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Note

Acute toxicity bioassay and tissue biochemical changes on sub-lethal exposure to the organo-phosphorus pesticide 'Phorate 10G' in the freshwater fish *Labeo rohita* (Hamilton, 1822)

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ABSTRACT

Toxicity of the organo-phosphorus pesticide, Phorate 10G was tested *in vivo* in the Indian major carp *Labeo rohita*. Based on the results of a range finding bioassay to assess the lethal dose range of 'Phorate 10G', various concentrations *viz*., 0.05, 0.1, 0.15, 0.20 and 0.25 mg l^{-1} were selected for estimation of LC_{50}. The LC_{50} value estimated by probit analysis was 0.137 mg l^{-1} for 96 h. Further, in order to assess the effect of sublethal toxicity, rohu fingerlings were exposed to sub-lethal concentrations of 0.05 and 0.075 mg l^{-1} of Phorate 10G for a total period of 30 days. At the end of exposure periods of 10, 20 and 30 days, 10 fingerlings each were sacrificed for carcass biochemical analysis of moisture, crude protein, crude fat and total ash contents. Moisture and total ash contents in rohu fingerlings exposed to the pesticide increased significantly, whereas crude protein and crude fat contents decreased with increase in concentrations as well as period of exposure ($p< 0.05$). Results of the study clearly indicated that 'Phorate 10G' affected the carcass proximate composition of rohu fingerlings which could be attributed to the energy requirement towards combating the toxic effects of pesticide exposure in the fingerlings of *L. rohita*.

Keywords: Biochemical composition**,** *Labeo rohita,* Organophosphate, Phorate 10G

Pesticides have substantially contributed to control of pests and increase of crop yields. But over the years, there is a growing concern about the indiscriminate use of pesticides in agriculture. Organo-phosphorous pesticides include some of the most commonly used pesticides globally. These are used in agriculture, home, gardens and veterinary practices. However, these pesticides entering natural water bodies can have adverse effects on the aquatic organisms, ranging from biochemical alterations at cellular levels to changes in whole populations. In general, the end points used in toxicity studies are mortality, survival and growth with acute toxicity tests. But these parameters are not appropriate, to assess the effect of long-term exposure to sub-lethal concentrations. Hence in the present study, proximate biochemical composition of the freshwater fish, rohu (*Labeo rohita*) was analysed to estimate the toxic effect of the organophosphate pesticide 'Phorate 10G', which is widely used in the paddy fields as well as in horticulture. The present study was planned to assess the acute toxicity as well as the effect of sub-lethal exposure to Phorate-10G in *L. rohita* which is a true denizen in the freshwater aquaculture. Rohu is the dominant species among the Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinius mrigala*) and contributes more than 80% of total inland fish production of India (Sulekha *et al.,* 1999).

Fingerlings of rohu, *L. rohita* were collected from the freshwater fish hatchery, Shramjivi Janata Sahayyak Mandal, Khaire-Valang, Mahad in Raigad District of Maharashtra and were brought to the laboratory. Fingerlings were treated with 0.05% $KMnO_4$ solution for 2 min to avoid any cutaneous infection. The disinfected fish stock was maintained in a 500 l plastic tank for 10 days to acclimatise under laboratory conditions. During acclimatisation, the fingerlings were fed twice a day with commercial pellet fish feed. For toxicity bioassays, fishes were stocked in all glass aquarium tanks (45 x 22.5 x 30 cm), each holding 20 l freshwater. Rohu fingerlings having an average weight of 4.67±0.23 g and an average length of 6.62±0.421 cm were used for the study.

Initially, range finding assay was conducted in five groups of fishes (each group in triplicate tanks with 10 numbers per tank) which were subjected to different concentrations of Phorate 10G (10% CG, Trade Name: THIMET) *viz.,* 0.002, 0.02, 0.2, 2 and 20 mg l^{-1} along with a control group without Phorate10 G treatment. The mortality of the fishes were recorded for a period of 24 h and the cumulative percentage mortalities were determined to estimate the lethal range of Phorate 10G concentration for *L. rohita* fingerlings.

Based on the results of the range finding assay, five concentrations *viz.,* 0.05, 0.1, 0.15, 0.2 and 0.25 mg l^{-1} of Phorate10 G were selected for estimation of 96 h LC_{50} as per the methods described by Reish and Oshida (1987). Five groups of fishes (each group in triplicate tanks with 10 numbers per tank) were subjected to different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 mg l^{-1}) of Phorate10 G along with a control group (without Phorat 10 G treatment) in triplicate. Mortality of the fishes were recorded for a period of 96 h and the LC_{50} value was estimated by probit analysis (Reish and Oshida, 1987).

For analysing the effect of sub-lethal toxicity of Phorate 10G, 30 numbers (in triplicate groups of 10 numbers each) of rohu fingerlings were exposed to sub-lethal concentrations of 0.05 and 0.075 mg l^{-1} of Phorate 10G along with a control group (in triplicate) without pesticide exposure, for a total period of 30 days. Fishes were fed with commercial fish feed, and the tanks were kept well aerated. Ten percent of the medium was replaced after every 24 h with freshwater having same concentration of the insecticide. At the end of exposure periods of 10, 20 and 30 days, 10 fingerlings from each group were sampled for carcass biochemical analysis. The protein content in the sample was estimated by the method of Lowry *et al.* (1951). Crude fat was determined as per the method described by Sadasivam and Manickam (1997). Moisture and ash contents were estimated following AOAC (2006). The experimental data were statistically analysed by SNK test (Fisher and Yates, 1963; Reigh and Oshida, 1987).

Observations on mortality of *L. rohita* fingerlings exposed to different concentrations of Phorate10G in the range finding assay are given in Table 1. In general, 0, 20, 60 and 76.67% mortality were observed in the concentrations of 0.002, 0.02, 0.2 and 2.0 mg l^{-1} respectively, whereas 100% mortality of fingerlings was observed in 20 mg l^{-1} concentration. The lethal concentration of Phorate10G for rohu fingerlings was observed to be between 0.02 and 0.2 mg l^{-1}.

The cumulative average percentage mortality of rohu fingerlings exposed for 96 h at various concentrations (0.05, 0.1, 0.15, 0.20 and 0.25 mg l^{-1}) of 'Phorate 10G' are shown in Fig 1. At the end of 96 h exposures, the total average percentage mortality of 23, 43, 53 and 70 was observed in 0.05, 0.1, 0.15 and 0.20 mg l^{-1} concentrations respectively. The 50% mortality of rohu fingerlings (LC_{50}) as obtained by probit analysis was observed at a concentration of 0.137 mg l^{-1} for 96 h. During the study, the rohu fingerlings were observed to be very sensitive to 'Phorate 10G' as evident from the behavioural responses such as erratic swimming, fall in opercular activity and increased gulping of air to meet the respiratory requirement.

In the present study, fish fingerlings were also exposed to sub-lethal concentrations in order to find out any possible carcass biochemical variations. The sub-lethal concentrations of 0.05 and 0.075 mg l^{-1} were selected based on the LC_{50} values of 'Phorate 10G' *i.e.* 0.137 mg l^{-1}. The results of the carcass biochemical analysis are presented in Table 2. Even at the lower concentration of 'Phorate 10G',

Table 1. Observations on percentage mortality of rohu fingerlings (n=10) in the range finding assay, after every 6 h post-exposure to different concentrations of 'Phorate 10G'.

Test concentrations (mg l^{-1})	Replications	Mortality of fingerlings observed at different time intervals (%)				Cumulative mortality (%)	Average mortality (%)
		6 h	12 h	18 h	24 h		
Control	1	0	0	0	0	0	0 ± 0
	2	0	0	0	0	0	
	3	0	0	0	0	0	
	1	0	0	0	0	0	0 ± 0
0.002	2	0	0	0	0	0	
	3	0	0	0	0	0	
	1	10	0	10	10	30	20.0 ± 1.8
0.02	2	0	10	0	10	20	
	3	0	0	0	10	10	
	1	10	10	0	20	40	60.0 ± 0.84
0.2	2	10	20	30	10	70	
	3	20	10	30	10	70	
	1	30	20	10	20	80	76.67 ± 1.18
2.0	2	10	10	20	30	70	
	3	10	20	30	20	80	
20.0	1	20	30	20	30	100	100 ± 0
	2	10	20	30	40	100	
	3	30	10	50	10	100	

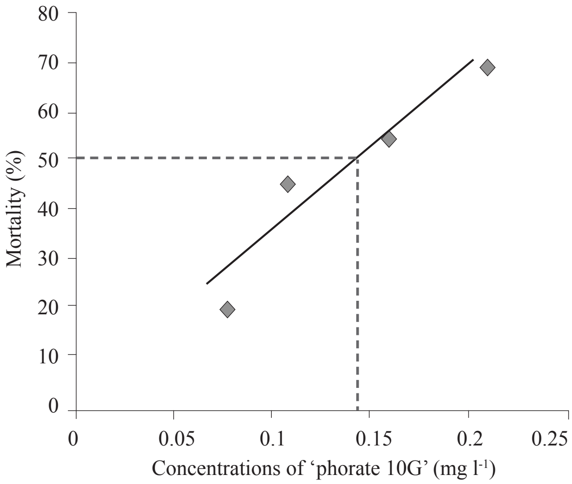

Fig. 1. Cumulative percentage mortality of rohu fingerlings exposed (96 h) to Phorate 10G

the values of protein and fat varied significantly ($p<0.05$) from those of control fish. Significant decrease ($p<0.05$) in the crude protein and crude fat contents of fingerlings were observed when exposed to different sub-lethal concentrations. Carcass proximate composition showed significant difference ($p<0.05$) between control fish and those of fish exposed to various sub-lethal concentrations of Phorate 10G, at the end of the 30 days exposure period (Table 2).

The fish body constituents such as protein, lipid and carbohydrate play an important role in energy metabolism. Any variations in the aquatic environment due to pollution are generally reflected on the body composition of aquatic animals. Palanicihamy *et al.* (1986) observed an increase in moisture and ash levels and decrease in crude protein and crude fat contents when exposed to sub-lethal concentrations of monocrotophos. Vutukuru (2005) reported appreciable decline in the biochemical profiles of total glycogen, total lipids and total protein contents of fish. However, the decrease in protein content was significant only at the end of 96 h. Palaniappan *et al*. (2009) observed a decrease in the nutritive value of muscle of *C. catla* fingerlings due to lead toxication. Reduction in protein content was observed when *Channa punctatus* (Bloch) was exposed to organophosphate insecticide chlorpyrifos (Jaroli and Sharma, 2005). They observed that the reduction in protein content might be due to increased utilisation of protein to meet out the energy demand under stress condition. Venkataramana *et al.* (2006) recorded significant decrease in protein in the gobiid fish, *Glossogobius giuris*, when subjected to malathion (0.5 ppm) for 96 h. In the present study, crude protein, crude fat, moisture and total ash contents showed sensitivity to the sublethal exposure of 'Phorate 10G'. Crude protein and crude fat content decreased while moisture and total ash contents increased with increased concentration and exposure period. Results of the present study clearly indicated that with increase in concentration and period of exposure, "Phorate 10G' affected the carcass proximate composition of rohu fingerlings which could be attributed to the energy requirement towards combating the toxic effects of pesticide exposure in the fingerlings of *L. rohita*.

Table 2. Biochemical composition of rohu fingerlings exposed to sub-lethal concentrations of 'Phorate 10G'

Proximate composition (%)	Initial	Phorate 10G concentration (mg l^{-1})								
		Control			0.05			0.075		
		10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Moisture	7.40±0.01 ^{d}	7.42±0.01 ^{d}	7.42±0.04 ^{d}	7.35±0.03 ^{c}	7.39±0.01 ^{b}	7.37±0.03 ^{b}	7.43±0.01 ^{ab}	7.86±0.01 ^{a}	7.91±0.01 ^{a}	7.90±0.05 ^{a}
Crude protein	66.08±0.002^{a}	66.10±0.01^{a}	66.09±0.04^{a}	66.08±0.03^{a}	65.86±0.03^{b}	64.62±0.25 ^{bc}	63.41±0.18^{c}	65.83±0.35^{b}	64.32±0.25 $^{b c}$	62.36±0.03^{d}
Crude fat	10.25±0.03 ^{a}	10.13±0.06^{a}	9.93±0.02^{a}	9.93±0.03^{a}	8.99±0.007^{b}	8.83±0.02^{b}	8.38±0.02^{c}	8.76±0.02^{b}	8.41±0.02^{c}	7.83±0.02^{d}
Total Ash	15.67±0.02 ^{d}	15.69±0.01 ^{d}	15.73±0.02 ^{cd}	15.80±0.03 ^{cd}	15.90±0.01^{c}	16.16±0.04 ^{bc}	16.16±0.02^{b}	16.07±0.03 ^{b}	16.23±0.01 ^{ab}	16.73±0.02 ^{a}

± : Standard error of mean, Values shown in rows that have different superscripts (a,b,c,d) differ significantly ($p< 0.05$).

References

AOAC 2006. *Official methods of analysis*, 18th edn. Association of Official Analytical Chemist, Washington, D. C., 1094 pp.

Fisher, R. and Yates, Y. 1963. *Statistical table for biological, agriculture and medical research*, 6th edn., Hingyip Printing Co., Hongkong, 146 pp.

Jaroli, D. P. and Sharma, B. L. 2005. *Effect of organophosphate insecticide on the organic constituents in liver of Channa punctatus*. *Asian J .Exp. Sci*., 19: 121-129.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randal, R. J. 1951. *Protein measurement with the Folin phenol reagent*. *J. Biol. Chem*., 193(1): 265-275.

Palaniappan, P., Vadivelu, M. and Vijayasundarm, F. 2009. Fourier transform Raman spectroscopic analysis of lead-exposed muscle tissue of *Catla catla*. *Romanian J. Biophys.,* 19(2): 117-125.

Palanichamy, S., Baskaran, P. and Balasubramanian, M. P. 1986. Sublethal effects of malathion, thiodon and ekalux on carbohydrate, protein and lipid contents on muscle and liver of *Oreochromis mossambicus*. *Proc. Symp. Pest. Resid. Env. Poll*., p. 97-102.

Reish, D. L. and Oshida, P. S. 1987. Manual of methods in aquatic environment research, Part 10, Short Term Static Bioassay. *FAO Fisheries Technical Paper,* FAO, Rome, p. 1-62.

Sadasivam, S. and Manickam A. 1997. *Biochemical methods,* 2nd edn., Newage International Publishers (P) Limited, New Delhi and Tamil Nadu Agriculture University, Coimbatore, 22 pp.

Sulekha, B. T., Anna Mercy, T. V. and Nair, J. R. 1999. Lethal toxicity of monocrotophos on the juveniles of rohu and mrigal. *Indian J. Fish*., 46(3): 319-321.

Venkataramana, G. V., Sandhya Rani, P. N., Murthy, P. S. 2006. Impact of malathion on the biochemical parameters of gobiid fish, *Glossogobius giuris* (Ham). *J. Environ. Biol*., 27(1): 119-122.

Vutukuru, S. S. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health*, 2(3): 456- 457.

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