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Analysis Of Biomagnification Of Pesticide, Hematological Biochemical Lethal Effcts On Pesticide Toxicity Fresh Water Streams Fingerlings Of *Punitus Denisinii (Day,1865)*

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ABSTRACT
The Indian fresh water stream fish *Punitus denisinii* was exposed cypermethrin pesticide. high usage of pesticide in the experiment were determined as sublethal and median lethal concentration at 96hr of exposer. These LC_{50}value indicate that cypermethrin is highly toxic to fish, significant changes observed like respiratory, hematological biochemical and enzymological parameter in fish were observed. The effect of cypermethrin the decrease in acid phosphatase in liver suggested the un coupling of phosphorylation by toxicity acid phosphatase were significant decrease in liver tissue when composed to those of muscle and gills in the fish *Punitus denisinii* collected from industrial polluted fresh water streams.

Key words: Indian major carp**,** pesticide**,** sub lethal and median lethal concentration, hematological parameter, cypermethrin.

INTRODUCTION

Western Ghats of South India is a renowned UNESCO world Heritage site and is one of the 'Hotspots" of biological diversity, popularly known as "Great Escarpment of India "Western Ghats of South India is well known for the rich freshwater fish fauna with a high level of endemism Pesticides are widely used in modern agriculture to aid in the production of high-quality food. However, some pesticides have the potential to cause serious health and environmental damage. Though the pesticides are applied to enhance agricultural production while the indiscriminate and contaminate the biota. Subsequent to the translocation of pesticides to aquatic environment the non-targets such as fish are exposed to low concentration over a long period and affect the efficiency of various life parameters and seem to produce many physiological and Biochemical changes in fish. Fish have been valued for many years as excellent indicators of water quality. High usage of pesticide in the field, it affects both biotic and abiotic environment. The oxygen consumption (biotic) is a very sensitive physiological process and the change in respiratory activity has been used as an indicator of stress in animals exposed to toxicants. A number of investigations on the effect of pesticides on the Oxygen Consumption of fish have been reported (Ram Nayan Singh et al., 2014; Mohammad Illiyas Hussain et al., 2015; Sivakumar 2015). Stresses and pollutants generally cause relatively rapid changes in Blood characteristics of fish (Kandeepan 2013; Deshmukh, 2016). A reduction in hemoglobin content and erythrocyte population resulting in anemia have also been suggested as reason for drop in Oxygen uptake in fish *Punitus denisinii* exposed to lethal Concentration of cypermethrin (Jayaprakash and Shettu, 2013). Though, the biochemical, Physiological and enzymatic parameters are the common biomarkers of exposed fish to toxicity of pollutant. Since blood glucose level is an important parameter to assess the stress condition of fish by pesticides. Enzymes play a significant role in food Utilization and Metabolism. Phosphatase plays an important role in synthesis and transport of metabolites across the membrane, secretary activity, and protein synthesis and glycogen metabolism. Pesticide pollution also affects the activity of enzymes and produce metabolic changes at cellular levels. The toxic effects of cypermethrin compounds on the activity of alkaline phosphatase in various tissues of fishes have been worked out by various workers. Dubey et al., (2014) reported significant inhibition of alkaline Phosphatase in liver, intestine and muscle tissues of *Punitus denisinii* when exposed to cypermethrin. The decrease in acid phosphatase in liver suggested the uncoupling of phosphorylation by toxicity. Acid phosphatase was significant decrease in liver tissue when compared to those of muscle and gills in the fish *Punitus denisinii* collected from Industrial polluted lake. The effect of Rogor on the activity of alkaline phosphatase was studied by Borah and Yadav (1996). In view of the paucity of information regarding the effects of pesticide, cypermethrin Respiratory, hematological, Biochemical and enzymological parameters in *Punitus denisinii* were made in this investigation.

MATERIAL METHORDS:

Commercially valuable and edible fresh water fish *Punitus denisinii* used in this experiment. The length and weight of the fishes ranged between 10-15 cm and 25-30 g, respectively, were acclimatized to laboratory conditions for 10 days and separated into groups (10 each). During the acclimatized period fishes were fed ad-libitum with rice bran (or) powdered oil cakes. The median lethal concentration (LC50) and sub lethal concentrations were found out by exposing the fish to different concentrations of Phosalone (1.5, 2, and 2.5 PPM) for 4 days and control group was also maintained separately. Pesticide, organophosphate represent one of the most widely used classes of pesticide with high potential for human exposure in field of cultivated area. Phosalone (C12H15ClNO4PS2) is a broad-spectrum organophosphate pesticide widely used to control pests in agricultural crops. It is commercially available organophosphate pesticide and is more toxic to living beings. Before starting the experiment, the Oxygen content of water used in the animal chamber was estimated by Winkler's method. Blood sample was collected from the control and experimental fishes by cardinal vein puncture using an insulin syringe containing 0.1ml of 0.2% EDTA of each group at 1st, 2nd, 3rd and 4th day of experiment. Hemoglobin was estimated by Darbkin’s method (Suganthi et al. 2015a). The blood sugar was estimated by O - toludine method. The alkaline phosphatase was estimated by using the method of Bergmeyer (1963) as modified by Butterworth and Probert (1970).

RESULTS AND DISCUSSION

Studies to evaluate the toxicity of cypermethrin to *Punitus denisinii* (3+0.5 g) exposed for 96 h showed nil mortality at 2.5 µg/l. The mortality rate increased with increase in the concentration of cypermethrin (Table.1). When percent mortality was plotted against log concentration of cypermethrin, a sigmoid curve was obtained (Fig. 1). The 96 h LC50 value was obtained from sigmoid curve was 4.0 µg/l. The percent mortality after transforming to probit mortality was plotted against log concentration of cypermethrin using probit method (Finney, 1971). In this a straight line was obtained and the LC value obtained from the graph was 4.0 µg/l also determined the 96 h LC50 value (Fig 2). The 96 h LC50 value was further verified by the method of Dragstcdt-Behren’s equation (Carpenter, 1975) and the value h LC50 value determined by the above three methods was 3.99g/l (Table 2). The upper and lower 95% confidence limits were found to be 4.231 ug/L and 3.668 ug/L, respectively (Table 3).

NORMAL FISH

Control fishes maintained a fairly compact school, covering about one third of the bottom during the first five days of the 15 days experiment. By fifth day, the school became less compact covering up to two-third of the tank area. Fishes were observed to scrap the bottom surface. When startled, they instantly formed a tight school that was maintained briefly. They were sensitive to light and moved to the bottom of the tank when light was passed into the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behavior was observed.

TREATED FISH

When the fish were exposed to the lethal concentration of cypermethrin, they migrated immediately to the bottom of the tank. The schooling behavior was observed to be disrupted on the first day itself and the fish occupied twice the area than that of the control group. They spread out and appeared to be swimming independent of one another. Irregular, erratic and darting movements followed this with imbalanced swimming activity and non-stop movement of pectoral fins (fanning). The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. On the 4 day they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom. Erection of dorsal and anal fins while motionless (Lateral display) They often barrel-rolled or spiraled at intervals and engulfed the air through mouth before respiration ceased. Prior to death, the pectoral and pelvic fins of affected fish were spread forward interiorly and movements and respiration rate declined. The fish eventually died with their mouth and operculum wide opened. A change in color of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was seen in dead fish. The rate of whole animal oxygen consumption of control and cypermethrin treated fishes are presented in Table 4 and Fig 3. Fish exposed to a lethal concentration depicted increased (Fig. 1) oxygen consumption on day 1 (8.597%) to day 2 (17.409%) and the increase was decreased (1.289%) on day 4 (Table 2). In the sub lethal concentration oxygen consumption increased (Fig. 1) on day 1, 5, 10 and 15 as compared to the control (Table 2) in the order of 1 (2.7942%) <5(17.141%) <10(16.497%) <15(22.515%). In the control fish tissue, maximum quantity of Ach was observed in brain followed by muscle, gill and liver (Table 5 and figure 4). The accumulation of Ach under the median lethal concentration of cypermethrin increased gradually up to 96 h in all the tissues namely gill, muscle and liver. Liver recorded the lowest concentration 18.28 µM/g wet wt., which is 9.11 percent over control at 96 h. A maximum increase of 55.41% was noted in the gill tissue at 72 h of exposure. The acute test for a long time has been a major component in toxicity testing (Braunbeck, 1998). In which acute chemical toxicity is determined as a 96 h LC50 value. However, the environmental significance of death of individuals after short term exposure to high concentration is questionable (Marigoudar, et. al 2009). In the science of aquatic toxicology, fish play an important role in toxicity testing and hazard evaluation, as do the white rat and guinea pig in mammalian toxicology. The bio-assessments of toxicity of cypermethrin with reference to aquatic biota, especially fish is crucial in establishing the toxicity evaluation. Toxicity of pyrethroids to different fish species varies between 0.001-71.5 ppm (Table 4). This data clearly indicates that cypermethrin is also toxic to *Punitus denisinii* as to other fish species mentioned in the table, since cypermethrin is readily taken up by aquatic organisms. Various symptoms of poisoning can be observed from studies involving the determination of LC50. In the present study, the fish maintained in normal freshwater behaved in usual manner i.e., they were very active with their well-coordinated movements. They were alert at slightest disturbance. But at the sublethal concentrations of cypermethrin they became irritable and hyper-excited. Jumping movements as well as restlessness were observed and finally the fish turned upside down. Mucus secretion and loss of equilibrium were also observed. They slowly became sluggish with short jerky movements, surfacing and gulping of air erratic circular movements. Finally, they settled down at the bottom with loss of equilibrium and rolling of the body, convulsions prior to death. The fish very often come to the surface in order to avoid toxic environment. Moreover, examination of the gill of dead fish revealed that the gill lamellate color was changed from red to brown. Tilapia exposed to lethal and sub lethal concentration of endosulfun and lidane exhibited abnormal behavior at lethal concentration, spiraling, tremors, jerky movements and rapid opercular movements. The fish struggled hard for breathing, often moved to the surface to engulf atmospheric air and tried to escape the toxic aquatic medium. After a few hours, equilibrium was lost and the fishes spiraled and slowly moved upward in a vertical position. Finally they lost equilibrium completely and were flat at the bottom.David (1995), Deva Prakasa Raju (2000), in *punitus denisinii* and Devario malbaricus(jerdon,1889) respectively, also observed similar symptoms, In the present study, the results showed a time-and concentration dependent inhibition of AChE activity by cypermethrin in the tissues of the fish*, punitus denisinii* (Table 5a and Figure 5). Inconsonance with the decrease in the AChE activity there is a corresponding increase in the Ach content of the tissues (Table 5 and Figure 4) suggesting decrease in the cholinergic transmission and consequent accumulation of Ach in the tissues. At lethal and sub lethal concentrations, cypermethrin produced grated inhibition of AChE activity in gill, liver and muscle tissues. Further, these effects are seen following both acute and sub-acute conditions. Inhibition of AChE results in nerve impulses as nerves become permeable to sodium, allowing sodium to flow into the nerve. Pyrethroids delay the closing of the gate that allows sodium flow (Vijverberg and Van den Bercken, 1990) and thus, multiple nerve impulses rather than the usual single one occur. In turn, these impulses release the neurotransmitter Ach, which stimulates other nerves (Eells, 1992), ultimately resulting in buildup of Ach within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (Mileson, et. Al., 1998). Similar results were obtained in tissues and other fish species (Rao, 2006; Chawanrat et. Al., 2007; Elif and Demet, 2007). Cypermethrin also affects the enzyme ATPase involved in cellular energy production, transport of metal atoms and muscle contraction (El-Toukhy and Girgis, 1993). Blood is a pathophysiological reflector of the whole body and, therefore, hematological components may be considered as promising bioindicators in insecticidal toxicosis as in diagnosing the structural and functional status of fish exposed to toxicants. Hematological study plays vital role in monitoring fish health, pollution load, stress and disease. Therefore, it is pertinent to study the impacts of pollutants on fishes. In the present investigation, fish *Punitus denisinii* on exposure to lethal and sub lethal concentrations of cypermethrin showed considerable alteration in the level of different blood parameters.

Table 1: Mortality of *Punitus denisinii* in different concentration of cypermethrin at 96 hours of exposure period

Sl.No.	Concentration of cypermethrin (µg/L)	Log concentration	No. of fish exposed	No. of fish alive	No. of fish dead	Percent mortality	Probit mortality
1	2.5	0.3979	10	10	0	0	0
2	3.0	0.4771	10	9	1	10	3.72
3	3.5	0.5440	10	8	2	20	4.16
4	4.0	0.6020	10	5	5	50	5.0
5	4.5	0.6532	10	4	6	60	5.25
6	5.0	0.6989	10	1	9	90	6.28
7	5.5	0.7403	10	0	10	100	8.09

Table 2: LC50 value of cypermethrin for *Punitus denisinii* after 96 hours of exposure

Sl.No.	Name of the Method	LC50 value (in µgram)
1	Percent mortality (Sigmoid curve)	4.0 µg/1
2	Probit mortality (Linear curve)	4.0 µg/1
3	Dragsted and Behren's method	3.97 µg/1
4	Mean	3.99 µg/1
5	Standard Deviation	0.0141

Table 3: 96 h LC50, slope and 95% confidence limits of cypermethrin in the freshwater stream fish, *Punitus denisinii*

Pesticide	96 h LC50 value (µg/L)	Slope	95% Confidence limits	
			Upper limit	Lower limit
Cypermethrin	4.0±0.03	1.289	4.231	3.668

Table 4: Oxygen consumption (ml of oxygen consumed/g wet wt of fish/h) of the fish, *Punitus denisinii following* exposure to lethal (4.0 µg/1) and sub lethal (0.4 µg/1) concentrations of cypermethrin.

Estimation	Control	Exposure Periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	5	10	15
Oxygen consumption	0.1861G	0.2021G	0.2185G	0.2005C	0.1885G	0.1913	0.2180C	0.2168A	0.228A
SD±	0.003	0.002	0.003	0.002	0.001	0.004	0.029	0.042	0.044
% Change	..	8.597	17.409	7.737	1.289	2.7942	17.141	16.497	22.515

Table 5: Ach level (µM/g wet wt.) in the tissues of the fish, *Punitus denisinii* on exposure to the lethal and sublethal concentrations of cypermethrin.

Tissue	Control	Exposure Periods							
		Lethal (h)				Sublethal (days)			
		24	48	72	96	1	5	10	15
Gill	31.10E	35.21C	42.20H	48.34A	45.67B	37.97D	32.08D	34.88C	34.49C
±SD	0.65	0.24	0.29	0.34	0.32	0.22	0.22	0.24	0.24
%Change	..	13.21	35.69	55.41	46.82	2.78	3.14	12.15	10.90
Muscle	35.42H	37.53F	40.61C	47.55A	44.76B	36.54G	38.94E	39.96D	39.51D
± SD	0.50	0.26	0.28	0.33	0.31	0.25	0.27	0.28	0.27
% Change	……	6.02	14.64	34.21	26.35	3.13	9.92	12.79	11.54
Liver	16.75F	17.29E	18.15B	18.75A	18.28B	17.36E	17.79D	18.07C	17.43D
± SD	0.23	0.12	0.12	0.13	0.12	0.12	0.12	0.12	0.12
% Change	..	3.20	8.34	11.91	9.11	3.62	6.23	7.89	4.08

Means are ± SD (n=6) for a parameter in a ow followed by the same letter are not significantly different (P≤0.05) from each other according to Duncan's multiple range test. Values are means ±SD (n=6) for oxygen consumption in a row followed by the same letters and are not significantly different (P≤0.05) from each other according to Duncan's multiple range test.

CONCLUSION

Cypermethrin in the aquatic medium is a major factor responsible for drastic changes in the fish blood raise and fall in RBC and WBC count Hb content, PCV or hematocrit and MCV value MCH and MCHC concentration in insecticide –exposed fish provide important information on the general physiology and health status of fish under Investec µg/l. action thus fish blood following exposure to contaminants is at the best suitable bio-indicators in the field of environment toxicology it may be added here that changes observed in the whole animal oxygen.

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