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Toxicity Assessment of Diazinon (Sabion 60 EC) on Some Local Fish Species of Dinajpur

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Abstract

Four different fish species such as Climbing perch (*Anabas testudineus*), Spotted snakehead (*Channa punctatus*), Stinging catfish (*Heteropneustes fossilis*) and Tangra (*Batasio tengana*) were exposed to various concentrations of Diazinon (Sabion 60 EC) to investigate the toxic level of Diazinon and mortality rate of fish species. The various concentrations in which the fish species were exposed as 100, 200, 300 and 400 ppb including a control. The median lethal concentration (LC_{50}) values of Diazinon (Sabion 60 EC) on Climbing perch, Spotted snakehead, Stinging catfish and Tangra were 167.45, 182.90, 217.59 and 190.29 ppb respectively for 96 hours of exposure. Remarkable behavioral abnormalities were found during the exposure. The study was also undertaken to investigate and to compare the percentages of protein and fat content of the fish species before and after application of pesticide. Some water quality parameters such as temperature, pH, dissolved oxygen, total dissolved solids and electrical conductivity were also measured during the experiments. In this experiment the pH value was in the range of 6.87-7.92 and temperature was in range of 26 to 29 °C in presence of pesticide. Fish species showed various physiological and behavioural alterations as rapid opercular movement, erratic swimming, gulping, mucus secretion in response to various concentrations of pesticide. These alterations were more in higher concentrations. The protein and fat percentages of the fish species were reduced after exposure of pesticide during this experiment.

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1. Introduction

Nowadays water pollution is a common phenomenon. Main sources of water pollution are drainage, surface runoff, industrial wastes and various chemical substances such as pesticides. (Maruthanayagam, 2004). Indiscriminate use of pesticides has been increased in the last few years and these also affects aquatic environment. In aquatic environment fish are the main victims of contamination. Fish are the main source of animal protein and it also helps in earning countries foreign exchange. But in aquatic environment pesticide contamination has harmful effects on fishes and other non-target organisms. Water is the culture environment for fish and other aquatic organisms. Fish and aquatic animals are exposed to insecticides in three primary ways as dermally, orally and by breathing (Mathur and Singh, 2006) . In water bodies pesticide contamination directly or indirectly causes fish killing and also reduces fish productivity (Talukdar et al., 2012). Synthetic pesticides are widely used for controlling pests in agriculture and they are one of the major causes of aquatic pollution. Among synthetic pesticides organophosphate insecticides are widely used in agriculture due to their high effectiveness for controlling insects.

Organophosphates constitute a heterogeneous category of chemicals specifically designed for the control of pests and in water bodies organophosphate causes acute and chronic toxicity to fish fauna (Rao et al., 2005). Among various forms of organophosphates, diazinon (0,0-diethyl 0-[6-methyl-2(1-methylethyl)-4-pyrimidinyl] is widely used for controlling pests. Diazinon has neurotoxic, hemotoxic, hepatotoxic, genotoxic and renal effects and is frequently found in point sources and nonpoint sources due to its chemical properties (EPA, 2003). Many studies on the effects of diazinon on various fish species has been conducted by various authors such as diazinon on *Lepomis macrohirus* (Dutta et al., 2003) and *Cyprinus carpio* (Cengiz et al. 2017), malafos on *Oreochromis niloticus* (Hasan et al., 2018) and sumithion on *Cyprinus carpio* (Sabrina et al., 2016). As Diazinon is a part of pest control in rice field, therefore it is necessary to evaluate the toxic effect of Diazinon. The fish species as Stinging catfish (*Heteropneustes fossilis*), Spotted snakehead (*Channa punctatus*), Climbing perch (*Anabas testudineus*) and Tangra (*Batasio tengana*) are very much popular fish species in Bangladesh. However, this study was undertaken to predict the applied doses of insecticide for safety of aquatic life especially fish population and to compare protein and fat content of fish species before and after application of pesticide.

2. Materials and methods

The experiment was performed in Agricultural Chemistry laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Four local fish species were used for this experiment. The average size of the fish species were Stinging catfish (*Heteropneustes fossilis)*- 15.40±0.60 cm Spotted snakehead (*Channa punctatus*), - 11.00±0.32 cm, Climbing perch (*Anabas testudineus*) - 12.60±0.51 cm, and Tangra (*Batasio tengana*) - 9.00±0.32cm. The fish species were collected from local market. Any injury to fish species was avoided during collection of fish species. Matured, healthy and any infection free fish species were used for this experiment. After collection the fish species were kept in a clean glass aquarium. Then experimental set up was done in the laboratory. Fifteen (15) plastic dishes of size 60cm x 30cm x 30cm which could contain 50 liters of tap water were used in the experiments.

Diazinon (Sabion 60 EC), an organophosphorus insecticide was collected from the authorized dealer of the insecticide in original sealed container from Dinajpur, Bangladesh. After collection, the desired concentration of the pesticide was prepared by adding requisite amount of water in a glass jar. Four doses of pesticide including a control dose with three replications were used for this experiment. The doses were 100, 200, 300 and 400 parts per billion. 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 fish were observed. Dead fish were removed and mortality was recorded at 6, 12, 24, 48 and 96 hours of exposure time. During the experiment the fish were in starved condition.

Some water quality parameters viz., temperature, dissolved oxygen, total dissolved solids, electrical conductivity and pH of the test media were recorded regularly. Temperature, dissolved oxygen and pH were measured by thermometer, DO meter (Model: DO-5509, TAIWAN) and pH meter (Model YSI58, USA). The method given by APHA (2005) was used to determine TDS. The conductivity meter (Model 470 cond. meter, UK) was used for measurement of EC values of water samples. LC_{50} 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

Protein and fat contents of the fish species were also determined in order to compare the percentage of the above content of fish species before and after application of pesticide. Protein content was determined by micro-kjeldahl method. Fat content was determined with the help of using Soxhlet apparatus.

3. Result and discussion

Mortality studies

Death is a decisive criterion in toxicity test because it is easy to determine and has obvious biological and ecological significance. It was estimated that with increasing the rate of concentration of the pesticide Diazinon, the mortality of fish increased that indicates a direct proportional relationship between mortality and concentration of Diazinon. No mortality was observed in the control during the experimental period. During this experiment the lethal concentrations (LC_{50}) for 96 hours of exposure time of the selected fish species estimated by probit analysis are shown in Table 1–4.

Table 1: Probit analysis on the effect of Diazinon (Sabion 60 EC) to Climbing perch at 96 hours of exposure

Concentration (ppb)	Log concentration	No. of organism	No. of organism dead	Percent kill	Probit	LC_{50} (ppb)	95% confidence Limit	
							Lower (ppb)	Upper (ppb)
00	-	10	0	00	-		94.11	230.21
100	2.00	10	03	30	4.48			
200	2.30	10	05	50	5.00	167.45		
300	2.48	10	07	70	5.52			
400	2.61	10	10	100	7.33			

Intercept (a) = -7.228, Regression coefficient (b) = 3.250, Heterogeneity (X^2) = 2.436 (Not significant), Probit = N.E.D. increased by 5, N.E.D.-Normal equivalent deviate

Table 2: Probit analysis on the effect of Diazinon (Sabion 60 EC) to Stinging Catfish at 96 hours of exposure

Concentration (ppb)	Log concentration	No. of organism	No. of organism dead	Percent kill	Probit	LC_{50} (ppb)	95% Confidence limit	
							Lower (ppb)	Upper (ppb)
00	_	10	00	00	_		164.68	275.63
100	2.00	10	01	10	3.72			
200	2.30	10	03	30	4.48	217.59		
300	2.48	10	07	70	5.52			
400	2.61	10	10	100	7.33			

Intercept (a) = -11.771, Regression coefficient (b) = 5.035, Heterogeneity (X^2) = 2.576 (Not significant), Probit = N.E.D. increased by 5, N.E.D.-Normal equivalent deviate

Table 3: Probit analysis on the effect of Diazinon (Sabion 60 EC) to Spotted Snakehead at 96 hours of exposure

Concentration (ppb)	Log concentration	No. of organism	No. of organism dead	Percent kill	Probit	LC_{50} (ppb)	95% Confidence Limit	
							Lower (ppb)	Upper (ppb)
00	-	10	00	00	-		122.19	241.56
100	2.00	10	02	20	4.16			
200	2.30	10	05	50	5.00	182.90		
300	2.48	10	07	70	5.52			
400	2.61	10	10	100	7.33			

Intercept (a) = - 8.791, Regression coefficient (b) = 3.886, Heterogeneity (X^2) = 1.935 (Not significant), Probit = N.E.D. increased by 5, N.E.D.-Normal equivalent deviate

Table 4: Probit analysis on the effect of Diazinon (Sabion 60 EC) to Tangra at 96 hours of exposure

Concentration (ppb)	Log Concentration	No. of organism	No. of dead organism	Percent Kill	Probit	LC_{50} (ppb)	95% Confidence Limit	
							Lower (ppb)	Upper (ppb)
00	-	10	00	00	-		140.46	238.76
100	2.00	10	01	10	3.72			
200	2.30	10	05	50	5.00	190.29		
300	2.48	10	07	70	5.52			
400	2.61	10	10	100	7.33			

Intercept (a) = - 11.807, Regression coefficient (b) = 5.180, Heterogeneity (X^2) =0 .848 (Not significant), Probit = N.E.D. increased by 5, N.E.D.-Normal equivalent deviate

From the above table it is shown that the probit is increasing with increasing rate of concentration. The lethal concentration (LC_{50}) was 167.45 ppb, 217.59 ppb, 182.90 ppb and 190.29 ppb for climbing perch (*A. testudineus*), stinging catfish (*H. fossilis*), spotted snakehead (*C. punctatus*) and tangra (*B. tengana*), respectively. The lower and upper limits were 94.11 ppb and 230.21ppb for *A. testudineus*, 164.68 ppb and 275.63 ppb for *H. fossilis*,122.19 ppb and 241.56 ppb for *C. punctatus*, and 140.46 ppb and 238.76 ppb for *B. tengana*, respectively at 95% confidence level. The results were recorded as 10% to 90% mortality during the experiment. Various biological and physicochemical factors are responsible for the variation of LC_{50} values of different fish species. The responses of the various fish species to the same toxicant are due to some experimental factors and some conditions of the test species.Various works have been performed by different authors on the effect of Diazinon on various fish species. The LC_{50} value of diazinon on the catfish, *Clarias gariepinus* was recorded as high as 7.3 mg/l for 96 hours (Al- Otaibi et al., 2019). The effect of diazinon (LC_{50}) 6.6 ppm was studied on African catfish, *Clarias gariepinus* (Olufemi et al., 2008).

Behavioral studies

Pesticide has long-term effects on fish body. The behavioral changes in fish species were more severe in higher dose and exposure time and these changes may be due to direct toxic effects of diazinon on the fish species. The behavioural changes observed against Phorate intoxication were increased opercular movement, respiratory distress such as gasping for air, loss of equilibrium and erratic swimming prior to mortality. In the treated fishes significant change in the colour of the gills from dark red to brownish black, high mucus films over surface of gills was also observed (Lakshmaiah and Indira, 2014). Due to acute toxicity of diazinon African catfish (*Clarias gariepinus*) showed restlessness, increased reaction to exogenous stimuli, incoordination of movement and postural orientation before death (Olufemi et al., 2008). The behavioral changes were also seen in the present investigation, over the duration of 96h of exposure to Diazinon (Sabion 60 EC). Haque et al. (1993) studied the toxicity of Diazinon to *Puntius gonionotus* and reported that gradually the fish lost equilibrium paralyzed and finally settled down to the bottom of the aquarium and remained at the same place till death. Similar behavioural pattern was also noticed in the present work at higher concentration.

The control group showed the normal behavior during the whole experiment and also normal responses was observed at the low concentration. After 24 h of exposure to Diazinon (Sabion 60 EC), significantly increased hyperactivity in terms of surfacing and scraping moments and schooling were observed in comparison to control. At 48 h of exposure, scraping moments decreased and other behaviors as hyper secretion of mucus, opening mouth for gasping, losing scales, hyperactivity were observed. After 72 h of exposure, all the fishes showed decreased surfacing and jerky movements and increased grasping movements, sank at bottom of the test chamber and independency in swimming. Normal shiny colour and behavior of test fish 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. Following the state of hyper excitability, the fish became inactive and loss of orientation. There was loss of equilibrium and paralysis which ultimately resolved in death of the fish.

Water quality parameters:

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

Table 5: Water quality parameters of the test media (Diazinon) on *Anabas testudineus* during the experimental period

Concentration (ppb)	Temperature (℃)	Dissolved oxyzen (ppm)	pH	Total dissolved solids (mgL^{-1})	Electrical conductivity (μScm^{-1})
00	26.83±1.11	7.77±0.63	6.87±0.11	107	160
100	27.00±0.93	7.57±0.02	7.08±0.10	113	170
200	27.10±1.12	6.53±0.02	7.11±0.11	121	180
300	27.47±0.92	6.27±0.32	7.35±0.10	134	200
400	27.57±1.07	5.12±1.15	7.68±0.09	140	230

Table 6: Water quality parameters of the test media (Diazinon) on *Heteropneustes fossilis* during the experimental period

Concentration (ppb)	Temperature (°C)	Dissolved oxyzen (ppm)	pH	Total dissolved solids (mgL^{-1})	Electrical conductivity (μScm^{-1})
00	28.08±0.21	8.26±1.67	7.62±0.07	113	232
100	28.23±0.41	7.19±1.68	7.66±0.07	123	237
200	28.38±0.37	7.08±1.60	7.67±0.06	128	242
300	28.80±0.39	6.86±1.38	7.22±0.07	143	241
400	28.85±0.44	6.72±1.40	7.78±0.06	156	247

Table 7: Water quality parameters of the test media (Diazinon) on *Channa punctatus* during the experimental period

Concentration (ppb)	Temperature (°C)	Dissolved oxygen (ppm)	pH	Total dissolved solids (mgL^{-1})	Electrical conductivity (μScm^{-1})
00	27.83±0.23	7.83±1.58	7.69±0.07	118	237
100	28.03±0.15	7.33±1.40	7.73±0.09	121	240
200	28.08±0.27	7.13±0.68	7.75±0.06	123	243
300	28.13±0.33	6.43±0.60	7.78±0.19	125	268
400	28.28±0.23	6.17±0.51	7.79±0.09	147	272

Table 8: Water quality parameters of the test media (Diazinon) on *Batasio tengana* during the experimental period

Concentration (ppb)	Temperature (ºC)	Dissolved oxygen (ppm)	pH	Total dissolved solids (mgL^{-1})	Electrical conductivity (μScm^{-1})
00	27.65±0.24	7.80±0.59	7.87±0.06	117	235
100	28.18±0.35	7.52±0.91	7.89±0.05	118	237
200	28.35±0.35	7.33±0.61	7.90±0.07	120	243
300	28.43±0.14	7.12±0.94	7.91±0.07	122	248
400	29.08±0.28	6.72±0.98	7.92±0.09	123	305

It was shown that temperature was usually unchanged. But dissolved oxygen was decreased with increasing rate of concentration while pH, total dissolved solids and electrical conductivity were increased with increasing rate of concentration.

Biochemical parameters

The changes in biochemical parameters such as carbohydrates, proteins and lipids are important to indicate the susceptibility of organ systems to pollutants by altering their function as indicated by Verma and Tonk (1983). The present investigation shows biochemical changes due to sub lethal concentration of Diazinon in total proteins and lipids in target organs and tissues significantly. Thus the pesticide has disturbed the normal functioning of cells with the resultant alterations in the fundamental biochemical mechanisms in fish. This would in turn result in the mortality of fish on chronic exposure to the pesticide. The biochemical parameters as protein and fat percentage before and after application of pesticide of the fish species are shown in the following Table 9-10.

Table 9: Percentage of protein before and after application of pesticide

Fish species	Percentage of protein before application of pesticide	Percentage of protein after application of pesticide
Channa punctatus	27.02 ± 0.37	18.14±0.13
Batasio tengana	20.5 ± 1.49	17.09±0.42
Heteropneustes fossilis	16.91 ± 1.06	16.10±0.44
Anabas Testudineus	16.03 ± 0.59	14.15±0.35

Table 10: Percentage of lipid before and after application of pesticide

Fish species	Percentage of lipid before application of pesticide	Percentage of lipid after application of pesticide
Channa punctatus	11.26 ± 0.87	4.44 ± 0.36
Batasio tengana	6.15 ± 1.32	3.44 ± 0.83
Heteropneustes fossilis	4.98 ± 1.49	3.30 ± 0.19
Anabas testudineus	2.86 ± 1.40	2.39 ± 0.43

It was shown that the protein and fat percentages of the fish species were reduced after application of pesticide.

4. Conclusions

Diazinon is a widely applied agricultural pesticide whose effect importantly on the environment and the possible contamination of surface waters has led to increased interest in toxicology. From the above discussion it was shown that with increased rate of concentration of pesticide the mortality rates of fish species were high. In field level higher concentration of pesticide is used for pest management. In this experiment lower concentrations of pesticide increased the mortality rate of fish species, so the higher concentration of pesticide in field level should cause various harmful and toxic effects to plant, animal and aquatic environment. Besides this after application of pesticide protein and fat percentages of fish species were greatly reduced. So to maintain sustainable environment we should try to use the minimal concentration of pesticide so that we can control the insects as well as can save the aquatic and terrestrial environment.

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