



“Effect of pesticides on survival behavior, feeding pattern, growth and mortality of the marine edible fish *Lepturacanthus savala*”

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Abstract

Pesticides get easily dissolved and transported in water to be readily taken up by the aquatic biota. It reaches the food chain causing various physical, biochemical and morphotoxicological changes. Fish are important source of food so, it is essential to protect the health of fish population. In the present investigation adult live specimen of marine edible fish *Lepturacanthus savala* were caught from the Khar-Danda sea coast. When the fish were exposed to two sub lethal concentration ie 1/10 or 2.5 % and 1/5 or 5% of the two pesticides, Rogor technical grade 30% EC., and Sevin 36% w/w (active ingredients) on different aspects of Ribbonfish, (*length:65.2±1.25cm and weight 130.5±0.5gms*) like survival behaviour, food intake, growth and mortality, it showed remarkable changes. In comparison to control the sub lethal effects of these pesticides led to decrease in the survival rate with the increase in concentrations. Respiratory disruption was observed as coughing and yawning because of toxic stress. There was decrease in the amount of food intake of the treated fish as compared to control. The weight, length and breadth showed a decreasing trend in both the concentrations of pesticides though there were variations in weight in terms of growth in the pesticides treated fish. Higher percentage of mortality occurred with the increase in concentration and exposure period.

Keywords : *Lepturacanthus savala , sub lethal concentrations, Pesticides Rogor, Sevin*

INTRODUCTION

Pesticides are highly effective when reaches the aquatic ecosystem, Agarwal,(1984) and Shrivastav,(2005)^[2] greatly influences the non-target organisms, especially fish (Herger *et al.*,1995).The injuries of pesticides to aquatic environments are incontestable, Albaster(1969)^[1]and the significant increase of these chemical discharge in the water bodies from surrounding area has led to deleterious effects for inhabiting aquatic organisms, Livingstone (2001), Herger W *et.al.*,(1995)^[19]. Pesticides not only effect the aquatic ecosystems but due to bio-accumulation reaches the human food chain.^[20] It causes multiple changes in the organism by altering the rate of growth^{[8][9]} and survival, nutrition, behavioural pattern etc, Al-Nahdi. *et.al.*,(2009)^{[4][5]}. As the major part of world is dependent on fish source as food, it is essential to protect the health of fish population, Austin B(1998)^[7].Among contaminants, pesticides have been recognized as strong biological poisons because of their persistent nature and cumulative action, Carson,R.(1962)"Silent spring. Among the four groups of pesticide, one pesticide from Organophosphates group and one from the Carbamate group were selected for the study . Rogor an organophosphate^{[28][26]} is more frequently used pesticide among the different classes because of its high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment (Freedman, 1995)^[17] while, Sevin or 1-naphthylmethyl a carbamate , Trade mark of Union Carbide is widely in use as insecticides. They are unstable and breakdown quickly to relatively harmless products. In this paper two sub lethal concentrations i.e. 2.5% and 5 % of Rogor and Sevin were taken to determine their effect on different aspects of marine edible fish, *Lepturacanthus savala* (*cuvier 1829, Taxonomioc no 172387*) like survival behaviour, feeding , growth and mortality.

II. MATERIALS AND METHODS

Experimental Fish

Test organism *Lepturacanthus savala* , commonly called as Ribbonfish,Rizvi (2001)^[30] average weight 130.5 ± 0.5 gms and length 65.2 ± 1.25 cm caught from Khar –Danda sea-coast,Bandra,Mumbai, Gupta.I *et.al.*,(2009)^[18]were adjusted to the laboratory conditions for 15 days before experiment and were maintained as control throughout the experiment.

Water parameters

The physiochemical features of the experimental water were analysed by maintaining water temperature at $28 \pm 2^{\circ}\text{C}$, dissolved oxygen at 37.5 to 4.10 mg/l and pH at 6.8 to 7.9 according to the method as described in APHA (1995).^{[6][10]},

Experimental toxicants :

Organophosphate Rogor (Dimethoate) an insecticide of technical grade 30% EC from, Cheminova India Ltd, Bharuch, Gujrat , India and Sevin/Carbaryl - an insecticides a carbamate, 1-naphthylmethyl, 36% active ingredients (w/w), K.S.Tilak *et.al*(1981)^[21] Bayer's India Ltd, Trade mark of Union Carbide were obtained.

Experimental design :

Toxicity tests were conducted in 30 litre water capacity, colourless, large nontoxic glass tank containing dechlorinated water. Fish were fed with natural feed of shrimps during and at the time of experiment. Dechlorinated tap water was used for making various concentrations of the pesticide. Brungs(1973)^[10] W.D & Ferguson, D.E(1968)^[11]. 1 ml of the pesticide selected was mixed with 99ml of water to prepare stock of 0.1% solution. A narrow range of 2-10 ml concentration was used to find the median lethal concentration using minimum 10 fish for each concentration. Mortality was recorded for every 24 hrs upto 96 hrs using probit analysis method(Finney 1961)

Fishes were exposed to the sublethal concentrations of Rogor 2.5% i.e., 0.0682 µg/L and Sevin 2.5% i.e., 0.0663 µg/L (one-tenth of LC50-96 hrs) and Rogor 5% i.e., 0.136 µg/L and Sevin 5% i.e., 0.132 µg/L (one-fifth of LC50-96 hrs)were chosen for the present study, Doudoroff et al.,(1951)^[15] and the fish were exposed to toxicant for 24, 48, 72 and 96 hr. One group was maintained as control group. Ten fishes were maintained in each group. Both control and treated fishes were observed after the end of each duration.

Experimental Observations

Behavioral changes:

When the fish were exposed to two sub lethal concentration ie 1/10 or 2.5 % and 1/5 or 5% of the two pesticides, they migrated to the bottom of the test chamber immediately. This is because of the toxic stress. Their schooling behavior was totally disturbed and they were swimming independently and this was followed by irregular, erratic and dangling movements with the imbalanced swimming activity.^[12] The swimming behavior was in a cork screw pattern and rotating along horizontal axis and followed by "S" jerks, sudden, rapid and non-directed sport of forward movement like busted swimming. They exhibited peculiar behavior that is the fish were trying to leap out from the test chamber which can be viewed as escape phenomenon. Behavioral changes are caused due to inactivation of (AChE) acetyl cholinesterase activity which results in excess accumulation of acetylcholine in the cholinergic synapses leading to hyper stimulation. Tables 1 and 2 given below shows the behavioral responses due to two different concentrations of Rogor and Sevin.

Table 1 – Behavioral changes at two different concentrations of Rogor

Symptoms Observed	Concentration of Rogor in $\mu\text{g/l}$							
	2.5%				5%			
	Time of Exposure							
	24 Hrs	48 hrs	72 hrs	96 Hrs	24 hrs	48 hrs	72 hrs	96 Hrs
Lethargy	+	++	M	M	++	+++	M	L
Increased Mucus	++	+++	+++	+++	+++	+++	+++	+++
Skin Discoloration	-	+	+	+	++	++	+++	+++
Muscle Fasciculation	+	++	++	+++	++	+++	+++	+++
Respiratory Distress	++	+++	+++	+++	+++	+++	+++	+++
Feeding Behavior	N	LA	LA	LA	LA	LA	LA	RF

Degree of intensity of symptoms

+ Low, ++ Moderate, +++ Severe

L - Lethal

N – Normal feeding

M- Moribund

LA – Loss of Apetite

RF – Refusal of Food

Table 2- Behavioral changes at two different concentrations of Sevin

Stmptoms Observed	Concentration of Sevin in $\mu\text{g/l}$							
	2.5%				5%			
	Time of Exposure							
	24 Hrs	48 Hrs	72 hrs	96 hrs	24 hrs	48 Hrs	72 hrs	96 hrs
Lethargy	+	++	++	+++	++	+++	M	L
Increased Mucus	++	+++	+++	+++	+++	+++	+++	+++
Skin Discolouration	-	+	+	+	+	++	+++	+++
Muscle Fasciculation	+	++	++	+++	++	++	+++	+++
Respiratory Distress	++	+++	+++	+++	+++	+++	+++	+++
Feeding Behavior	N	N	LA	LA	LA	LA	RF	RF

Degree of intensity of symptoms

+ Low, ++ Moderate, +++ Severe

L- Lethal**N – Normal feeding****M- Moribund****LA – Loss of Appetite****RF – Refusal of Food**

Respiratory Changes :

Respiratory disruption was observed due to cough and yawning this is because of toxic stress. Fishes often barrel rolled or spiraled at regular intervals and engulfed the air through mouth before respiration ceased. The initial elevation in the oxygen consumption could be explained in terms of acceleration of oxidative metabolism during the initial hours of exposure, as a result of sudden response to the toxic stimulus of pesticide. The study reveals that both the pesticides are toxic to the fish. In *Tilapia mossambica* and even low concentration of dimethoate/Rogor^[36] stress create respiratory disturbance which ultimately leads to the deterioration of general health of the fish , Shereena et al.

A change in colour of the gill lamellae from reddish to light brown with coagulation of excess mucous on the gill lamellae were observed.

Effect on Feeding :

Feeding behavior was studied to find out the effect of different concentrations of pesticides on their food consumption, Portsev,P.I(1980)^[27].For this the fish were fed with definite weighed quantity of shrimps and prawns between 10.30 a.m and 11.30 a.m. After about three hours, the unconsumed food were siphoned out, lightly blotted with absorbent paper and weighed. The difference in the two weights were taken as the quantity consumed. The quantity consumed per gram weight were determined. Changes in food intake due to the effect of two different concentration of the two pesticides are presented in Table 3 and 4 respectively.

Table- 3 Food intake in grams/week in *Lepturacanthus savala* exposed to the two concentrations of Rogor pesticide

Time period	Control	Rogor 2.5% (1/10)	Rogor 5 % (1/5)
1 st week	21.23gms	9.56gms	17.31gms
2 nd week	23.98 gms	17.34gms	16.12gms
3 rd week	26.67gms	14.89gms	12.88gms
4 th week	29.18gms	12.51gms	11.06gms

Table - 4 Food intake in grams/week in *Lepturacanthus savala* on two concentrations of Sevin/Carbyl pesticide

Time period	Control	Sevin 2.5% (1/10)	Sevin 5 % (1/5)
1 st week	21.23gms	20.01gms	18.89gms
2 nd week	23.98 gms	18.64gms	16.34gms
3 rd week	26.67gms	16.75gms	14.08gms
4 th week	29.18gms	13.53gms	11.32gms

Effect on growth :

The growth was measured in terms of increase in the weight per fish. Observations were made once in a week and recorded till the end of the experiment(4 weeks),Kwok .K.Y et.al(2000)^{[22][23]}

A beaker with known quantity of water was taken, weighed and to this weighed beaker the test fish were added and weighed again. The difference in the two successive weights were taken as increase or decrease in weight in particular group during every week, recorded in the present study. The data on growth analysis is as per the method of Webb and Brett (1972).

The effects of two sub lethal concentrations of the two pesticides on the growth Md.A.Sweilum,(2006)^[26]were observed. The test fish, *Lepturacanthus savala* were exposed to two sub-lethal concentrations, i.e 1/10 or 2. 5% and 1/5 or 5% of the 96 hrs LC50 value of of Rogor and Sevin.

Table-5 Weight in grams/week in *Lepturacanthus savala* exposed to the two concentrations of Rogor pesticide

Time period	Control	Rogor 2.5% (1/10)	Rogor 5 % (1/5)
1 st week	135±0.42 gms	134±8.06 gms	133±2.03 gms
2 nd week	135±9.02 gms	133 ± 0.86gms	131±7.05 gms
3 rd week	136±8.64 gms	132±5.86 gms	130±4.28gms
4 th week	137±0.34 gms	134 ± 4.43gms	130±1.95gms

Table - 6 Weight in grams/week in *Lepturacanthus savala* on two concentrations of Sevin/Carbaryl pesticide

Time period	Control	Sevin 2.5% (1/10)	Sevin 5 % (1/5)
1 st week	135±0.42 gms	134±9.10 gms	134±0.06 gms
2 nd week	135±9.02 gms	133±8.32 gms	132±2.38 gms
3 rd week	136±8.64 gms	133±2.03 Gms	131±5.07 gms
4 th week	137±0.34 gms	133±0.05 gms	131±0.02 gms

Effect on mortality :

The LC50 value, four(4) different concentrations of Rogor and Sevin i.e., 0.5 ppm, 1.00 ppm, 1.50 ppm, 2.00 ppm were determined, Tiwari, et.al(2004)^[34]. A set of 20 acclimatized fishes were chosen randomly for toxicity determination and mortality rate was determined at the end of 24, 48 and 96 hrs. (Table-7& 8).

Table-7 Mortality due to ROGOR and SEVIN at 4 different concentrations of LC50

Concentration of pesticide	LC50 (Lethal concentration)	Rogor $\mu\text{g/l}$	Sevin $\mu\text{g/l}$
0.5 ppm	24hrs	0.898	0.865
	48hrs	0.993	0.906
	96hrs	1.325	1.509
1.00 ppm	24hrs	1.816	1.731
	48hrs	1.994	1.824
	96hrs	2.731	2.652
1.5 ppm	24hrs	1.724	2.596
	48hrs	2.987	2.730
	96hrs	4.056	4.161
2 ppm	24hrs	3.632	3.442
	48hrs	3.978	3.658
	96hrs	5.438	5.310

Percentage mortality

A separate group of 16 fish were set as control. The volume acetone in control were same the volume of pesticide of different concentration mixed with tap water. Mortality of fish per week for different groups were recorded during the test period. From this percentage of dead fish at the end of the test were calculated and used as the measure of the toxicant induced mortality. The observations were made at every week to note the number of fishes survived in particular concentration. Similar

observations were continued for 4 weeks exposure. From this percentage of mortality in the two sub-lethal concentrations were calculated at the end of the chronic exposure of 4 weeks. (Table-7 & 8)

Table 8: Percentage Mortality at various concentrations of Rogor and Sevin pesticide on *Lepturacanthus savala* of 96 hrs of LC50

Concen-tration of pesticide in mg/l	Number of fishes	Number of Mortality for Rogor	% Morta-lity	Number of Mortality for Sevin	% Morta-lity
0.0	16	0	0%	0	0%
0.5	16	0	0%	0	0%
0.75	16	2	12.5%	1	6.25%
1.0	16	5	31.2%	4	25 %
1.5	16	8	50%	6	37.5%
2.0	16	11	68.7%	9	56.25%
2.5	16	13	81.2%	11	68.75%
3.0	16	13	81.2%	12	75%
3.5	16	14	87.5%	13	81.25%
4.0	16	16	100%	14	87.5%

III. RESULT AND DISCUSSION

The toxicity is noticed by some specific behavioral characteristics during the experimentation periods in different time intervals, Satyavardhan.K (2013)^[32] The behavioral symptoms in the fish included loss of schooling behavior, swimming near the upper surface, hyper activity, zigzag movement, loss of buoyancy. The respiratory symptoms included elevated cough, increased gill mucous secretion, flaring of the gill arches, head shaking and restlessness before death. Some of the behavioral changes recorded are presented in Table-1and 2.

The changes in the amount of food intake due to two different concentration of the two pesticides as presented in Table 3 and 4 respectively showed that there was decrease in the amount of food intake of the treated fish as compared to the control, Zang,B(2004)^[34]The amount of feed declined in the 5% pesticide concentration

to 2.5 % pesticide concentration treated fish. So there is decline in food intake with the increase in pesticide concentration. Table – 3 & 4

The weight, length and breadth showed a decreasing trend in both the concentrations of pesticides though there were variations in weight in terms of growth in the pesticides treated fish. Table -5 & 6

The relation between the pesticides Rogor and Sevin concentration and mortality rate of Ribbonfish *Lepturacanthus savala* are shown in the Table- 7 and 8. The results indicated different mortality rate of fishes which increased with the corresponding increase in concentration of the pesticides. The mortality in control and treated fish for 0.5 mg/l concentration of pesticides were virtually absent. So it is concluded that higher percentage of mortality occurred with the increase in concentration and exposure period. Susceptibility of *Lepturacanthus savala* to the different doses of pesticides were duration and concentration dependent as mortality increased with an increase in concentrations. Almost same result was observed by Srivastav et. al., (2012) toxicology of Chlorpyrifos to a freshwater catfish, *Mystus seenghala*. The results of this study will be helpful to understand the toxicity of pesticide to fish and could be an indication of early warning signs of pesticide toxicity.

IV. SUMMARY AND CONCLUSION :

There are large variations in the safe levels estimated by different methods for the pesticides. In addition to dose and dose-time dependent increase in mortality rate, stress signs in the form of behavioral changes were observed in response to the test pesticides. Loss in weight was probably to cope up with the increasing doses so that the fish could overcome the toxic effect of pesticide to make a balance between growth and metabolism. This was in conformity with the effects of thiodon pesticides on *Clarias gariepinus*. There were increased mortality with an increase in concentration and exposure period of pesticides. The study on the survival data is helpful to determine the toxicity of pesticides in relation to time. The toxicity of the carbamate insecticide Sevin/carbaryl and its metabolite, 1-naphthol, have also been studied by Tilak et al.^[21]. The degradation product of the insecticide was found to be more toxic than the parent compound, to all the fish species.

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