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Effects of Formalin and Some Agrochemicals on the Behavior and Mortality of *Channa Panctatus* (Bloch, 1793)

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Abstract: An experiment was carried out using four chemicals (formalin, urea, clear, potent) with a view to find out a suitable range to be used in actual lethality trials. The experimental trial was conducted for a period of 7 days. The mortality of fish was recorded at 1-day, 2-day, 3-day, 5-day and 7-day in urea and formalin treatment and 12h, 24h and 36h in clear (herbicide) and potent (insecticides) treatment of exposure. Hundred percent mortality of *Channa panctatus* was recorded in formalin, clear and potent treatments at the end of experimental period. Mortality of *Channa* was recorded 66.67% at the concentration of 2g/l of urea and another dose was 100%. After treatment sometime the fish settle down to the bottom of the bowl and then gradually fall into death. At low concentration the mortality occurred slowly. The fish showed various abnormal behavours. The fish were restless, occasionally jumped up with to and fro movement, having less equilibrium and frequent opercular activity, became paralysed after a time and ultimately died.

Key word: Behavior, Mortality.

Introduction

Bangladesh, an agro-based riverine country blessed with a vast area of both fresh water and marine environment, and is one of the richest in Asia in respect of fresh water fishery. *Channa punctatus* is an endemic fresh water fish found everywhere in Bangladesh. These fishes are commonly called snake-headed fish. The Bengali name of this fish is Taki. The fish is under the family-Channidae.

Most of the farmers of Bangladesh are illiterate. They are not conscious about the effect of toxic agro chemicals on life and environment. Recommend doses and use of chemicals is not clearly known to the farmers. The farmers are still using deadly hazard chemicals, which are banned nationally as well as internationally. A survey conducted by Bangladesh Fisheries Research Institute, Riverine Station, Chandpur reported that about 34% of the farmers applied chemicals in over doses 76% of the farmers have little or no idea about the methods of chemicals application and 70% observed fish mortality after the use of chemicals in their crop fields (FRI, 1991).

Formalin is a solution of about 40% by weight of formaldehyde (HCHO) gas in water (40g of formaldehyde in 100 ml of solution). It has an odor that is pungent and suffocating. It is well known as a preservative in medical laboratories, as an in balm fluid and as a sterilizer. Formalin (heavily diluted form) is used as an aquatic chemo therapeutant (i.e. treatment against fish diseases caused by protozoa, fungi). The US Food and Drug Administration approved three commercial formalin products for use in US food fish aquaculture as parasitoids and fungicides. It is how ever not approved for usage in aquaculture in Australia, Europe and Japan because of its association with oncogenesis (tumor development).

The hazards of chemical contaminants have been known since 1930. Toxicity of spray or dust from chemicals in the aquatic environment has been well known documented by Henderson *et al*. (1959), Katz (1961), Soon (1970), Krames and Plapp (1972), Davey et at. (1976). Chemical may enter natural water through different sources (i) Direct application for control of aquatic weed, weed fish and aquatic insect. (ii) through run off from agricultural land, (iii) industrial effluent, (iv) drift from aerial and land application.

The effect of Agro chemicals of fish may be grouped in acute and chronic effects. Acute effects are those that occur rapidly as a result of short term exposure to a chemical. Acute toxicity of several pesticides on fish has been exclusively reviewed (Jonson and Finley, 1980; Mehrle and Mayer, 1985). Chronic effect may occur when the test animals are exposed to the chemicals for a

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long period. Fish may die when large quantities of toxic substances from agricultural run off and industrial discharges enter into the rivers. As a result of accumulation of some chemicals in the fish body, they may lose their reproductive capacity and other physiological activities like growth oxygen consumption etc. (reviewed by Anonymous, 1994). Toxicity of chemicals may destroy spawning grounds and feeding areas, restrict migration of fish, reduce resistance to disease and deteriorate the quantity of fish produce (Metelev *et al.*, 1983).

Materials and Methods

The experiment was conducted from July 2011 to March 2012 at the Fisheries Research Laboratory. Department of Zoology, University of Rajshahi. In laboratory the fishes were immediately transferred to plastic bowl containing tap water and maintained for about 7 days in static condition, and were fed with small pellets of artificial feed (flour dough), twice a day. The pH value was measured by a digital pH meter. Dissolve oxygen measured by Winkler method (APHA,1 992) and free carbon dioxide (mg/l) was measured by titration of water samples with N/44 NaOH solution and phenolphthalein as an indicator (Walch, 1948).

Experimental chemicals

1. Formalin, 2. Urea (Nitrogenous fertilizer), 3.Clear (500EC Protilaclor), 4.Potent(250 EC Propiconajol).

Treatments

Three concentrations of formalin were treated against the fish. The concentrations were 0.25, 0.50 and 1ml/l formalin, clear and potent separately in water three concentration of urea were treated against the fish. The concentration were 1, 2 and 3 g/l urea in water.

Experimental procedure

A series of experimental bowls were set, which contained water mined with different concentrations of formalin, urea, clear and potent separately. These experimental concentrations were chosen from pilot experiments using different concentrations of formalin, urea, clear and potent separately against the fish, *C. panctatus* The behavior of the fishes were observed and based on mutuality within 12 hours, sub-lethal concentrations (as mentioned) of those compounds were selected for the main experiment. Three randomly selected fishes were transferred to each treatment bowl. The fishes were not fed during experimental trials. The concentration of urea used in the experiment for *C. panctatus* were 1, 2 and 3g/l were maintained. In this experiment the concentration of formalin, clear and potent were 1ml/l, 0.250ml/l and 0.25ml/l in water. In urea and formalin treatment the observation were made at firstly 25-30 minutes and in potent treatments the observation were made at firstly 20-25 minutes and then 12-36 hours. During the period of acclimatization dead as stressed were discarded. At that time of overall treatment with chemicals the behavior and mortality of fishes was noted carefully to determine the differences between controlled and treated. In that period dead fishes were recorded and removed to prevent the deterioration of water quality.

Results and Discussion

The mean values of water temperature, dissolved oxygen (DO), carbon-dioxide (CO_2) and pH values are 27.9±0.3°C, 7.92±0.17 3.6±0.7 mg/l and 7.92±0.17mg/l respectively.

Effect of chemical treatment on behavior

In experimental period the fish showed abnormal behavior, quickly after treatment. Finally after some time the fish settle down to the bottom of the bowl and then gradually fell into death. But during the experiment it was observed that when concentration was low then slowly induce mortality of the fish. The fish demonstrated various abnormal behavior shown by the fish were restless, to and fro movement, loss of equilibrium, jumping, increase opercular activity, paralysis. Behavior of the test fishes was observed during the experimental period. The fishes were shown reaction within 20-25 minutes after treatment.

In Urea treatment: In experiment the doses of urea were 1g/l, 2g/l and 3g/l. After addition of urea in the experimental bowl, the fishes were normal. But the exposed fishes showed some abnormal activities after 1 day. At that time the fishes were not taken any food. They were weak and moved slowly. Their scales were protruded near tail in that time. After 3 days most of the fishes were weak and first fish was dead which was in concentration 3g/l, then 2g/l, 1g/l was also dead. The control fishes (i.e. without urea treatment) remained alive and active throughout the experimental period.

Table 1. Average total length (TL) and total weight (TW) of *C. punctatus* and the death time of them by Urea

Concentration	TL (cm)	TW (g)	After 1st day	After 2nd day	After 3rd day	After 7th day	Total death
1g/l	12	18.5	1	0	0	0	3
	14	30.	0	0	1	0	
	14.5	27	0	0	1	0	
Mean ± SD	13.50± 1.08	25.16± 4.87					
2gm/l	11	20	0	1	0	0	2
	12	23	0	1	0	0	
	13.5	17.5	0	0	0	0	
Mean ± SD	12.17 ± 1.03	20.17± 2.25					
3g/l	13	25	0	1	0	0	3
	11.7	20	1	0	0	0	
	11	20	0	1	0	0	
Mean ± SD	11.9 ± 0.82	21.67 ± 2.36					

In formalin treatment: In experiment the doses of formalin were 1ml/l, 0.50 ml/l and 0.25ml/l. In first concentration of experiment pectoral fin of a fish was despoiled. When formalin was added in experimental bowl the affected fish started Jumping. The fishes of 0.50 ml/l,0.25ml/l concentration was Jumping after 5-7 minutes. Within 20 minutes the fish completely relaxed at the bottom and tried to swim in the water surface. This period they lost of equilibrium and became paralyzed. As the fish reached towards moribund stage they were found to become non-sensitive to touch and sound. Finally the fish settle down to the bottom of the bowl and fell into death with their bad smell. The control fish was alive throughout the experimental period.

Table 2. Average total length (TL) and total weight (TW) of *C. punctatus* and the death time of them by Formalin

Concentration	TL (cm)	TW (g)	After 1st day	After 2nd day	After 3rd day	After 7th day	Total death
1ml/l	13	30	1	0	0	0	3
	12.7	25	1	0	0	0	
	17	46	1	0	0	0	
Mean ± SD	14.23±1.95	33.67± 8.96					
0.50 ml/l	13.4	34.5	1	0	0	0	3
	11.5	17.5	1	0	0	0	
	12	15	1	0	0	0	
Mean ± SD	12.30± 0.80	22.34± 8.66					
0.25 ml/l	10	15	0	1	0	0	3
	12.5	25	0	1	0	0	
	11.5	20.5	0	1	0	0	
Mean ± SD	11.34± 1.02	20.07± 4.09					

In clear and potent treatment: After addition of clear and potent in the experimental period the fishes exhibited various signs of distress. After some time the fish came to the upper surface of water for 1 to 2 minutes. The fishes were found to keep their mouth wide open and they were jumping. They jumped strongly and tried to reach the upper surface of water to escape from the toxic environment. At that time, fish swam rapidly with increased rate of opercular movement. Subsequently, a vigorous spastic and mostly superficial movement of the fins set in and respiration become rapid. Within 10 minutes the fish relaxed at the bottom and tried to swim to the water surface after each brief quiescent period accompanied by partial loss of equilibrium and become paralyzed. This was followed by complete loss of equilibrium with the fish swimming on their backs and slow operculum activities. As the fish reached towards moribund stage, they were found to become non-sensitive to touch and sound. Finally the fish settle down to the bottom of the bowl. Body colour become slightly fade than the control fish and then gradually fell into death with their bad smell. The control fishes i.e. without clear and potent treatment remained alive and active throughout the experimental period. In Table 3 and 4 the time was taken to become senseless and that to death *C. panctatus* are shown against different concentrations of clear and potent.

Table 3. Average total length (TL) and total weight (TW) of *C. punctatus* and the death time of them by Clear (Herbicide)

Concentration (ml/l	TL (cm)	TW (g)	After 12hours	After 24 hours	After 36 hours	Total death
	12.7	20.5	1	0	0	
1	13	24.4	1	0	0	3
	11.5	13	1	0	0	
Mean ± SD	12.40 ± 0.65	19.30±4.73				
	13	22	1	0	0	
0.50	11	24	1	0	0	3
	14.5	23.5	1	0	0	
Mean ± SD	12.84±1.43	23.17±0.85				
	9.5	10	1	0	0	
0.25	12	13	1	0	0	3
	14.5	25	1	0	0	
Mean ± SD	12± 2.04	19.34± 6.65				

Table 4. Average total length (TL) and total weight (TW) of *C. punctatus* and the death time of them by Potent (Insecticide)

Concentration (ml/l)	TL (cm)	TW (g)	After 12 hours	After 24 hours	After 36 hours	Total death
	12.5	19	1	0	0	
1	11	13.5	1	0	0	3
	13	22	1	0	0	
Mean ± SD	12.17 ± 0.85	18.17± 3.52				
	13	23.5	1	0	0	
0.50	13.7	24	1	0	0	3
	17	30	1	0	0	
Mean ± SD	14.57± 1.74	25.84 ± 2.95				
	12	20	1	0	0	
0.25	14.5	25	1	0	0	3
	10	15	1	0	0	
Mean ± SD	12.17 ± 1.84	19.67 ± 3.68				

Mortality

The experiment was carried out with chemical with a view to find out a suitable range to be used in actual lethality trials. The experimental trial was conducted for 7-days. The mortality of fish was recorded at 1-day, 2-day, 3-day, 4-day 7-day in urea and formalin treatment and 12h, 24h and 36h in clear and potent (insecticides) treatment of exposure.

66.67% mortality of *C. panctatus* was recorded at the concentration of 2g/l of urea and another done mortality was 100%. 100% mortality of *C. panctatus* was recorded at formalin, clear and potent treatment at the end of experimental period.

The effect of different concentration and exposure time of urea, formalin, clear and potent as shown by the mortality of *C. panctatus* is given in Table 5.

Table 5. Mortality of *C. punctatus* due to urea, formalin, clear and potent treatment

Treatment name	Concentration	No. of fish used in treatment	No. of dead fish	% Mortality	No. of fish in control	% Mortality
	1g/l	3	3	100%	1	0
Urea	2g/l	3	2	66.67%	1	0
	3g/l	3	3	100%	1	100%
	1ml/l	3	3	100%	1	0
Formalin	0.50ml/l	3	3	100%	1	0
	0.25ml/l	3	3	100%	1	100%
	1ml/l	3	3	100%	1	0
Clear	0.50ml/l	3	3	100%	1	0
	0.25ml/l	3	3	100%	1	0
	1ml/l	3	3	100%	1	0
Potent	0.50ml/l	3	3	100%	1	0
	0.25ml/l	3	3	100%	1	0

Table 6. Correlation between concentration of Potent and time taken to senseless and death in *C. punctatus*

Concentration	Mean ± SD			
	TL (cm)	TW (g)	Time for senseless (min)	Time taken to die (min)
1ml/l	12.17 ± 0.85	18.17 ± 3.52	9 ± 1.41	13.34 ± 2.35
0.50ml/l	14.57 ± 1.74	25.84 ± 2.95	15± 4.08	21.67±2.36
0.25ml/l	12.17 ± 1.84	19.67 ± 3.68	16.67± 4.72	23.34±6.23
r-value			0.77	0.62

A 5% level of significance tabulated is value for 2 df 0.950 an our calculated correlation coefficient value are r = 0.77 (time taken to senseless) and r = 0.62 (time taken to die). Both value is less than the tabulated value. So we can say that the time taken to brought the effects were positively correlated with the concentration.

The toxicity of a substance in aquatic medium is generally influenced by the variation of physical, chemical and biological factors. Temperature, pH, alkalinity have profound effects on the toxicity of agro-chemicals (Boyd 1979; Rand and Petrocelli, 1985). The toxicity of organophophorus compounds when used as insecticides largely depends upon the pH. alkalinity, hardness and different dilution of water (Henderson and Pickering, 1959; Rand and Petrocelli, 1985). According to APHA (1980), fluctuation in temperature should not exceed 40°C and similarly oxygen content must not fall below 4mg/for the warm water fish. From the Table 1 it is also evident that physical and chemical properties of the bowl water were within the desirable range for fish culture which correlated with the work of Boyd (1979).

Various aspects of behavior resulting from intoxication of the fished observed in the present study and anomalies that were in comformity with other researchers. Behavioral change may in fact be the first response of an organism to environmental perturbation (Slobokin, 1968). The abnormal behavior of experimental fished exposed to formalin such as to and fro movement, jumping, violet spasm, loss of equilibrium, rapid movement of operculum and lateral swimming are supported by other worker who performed experiment with various toxicant (Henderson *et al*., 1959, Andrews *et al*., 1966). It is an indication that the effects of chemical are species specific. In the present study hyperactivity was observed at early stage of exposure, rather the fish become lethargic during the later stage of exposure. Similar observation was also made by Javaid and Waiz, (1972) by endrin, dieldrin, DDT, aldrin and BHT to *channa punctatus.* Increased opercular activities and oxygen demand caused by the toxicant is consistent with observation made by Kadama *et al*. (1955). The slow movement and ignorance to prodding might also be due to main function of the nervous system. According to Holden (1962) death of the fish under organophosphorus and organochlorine treatment caused through paralysis of the fish. Loss of equilibrium observed in the present study may be due to consequences of inpairment in nerve function caused by the applied toxicant. Saxena and sehgal. (1986) also conducted laboratory investigation to toxicity bioasay on *Channa punctatus* with some pesticides like nuvan, dimecron, aldorisam etc. According to Metelev *et al*. (1983) organophorus compounds are poisons with aneuroparalytic and enzymatic action. Investigation of Gardner and Laroche (1972) suggested that neurotoxic effect and consequent irritation to the perceptive system induce behavioral reaction to teleosts. Holden (1962) found DDT accumulating in external mucous of brown trout and pointed out of possibility of its entry into the fish through the skin. The lighter colour of the integument may be fade due to absorption of the toxicant/chemical through the skin, which affected the pigment. The present experiment showed with the increased rate of opercular movement, loss of equilibrium, become paralyzed and finally settle down to the bottom of bowl and fell into death. Similar views were expressed by Anees (1976); Sastry and Sharma (1979); AL-Arabi *et al*., (1995) reported that after application of chemical (e.g-diazinon, melhyl parathion, malathion, endrin, sumicidin etc.) on *Channa puctutas.* Malathion treatment on behaviour and mortality of *channa punctatus* by Nesa (2000).

Conclusion

Formalin is used to disinfect water of the fish culture ponds during the outbreak of parasites and some diseases. Care should be taken that the concentration of must remain within the range of prescribed concentrations. So, frequent use of urea at high concentration should be restricted in the crop fields. Sometime urea is used in the pond during pre-stocking period, for better production of

phytoplanktons. Clear and potent were found to be infective to kill *Channa punctatus* at or bellow 1ml/l. The fishes demonstrated various abnormal behavior.

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