International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 9 [4] December 2018 : 01-05
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www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA **ORIGINAL ARTICLE**

DOI: .10.15515/iaast.0976-4828.9.4.15

Toxic Effect of Phosalone an Organophosphate pesticide on Protein Levels in Some Tissues of Fresh Water Fish *Channa punctatus*

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ABSTRACT
The objective of present study is to evaluate the effect Phosalone on proteins metabolism in fingerlings of Channa Punctatus. Based on the acute toxicity study the lethal concentration (LC_{50}) of Phosalone for Channa punctatus has been calculated as 0.309 mg/L at 96 h. The fish was exposed to sub lethal concentration of 0.038 and 0.077 mg/L for a period of 10, 20 and 30 days. The sublethal effect of pesticide was investigate in liver, muscle and intestine tissues during exposure period of 10, 20 and 30 days. The treatment reduced the decrease in the total protein level in the all the organs and maximum reduction was recorded in liver than other organs. The decreasing protein concentration was directly related to duration of the exposure period. The sublethal effect of Phosalone, significantly reduced the protein content of fish.
Key words: *Channa punctatus,Phosalone, Acute toxicity.*

Received 22/07/2018 Revised 20/08/2018 Accepted 09/09/2018

Citation of this article
M.Sudha, M. Ahamed Basheer, M.Raffiq Hussain.. Toxic Effect of Phosalone an Organophosphate pesticide on Protein Levels in Some Tissues of Fresh Water Fish *Channa punctatus*. Int. Arch. App. Sci. Technol; Vol 9 [4] December 2018. 01-05.

INTRODUCTION

Many chemicals pollutant from electroplating, paint, pharmaceutical, leather, paper mills and agrochemicals industries were discharges into water, it leads to serious environmental problem in past few decades. These chemicals, affect the physico chemical properties of aquatic ecosystem. Among the chemicals, pesticides are highly toxic to living organisms, also it easily reached to aquatic and terrestrial environment by agricultural and household practice. Recent years, the pesticide consumption was drastically increased for high productivity of plant yield to provide the adequate food to fast growing population.

These pesticides enter into aquatic environment, it is great threat to aquatic fauna especially fishes, which constitute one of the major sources of protein rich food for mankind [1]; also it toxic to *Daphnia*,alga, *Lemna* (2-4), it leads to affect the food chain of the aquatic organism. The agrochemicals might be accumulating in fish and carry over to human being and caused health hazards [5]. Monocrotophos and lambda cyhalothrin [6], Phenthoate [7] were showed toxicity and biochemicals changes on *Labeorohita*. According to Alishahi et al. [8] agrochemical diazinon exhibited acute toxicity against *Barbus sharpeyi,* also sublethal effects such as reduce the haematological parameter of RBC, WBC,Hb and globulin.The agro chemicals, chlorpyrifos, endosulfan, and bifenthrin showed toxicity to common carp and *Ctenopharyngodon idella* at µg/L concentration [9].The accumulation of pesticides

produces some physiological, biochemical and morphological changes in the freshwater fauna [10]**.** Generally, agrochemicals are highly toxic to fish at lower concentration. Hence, the present study was investigated to evaluate the impact of Phosalone on protein content in various tissues of the fish *Channa punctatus*.

MATERIAL AND METHODS

Collection of fish

The live fish *Channa punctatus* (10 ± 5 g) were collected from local pond, Thiruvithancode, Kanyakumari District. The fish was selected without any pathological infection, by visual examination. Care was taken, while carrying fish to the laboratory for reduce hyperactivity, physical injuries; finally the fish washed with disinfectant (0.1% potassium permanganate ($KMnO_4$) solution). After that the fish was acclimatized into large cement tank containing chlorine free, bore well water for 10 days under normal temperature. Before released the fish, the tank washed with 1% $KMnO_4$ to avoid the fungal infection. Water was changed in alternate days. The fish were fed *ad libitum* with the formulated fish diet prepared from ground oil cake and rice bran in the laboratory. All the physicochemical parameter was maintained with in the limit during the acclimation and experimental period.

Experimental Design

Acute toxicity

Healthy fish, *Channa punctatus,* was chosen at random from the acclimatized tanks.Ten number of fishes were introduced into eachtreatments *i.e* 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5% concentrations and control for a period of 96 hrs.

Sublethal effects

Based on the acute toxicity result, sub-lethal concentrations, *viz.,* 0.077($1/4^{th}$) mg/L and 0.038 mg/L ($1/8^{th}$) were chosen to expose the fish for biochemical studies. The fishes were kept at each concentration for the period of 30 days. All the fishes were regularly fed with formulated fish diet prepared from ground oil cake and rice bran during the experimental period.

Protein estimation

After 10, 20, and 30 days exposure, the control and treated fishes were taken out and the following tissues, viz liver, muscle and intestine, were dissected out under aseptic condition. The wet sample were used for total protein estimation by the methods of Lowry *et al.* (1951). Four replicates were maintained for each concentration and control.

RESULTS AND DISCUSSION

The physicochemical parameter of the water (saturated oxygen > 70%, pH 7.2-7.9, temperature 23.5-24.8 °C, Hardness 140 mg/L and photoperiod 12: 12 h dark and light cycle) were maintained during acclimation and experimental period. Present study, the different concentration of the pesticide exhibited 10-100% mortality during the exposure period (Table 1). LC_{50} values were found to be 0.778, 0.633, 0.554, 0.416, 0.351, 0.309 and 0.261 mg/L for 12, 18, 24, 48, 72, 96 and 120 hours respectively(Table 2). Similarly,the acute toxicity of pesticides in different fish were studies by many researchers; *Brycon amazonicus* [12], *Nemacheilus botia* [13], *Xiphophorus helleri*[14], *Oreochromis niloticus*[15] and *Colossoma macropomum*[16].In the present study, duration of the pesticide treatment increased the LC_{50} value was decreased. Similarly,Gavit and Patil [17] who stated that, the agrochemical acephate toxicity was increased (based on the LC_{50}) when duration of the exposure period increased.

The result of the present study showed significant decrease in protein content in the tissues. The protein content in all the control tissue samples showed an increasing trend with increasing exposure period and in treated tissue samples there is a decreasing trend with increasing exposure period.In the exposed fish, the total protein in the muscle, liver and intestine showed a decreasing trend from the beginning of the exposure (Table 3). The results revealed that liver contained the maximum amount of protein (10.88 – 12.04 mg/100 mg wet tissue) and it was followed by muscle (10.47 – 11.62 mg/100 mg wet tissue) and intestine (8.29 – 9.12 mg/100 mg wet tissue) in control fish. The percentage of reduction in the muscle protein content over the control at 0.038 mg/L was 27.12, 35.24 and 46.38%.Whereas at 0.077 mg/L, it was 30.47,42.44 and 55.42% for 10, 20 and 30 days of exposure respectively. The decline in the protein level was higher in the liver tissue

than in the muscle and intestine. In the liver, the protein level was reduced to 8.97 mg/100mg wet tissue in the lowest concentration (0.038 mg/L) on 10th day and it was found to have been decreased further to 5.91 mg/100 mg wet tissue after 30 days at the same concentration and the decrease was equivalent to 50.91 % reduced over control. A maximum percentage of reduction (62.71%) was observed after 30 days at 0.077mg/L. In the present study, duration of the exposure period was increased in sublethal toxicity, the protein concentration of liver, muscle and intestine was decreased.

Table 1. Acute toxicity effect of Phosalone on *Channa punctatus*

Pesticide concentration mg/L	Percentage of mortality						
	12 hr	18 hr	24 hr	48 hr	72 hr	96 hr	120 hr
1	_	_	_	_	_	_	_
1.5	_	_	_	_	_	_	10
2	_	_	_	_	_	10	20
2.5	_	_	_	_	10	30	40
3	_	_	_	10	30	40	60
3.5	_	_	10	30	50	60	70
4	_	_	10	40	70	80	90
4.5	_	_	20	60	80	90	100
5	_	10	20	80	90	100	
5.5	_	20	40	80	100		
6	10	40	60	100			
6.5	20	60	80				
7	30	70	90				
7.5	40	80	90				

Table 2. Calculation of log – dose probit Regression line for mortality experiments (96 hours) in which same sized *Channa punctatus* were exposed to different concentration of the Phosalone pesticide in the Basvine technique .

(1) Dose %	(2) No.	(5) Mor. %	(6) Log dose	(7) Emp. Pro.	(8) Exp. Pro.	(9) Work Pro.	(10) Wt. Coef.	(11) Weight W	(12) wx	(13) wy	(14) y
0.20	10	10.00	0.30	3.72	3.53	3.75	0.27	2.69	0.81	10.09	3.45
0.25	10	30.00	0.40	4.48	4.33	4.49	0.53	5.32	2.12	23.89	4.25
0.30	10	40.00	0.48	4.75	4.98	4.75	0.64	6.37	3.04	30.28	4.90
0.35	10	60.00	0.54	5.25	5.54	5.24	0.58	5.81	3.16	30.47	5.45
0.40	10	80.00	0.60	5.84	6.02	5.82	0.44	4.39	2.64	25.57	5.93
0.45	10	90.00	0.65	6.28	6.44	6.27	0.30	3.02	1.97	18.94	6.35
0.50	10	100.00	0.70	7.33	6.82	7.13	0.18	1.80	1.26	12.84	6.73

STATISTICS

SW = 29.400 SWX= 15.004 X Bar= 0.510 SWY=152.079 Y Bar= 5.173
SWX*X= 8.018 SWY*Y= 808.154 SWXY = 80.322
b Value = 8.257
Regression Equation y = 8.257x + 0.96
If y=5.0 then x = 0.489 This corresponds to dose of 0.309
Variance 0.0006 Chi-square 1.12 (with 5 Deg. of freedom p)
Lower Limit 0.4404 Log Dose 0.4894 Upper Limit 0.5384

Our result corroborates with earlier finding of Kumar et al. [18] who stated that sublethal concentration of thiamethoxam reduced the protein level of *C. punctatus*.Pesticide treated *C. punctatus*, brain, muscle and gills enzymes of acetylcholinesterase activity was decreased

dose dependably [19]. Thoker [20] who has studied that Carbofuran and Malathion, reduced the total protein concentration of liver of*C. punctatus* at different interval period of 7, 4, 21 and 28 day after treatments. Mirghaed et al [21] reported that indoxacarb treated common carp gill and kidney were severely damaged and also, they found biochemical alteration in treated fish. The decrease in protein content of *Channa punctatus* intoxicated fish in the present study also indicates the physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress the animals, require high energy. This energy demand might have led to the stimulation of protein catabolism. Similar change was observed in *C. punctatus* exposed to technical grade malathion by Agrhariet al[22] and Tilak et al [23] explained the reduction of protein content of liver, brain and ovary *of* C. *punctatus* exposed to fenvalerate. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress [24]. Such a decrease in the protein content of fish when it is under chronic stress of acetamiprid could be due to the degradation of proteins as a source of energy to meet the extra energy demand for its maintenance at the time of stress [25]. Sandhya et al [26] reported that reduction in protein content could be due to its utilization to mitigate the energy demand when the fish are under stress.The present study revealed the reduction in protein levels in the tissues of *Channa punctatus* by following acute exposure of toxicant Phosalone. The present work indicates that Phosalone causes alterations in the protein metabolism of fresh water fish *Channa punctatus*.

Table 3 Changes in the level of Protein content (mg / 100 mg wet tissue) in the selected tissues (Liver, muscle and intestine) of *Channa Punctatus* exposed to sub lethal concentrations of Phosalone

Organs	Days	Control	Conc. of pesticide(mg/L)	
			0.038	0.077
Liver	10	10.88±0.01	8.97±0.02 (17.55%)	7.41±0.01 (31.89%)
	20	11.17±0.01	7.62±0.03 (31.78%)	6.52± 0.01 (41.63%)
	30	12.04±0.02	5.91±0.02 (50.91)	4.49 ± 0.01 (62.71%)
Muscle	10	10.47±0.02	7.63±0.02 (27.12%)	7.28±0.01 (30.47%)
	20	10.98±0.03	7.11±0.03 (35.24%)	6.32± 0.02 (42.44%)
	30	11.62±0.02	6.21±0.03 (46.38)	5.18 ± 0.03 (55.42%)
Intestine	10	8.29±0.01	6.51±0.02 (21.47%)	5.04±0.01 (39.21%)
	20	8.61±0.01	4.82±0.03 (44.02%)	4.02± 0.01 (53.31%)
	30	9.12±0.02	4.36±0.02 (52.19%)	3.61 ± 0.01 (60.42%)

All the values are <0.05 level significance

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