confidence limit	
							Lower (ppm)	Upper (ppm)
00	-	10	00	00	-	2.26	1.41	3.19
01	00	10	02	20	2.67			
02	0.30	10	04	40	2.95			
04	0.60	10	07	70	3.25			
06	0.78	10	09	90	3.45			
10	01	10	10	100	3.72			

Intercept (a) = -1.021, Regression co-efficient (b) = 2.893, Heterogenicity (X^2) = 0.786 (Not significant), Probit = N.E.D. increased by, N.E.D. Normal equivalent deviate.

Table 3. Probit analysis on the effect of Cartap (Suntap 50 SP) to Climbing perch at 96 hours of exposure

Concentration (ppm)	Log concentration	No. of Organism	No. of Organism dead	Percent kill	Probit	LC_{50} (ppm)	95% confidence Limit	
							Lower (ppm)	Upper (ppm)
00	-	10	00	00	-	3.48	2.45	4.89
1	0	10	01	10	2.67			
2	0.30	10	02	20	2.95			
4	0.60	10	04	40	3.25			
6	0.78	10	08	80	3.45			
10	1	10	10	100	3.72			

Intercept (a) = -1.727, Regression co-efficient (b) = 3.188, Heterogenicity (X^2) = 2.945 (Not significant), Probit = N.E.D. increased by, N.E.D. Normal equivalent deviate.

Table 4. Probit analysis on the effect of Cartap (Suntap 50 SP) to Tangra at 96 hours of exposure

Concentration (ppm)	Log concentration	Number. of Organism	No. of Organism dead	Percent kill	Probit	LC50 (ppm)	95% confidence Limit	
							Lower (ppm)	Upper (ppm)
00	-	10	00	00	-	2.69	1.70	3.90
01	00	10	02	20	2.67			
02	0.30	10	03	40	2.95			
04	0.60	10	06	60	3.25			
06	0.78	10	08	08	3.45			
10	01	10	10	100	3.72			

Intercept (a) = -1.134, Regression co-efficient (b) = 2.640, Heterogenicity (X^2) = 1.652 (Not significant), Probit = N.E.D. increased by, N.E.D. Normal equivalent deviate.

Table 5. Water quality parameters of the test media Cartap (Suntap 50 SP) on stinging catfish during the experimental period

Concentration (ppm)	pH	Dissolved oxygen (ppm)	Temperature (°C)	Electrical conductivity (μScm^{-1})	Total dissolved solids (mgL^{-1})
00	6.79 ± 0.25	3.44 ± 0.20	22.01 ± 0.22	252	192
1	6.81 ± 0.24	3.15 ± 0.80	22.09 ± 0.35	255	197
02	6.86 ± 0.36	3.08 ± 0.60	22.12 ± 0.50	262	202
04	6.87 ± 0.31	3.04 ± 0.40	22.18 ± 0.55	265	207
06	6.89 ± 0.35	3.01 ± 0.40	22.21 ± 0.65	271	210
10	6.96 ± 0.30	2.94 ± 0.60	22.32 ± 0.60	282	212

Table 6. Water quality parameters of the test media Cartap (Suntap 50 SP) on Spotted snakehead during the experimental period

Concentration (ppm)	pH	Dissolved oxygen (ppm)	Temperature (°C)	Electrical conductivity (μScm^{-1})	Total dissolved solids (mgL^{-1})
00	6.29 ± 0.25	3.45 ± 0.10	23.91 ± 0.50	251	195
01	6.37 ± 0.25	3.90 ± 0.10	23.92 ± 0.55	256	197
02	6.42 ± 0.36	3.70 ± 0.30	23.96 ± 0.42	264	199
04	6.47 ± 0.31	3.40 ± 0.40	23.97 ± 0.30	271	203
06	6.55 ± 0.35	3.10 ± 0.80	23.96 ± 0.52	276	206
10	6.66 ± 0.30	2.45 ± 0.50	23.99 ± 0.25	279	213

Table 7. Water quality parameters of the test media Cartap (Suntap 50 SP) on Climbing perch during the experimental period

Concentration (ppm)	pH	Dissolved oxygen (ppm)	Temperature (°C)	Electrical conductivity (μScm^{-1})	Total dissolved solids (mgL^{-1})
00	6.39 ± 0.01	3.54 ± 0.30	23.87 ± 0.50	255	190
01	6.46 ± 0.02	3.48 ± 0.60	23.89 ± 0.60	258	194
02	6.52 ± 0.05	3.42 ± 0.50	23.91 ± 0.80	265	197
04	6.56 ± 0.09	3.41 ± 0.40	23.94 ± 0.35	269	202
06	6.67 ± 0.02	3.38 ± 0.50	23.97 ± 0.20	275	206
10	6.82 ± 0.05	3.35 ± 0.70	23.99 ± 0.10	287	210

Table 8. Water quality parameters of the test media Cartap (Suntap 50 SP) on Tangra during the experimental period

Concentration (ppm)	pH	Dissolved oxygen (ppm)	Temperature (°C)	Electrical conductivity (μScm^{-1})	Total dissolved solids (mgL^{-1})
00	6.79 ± 0.25	3.48 ± 0.40	22.47 ± 0.40	253	185
01	6.93 ± 0.13	3.27 ± 0.60	22.66 ± 0.30	261	189
02	6.86 ± 0.36	3.29 ± 0.82	22.72 ± 0.30	265	193
04	6.87 ± 0.31	3.16 ± 0.25	22.81 ± 0.20	269	195
06	6.89 ± 0.35	2.98 ± 0.26	22.85 ± 0.50	274	200
10	6.96 ± 0.30	2.79 ± 0.60	22.93 ± 0.25	278	207

Cite this article:

Haque, M. N., Islam, M. J., Sarker, B. C., Pramanik, S. K. Zahan, M. N. & Mahajebin, T. (2018). Response of Four Local Freshwater Fish Species to The Toxicity of Thiocarbamate Insecticide Cartap (Suntap 50 SP. ***International Journal of Science and Business, 2*(3), 432-442.**
doi: https://doi.org/10.5281/zenodo.1324445

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