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PHYSIOLOGICAL RESPONSES OF FISH TO ROGOR AND ALACHLOR PART-I. GENERAL IMPACT ON *HETEROPNEUSTES FOSSILIS*

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For *H. fossilis* 50% lethal concentration values ((LC_{50}) of rogor were found to be 12.88, 12.13, 11.40 and 10.53 ppm for 12 h, 24 h, 48 h and 96 h respectively. In case of alachlor, these were 4.717, 4.430, 4.162 and 3.732 ppm, respectively for the same duration. Relative potencies of alachlor in comparision to rogor for different time assays are 3.7305, 2.7381, 3.7391 and 3.8215. The data obtained clearly indicate that alachlor is more toxic than rogor. The fish showed specific behavioural responses to different concentrations of pesticides while prompt behavioural changes were noticed soon after the fish was introduced to the test concentration, these markedly decreased as time passed. Mortality data was plotted on a probit log paper and a straight line relationship between concentration and probit of kill was established for different time assays.

INTRODUCTION

Toxicity of the environmental pollutants to the aquatic fauna and their role in changing the aquatic ecosystem are almost perennial. Advances in the field of agricultural sciences and latest techniques involving effective synthetic phytopharmaceutical compounds in the form of biocides, have endangered the existence of aquatic biolife. The outflow of pesticides causes great pollution in inland water bodies resulting in a huge mortification of inhabiting flora and fauna including palatable fish. Therefore, the study of toxicity to an organism is an essential first step towards evaluating the impact of pesticides on the freshwater environment, by determining the safe concentration and formulating the safe application rate of an insecticide (Sprague, 1969, 1970 & 1973).

MATERIAL AND METHODS

Heteropneustes fossilis (commonly called *Singhi*) were collected from local fresh water habitats and acclimatized to the laboratory conditions (in a tank) for a minimum period of one week during which they were treated with an antibiotic (6 ppm Ampicillin solution) to avoid any possible bacterial infection (Dubale & Singh, 1979). The fishes were fed *ad libitum* with chopped goat liver before their transfer to experimental containers on random basis (Gaddum, 1953; Finney, 1971). Ten visibly healthy fishes (wt. 20-40 gms; length 17-25 cm) were exposed to each concentration irrespective of their sex. The dilution water was analysed according to the standard methods published by APHA *et al.* (1976) and it was found to be quite satisfactory for maintaining the fish.

The insecticides used in experimentation were rogor 30 EC (Organophosphate) and alachlor 50 EC (Organochlorine). Commercial grade rogor and alachlor were used directly as stock solution. The fishes were exposed to each concentration for a maximum period of 96 hrs and a control experiment was also set up side by side for each concentration. Preliminary tests were carried out to estimate the LC_{50} and LC_{100} values, followed by 96 hrs exposure of 10 fishes in each concentration. The mortality of fish in each test concentration was recorded. The bioassays were repeated 5 times, so that in all 50 fishes (10 x 5) were exposed to each test concentration.

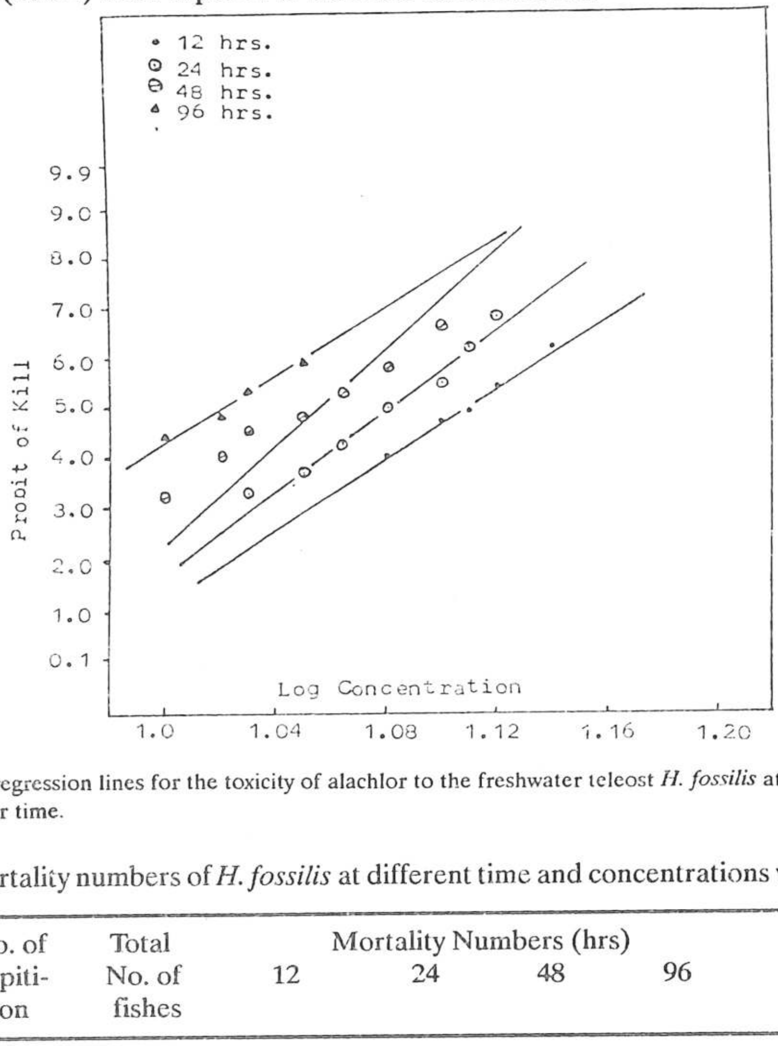

Fig. 1. Probit regression lines for the toxicity of alachlor to the freshwater teleost *H. fossilis* at 12 hr, 24 hr, 48 hr and 96 hr time.

Table I. Mortality numbers of *H. fossilis* at different time and concentrations with rogor.

Conc. in mg/l	No. of Repiti-tion	Total No. of fishes	Mortality Numbers (hrs) 12	24	48	96	Total
10.0	5	50	-	-	2	14	16
10.4	5	50	-	-	8	21	29
10.8	5	50	-	2	15	30	47
11.2	5	50	-	5	21	41	67
11.7	5	50	1	10	30	49	90
12.1	5	50	8	25	39	50	122
12.5	5	50	19	34	47	50	150
12.9	5	50	24	44	50	50	168
13.3	5	50	33	48	50	50	181
13.7	5	50	44	50	50	50	194
Total			129	218	312	405	1064

Table II. Test of significance applied to mortality rates in different times and concentrations of rogor with *H. fossilis*.

Source of variation	D.F.	S.S.	M.S.S.	F	at 5%
Concentration	9	9672.6	1074.7333	20.24	8.79
Time	3	4251	1417	26.68	
Error	27	1434		53.11	
Total	39	15357.6			

The mortality numbers were recorded for each concentration at 12, 24, 48 and 96 hrs after applying Abotts (1975) formula :

CM = % Test mortality - % Control mortality / (100 - % Control morality)

Significance of the data was tested by using the formula given by Snedecore (1967). Discrepencies (chi^2) between observed and exposed mortality numbers, were tested with the help of (chi^2) test and a straight line relationship between probit of kill and log concentrations was established. An estimation of the presumably for harmless (Safe) concentration of the insecticide, was also calculated according to Hart *et al.* (1945). Student *t* test (Fisher, 1950) was applied to evaluate the level of significance at 0.01 and 0.05% levels.

Table III. Mortality numbers of *H. fossilis* at different time and concentrations with alachlor.

Conc.in mg/l	No. of Repiti-tion	Total No. of fishes	Mortality Numbers (hrs) 12	24	48	96	Total
3.35	5	50	-	-	-	1	1
3.50	5	50	-	-	-	4	4
3.65	5	50	-	-	8	8	8
3.80	5	50	-	-	1	18	19
3.95	5	50	-	-	12	25	37
4.10	5	50	-	4	22	38	64
4.25	5	50	-	12	29	50	91
4.40	5	50	7	24	42	50	123
4.55	5	50	16	33	47	50	146
4.70	5	50	24	43	50	50	167
4.85	5	50	33	49	50	50	182
5.00	5	50	45	50	50	50	195
Total			125	215	303	394	1037

RESULTS

The behavioural responses of the fish varied according to test concentrations and length of exposure. The fishes exposed to the toxicant show undulation of body, rapid and irregular opercular movements, increase in surface hyper-excitability and jerky movements followed by occasional somersaults. When the toxic effects became more acute, the fish lost equilibrium and started floating on the surface latero- horizontally and showed aerial mode of respiration. Another remarkable change observed was swollen belly and heavy secretion of mucous over the body surface. Fish also showed varied floating and a few frequently dashed against the walls of container finally sinking down at the bottom and died in higher concentration. Fishes were frequently observed to gulp the atmospheric air directly while some developed tremors, convulsions and depigmentation. A close examination of dead fish exhibited open opercula, body torsion, depigmented body and whitish gills with ample mucous, occasionally with sign of petechial bleeding.

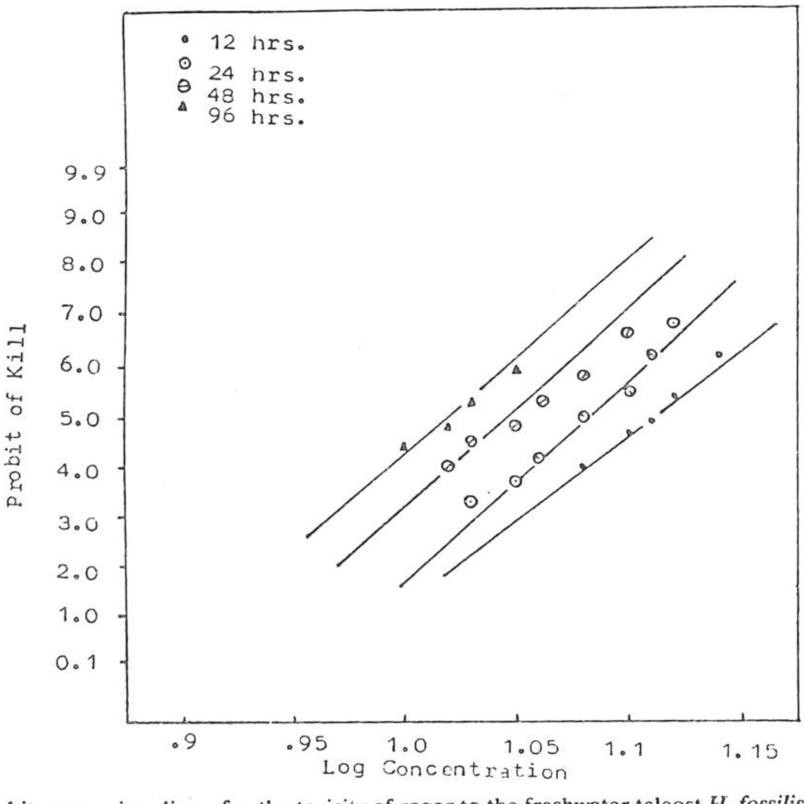

Fig. 2. Probit regression lines for the toxicity of rogor to the freshwater teleost *H. fossilis* at 12 hr, 24 hr, 48 hr and 96 hr time.

The present data reveal (Table V) that LC_{50} value of rogor for *H. fossilis* at 12 hr was 12.88 ppm. Lower and upper limits of the concentrations were found to range from 12.75 to 13.02 ppm. A slope function of 1.0677 was obtained. For 24hr, LC_{50} value, the lower and upper limits were 12.13, 12.01 and 12.24, respectively. The value of the slope function decreased to 1.0595. At 48hr, the LC_{50} value of 11.40 was recorded with lower and upper limits of the concentrations were determined as 10.55 to 10.82, respectively. At 96hr the value of slope function increased upto 1.0702.

It was further observed that the trend of fluctuations in LC_{50} values with the period of exposure showed similar pattern with alachlor. The 12hr LC_{50} value was 4.717 ppm and the lower and upper limits were 4.662 to 4.771 ppm, respectively with a slope function of 1.0671. The LC_{50} values of 4.430 ppm was recorded at 24hr with a lower and upper limits 4.375 to 4.467 ppm. The value of slope function decreased to 1.0614. The 48hr,

LC_{50} with its lower and upper limits were determined, the values being 4.162, 4.123 & 4.202 ppm, respectively. The values of slope function was further decreased to 1.0589. At 96hr the LC_{50} value was estimated as 3.732 ppm with a lower and upper limits of 3.686 and 3.766 ppm. The variation in susceptability as indicated by the slope function was 1.0747.

Relative potencies of the pesticides measured at selected levels of kill showed that alachlor was 2.7305 to 2.8215 times more toxic when compared to rogor depending upon the time of exposure. Thus the toxic effect of these pesticides were found in the order alachlor > rogor.

Safe level concentrations of rogor and alachlor to *H. fossilis* have been determined (using different application factors) and are 3.021 ppm and 1.102 ppm, respectively which are presumably harmless and acceptable safe levels.

Table IV. Test of significance applied to mortality rates in different times and concentrations of alachlor with *H. fossilis*.

Source of variation	D.F.	S.S.	M.S.S.	F	at 5%
Concentration	11	15014.23	1364.93	20.184762	8.765
Time	3	3337.73	1112.5766	16.452927	
Error	33	2231.52	67.6218		
Total	47	20583.48			

DISCUSSION

Certain aspects of chemical ecology of fish and the importance of chemical communication in maintaining the behaviour pattern of fish as well as other marine organisms has been reviewed by Hasler (1979). According to Muirhead-Thomson (1971), "there is increasing realization that the effect of pesticides on the reactions of fish, other than the easily observable mortality effects, must be taken into account in evaluating the complete ecological impact of a contaminating substance". The reaction of fish to various organophosphorus and organochlorine pesticides can be in general characterized by erratic movements, muscle tetanus, paralysis and flexed opercula. The subjective observations on behavioural responses of *H. fossilis* to the lethal and sublethal concentrations of rogor and alachlor are in agreement with those in *Ictalurus punctatus* (Carter, 1971), *Psudorasbora parva* (Kanazawa, 1975), *Channa punctatus* (Annes, 1975; Srivastava & Srivastava, 1981), *Tricogaster fasciatus* (Gupta & Singh, 1981), *Colisa fascitus* (Sharma & Gupta, 1983) exposed to different pesticides. Cutcomp *et al.* (1971), Dikshit *et al.* (1978), Chambers (1976), Negherborn (1959) reported that organophosphorus and organochlorinated pesticides inhibit acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) system which results in the abnormal behaviour and death of the organism. Wildish *et al.* (1971), Alsen *et al.* (1973) and Verma *et al.* (1979) could not establish a linear relationship between the death of fish and degree of brain and serum cholinesterase inhibition. The abnormal behaviour due to toxic effect of pesticides have been reported by Singh *et al.* (1965), Saunders (1969) and Carter (1971) telly with the present observations. Besides, an interesting observation was that fish showed a change of aquatic to aerial mode of respiration during the exposure period. This is attributed to the toxic effect on the metabolism. Heavy exudation of mucous over the body surface is attributed to the acute effect of the toxicant on the physiology of the fish. It is presumed that the toxicant affected the chromatophores of the fish so that significant change was observed in the body colour. In the present study also a few crippled *H. fossilis* were observed. Annes (1975) reported that the physiological events

Table V. LC_{16}, LC_{50}, LC_{84}, values with 95% fiducial limits, slope function and probit regression equations fo r toxicity of rogor to *H. fossilis*.

Duration (hrs)	LC_{16} Value	LC_{16} 95% Fiducial limits Upper	LC_{16} 95% Fiducial limits lower	LC_{50} Value	LC_{50} 95% Fiducial limits Upper	LC_{50} 95% Fiducial limits lower	LC_{84} Value	LC_{84} 95% Feducial limits Upper	LC_{84} 95% Feducial limits lower	Slope probit Regression function Equation
12	12.07	12.29	11.84	12.88	13.02	12.75	13.76	13.02	13.51	1.0677-33.888 + 35.031X = Y
24	11.43	11.57	11.29	12.13	12.24	12.01	12.83	13.01	12.67	1.0595-37.722 + 39.438X = Y
48	10.87	11.01	10.71	11.40	11.49	11.31	11.96	12.10	11.85	1.0489-46.111 + 48.348X = Y
96	9.84	10.03	9.55	10.53	10.82	10.55	11.27	11.52	11.36	1.0702-29.531 + 33.778X = Y

All values except slope function (s) and regression equations are given in mg/l.

Table VI. LC_{16}, LC_{50}, LC_{84}, values with 95% fiducial limits, slope function and probit regression equations for toxicity of alachlor to *H. fossilis*.

Duration (hrs)	LC_{16} Value	95% Fiducial limits Upper	lower	LC_{50} Value	95% Fiducial limits Upper	lower	LC_{84} Value	95% Feducial limits Upper	lower	Slope probit Regression function Equation
12	4.419	4.490	4.312	4.717	4.771	4.662	5.032	5.518	4.951	1.0671-53.884 + 35.185X = Y
24	4.164	4.220	4.086	4.430	4.467	4.375	4.691	4.745	4.643	1.0614-58.169 + 38.391X = Y
48	3.931	3.988	3.873	4.162	4.202	4.123	4.408	4.469	4.348	1.0589-59.858 + 40.052X = Y
96	3.472	3.530	3.414	3.732	3.766	3.686	4.010	4.085	3.938	1.0747-45.006 + 31.813X = Y

All values except slope function (s) and regression equations are given in mg/l.

leading to death in fish by lethal exposure to pesticides are not well understood. The present study was indicative of the general toxic effect produced by most of the organophosphorus and organochlorine pesticides.

In the present investigation it was observed that the system of *H. fossilis* can function within a broad range of pollution by rogor and alachlor and the toxicity response of the fish towards rogor and alachlor is a function of concentrations and duration of exposure. The median tolerance limit for this fish to various pesticides was worked out by Gouda *et al.* (1981), Dubale & Awasthi (1982), Verma *et al.* (1979), Basak & Konar (1977), Verma *et al.* (1977 & 1981), Singh & Singh (1977) and Choudhary *et al.* (1981). According to Muirhead-Thomson (1971) all organochlorine pesticides are highly toxic to fish than the organophosphate compounds under the same experimental conditions. Our findings support their observations.

The 96hr LC_{50} values of rogor to *H. fossilis* has been worked out by Dubale & Awasthi (1982) as 24 ppm which is not in accordance with the present findings. This difference in LC_{50} values may be due to the fact that determination of LC_{50} is influenced by various factors like temperature, salinity and pH of the medium, type and concentration of chemical and duration of exposure (Eisler, 1968; Schoettger, 1970). It is inferred that with the increase in the duration of exposure, the variability in susceptability is increased (Macak & Mcallister, 1970). Sparks *et al.* (1982) reported that the temperature and toxicity are positively correlated for most organophosphorus and carbamate pesticides and stated that toxicity increases with an increase in temperature. Conversely many pyrethroids and organochlorine pesticides exhibited a negative temperature coefficient and thus greater toxicity was present at lower temperature (Guthrie, 1950; Blum & Kearns, 1956).

Determination of LC_{50} values is useful since it provides fundamental data for the design of complex models and is also useful as a preliminary data for other studies involving more frequent dosing (Cutcomp *et al.*, 1971). Moreover, these values are useful in the evaluation of safe level or tolerance level of a pollutant to the aquatic biotype for establishing limits and levels of acceptability of pollutant by an animal. Such study is also helpful in designing the preferential aquaculture programme based on the quality of the environment and quantity of pollutant in it (Dikshit *et al.*, 1978).

Concentration of a chemical, harmless to the greater part of a fish population even with prolonged exposure, is known as safe concentration (FAO/EIFAC, 1983). The safe concentration was estimated by the widely accepted method of Hart *et al.* (1945). Bender (1969a, & b) studied that there exists a synergistic effect between hydrolysis product present in the basic aqueous solutions of pesticides and the parent compound may not be correct, as a complex system of grades may be produced from the hydrolysis or metabolism of pesticides, which could result in synergism playing significant role in mortality. It was further strengthened by APHA (1976) that safe values certainly do not represent the concentration that are safe in natural waters. Long term exposures to much lower concentrations may be lethal to fishes and other organism and still lower concentrations may cause nonlethal impairment of their function, but in general the concentration considered safe in the present study may help in determining the water quality critaria and standards for aquatic environmental protection works and management.

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