



EFFECT OF ENDOHYPER ON BIOCHEMICAL CONSTITUENTS OF FRESHWATER FISH *MYSTUS VITTATUS*

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Abstract: Sublethal effects of endohyper on biochemical constituents were studied in *Mystus vittatus* for the period of ten days. *M.vittatus* exhibited maximum tolerance up to 0.006ppm concentration. In the sublethal concentration (0.002ppm) protein, carbohydrate, lipid content of muscle and liver were reduced and acid and alkaline phosphatase enzyme activity were increased when the days of exposure increased.

Key words - *Mystus vittatus*, Endohyper, Protein, Carbohydrate, Lipid.

I. INTRODUCTION

Insecticide Endohyper is a class of chlorinated hydrocarbon, that's widely recognized for its insecticidal activity and is getting used broadly in agriculture operations. Several workers were reported their impact on non-target organisms including fish (Bharti, and Rasool, 2021; Olsvik *et al.*, 2020; Garima, and Neera, 2018; Pallavi, *et al.*, 2016; Kandeepan, 2014). Similarly, Malathion is noticeably poisonous to *Channa punctatus* and observed behavioural and biochemical changes in fish (Islam, et al., 2019), *Channa punctatus* behavioural changes have been observed by means of the exposure of endosulfan (Garima, and Neera, 2018). Also, significant changes were occurring in respiratory, haematological, biochemical, and enzymological parameters in fish were observed by Kalaimani and Kandeepan, (2017). The present investigation was carried out on the biochemical changes in the quantity of carbohydrate, protein, lipid, and acid and alkaline phosphatase enzyme activity of *M.vittatus* due to its exposure to endohyper.

II. MATERIALS AND METHODS

M.vittatus fishes (3.0±0.25g) were collected from local ponds near Palani, Tamilnadu. They were transported to the laboratory in polythene bags containing oxygenated water. The fish were acclimated for seven days at 28 + 0.5°C in the laboratory condition feeding on goat liver. From this stock, fish were recruited for the experiment. Endohyper (Endosulfan 35% + Cypermethrin 5%) pesticide purchased from a local shop at Palani. Toxic, median, lethal (LC50), and sublethal concentrations of endohyper were determined by exposing the fish to different concentrations for 96 h. The experimental media were changed every 24h. The LC₅₀ value was estimated by the probit analysis method (Finney, 1978).

A. Experimental design

A group of ten individuals was introduced in glass aquaria containing 10 liters sublethal media (0.002ppm concentration) of endohyper (Treated) along with a control group with pesticide-free water (Control). In each case, triplicate was maintained with an ad-libitum diet. The rearing media was changed daily and experiments were carried out for ten days. Everyday fish was sacrificed and removed the muscle and liver for the estimation of carbohydrates (Roe, 1955); Protein (Lowery *et al*, 1951); fat (Folch *et al.*, 1957); and acid & alkaline phosphatase activity (Bergmeyer 1963).

III. RESULTS AND DISCUSSION

The percent mortality of the fish *M.vittatus*, when exposed to different concentrations of endohyper as a function of exposure time, revealed that a concentration of 0.006ppm was found as median lethal concentration (LC50) at 96h exposure (Table1). Previous reports suggested that 1.5PPM of Phosalone was the LC50 for the fish *Labeo rohita* and 8 ppm of malathion for *C. punctatus* (Bharti, and Rasool, 2021; Kalaimani and Kandeepan, 2017). The LC50 values at 96 hours of exposure of Chlorpyrifos were estimated to be 23.10 ppm, 20.32 ppm, 16.61 ppm, and 13.90 ppm for stinging catfish (*H. fossilis*), spotted snakehead (*C. punctatus*), climbing perch (*A. testudineus*), and tangra (*B. tengana*), respectively (Zahan *et al.*, 2019). So the different fishes have different tolerance range against the toxic effects of the same pesticide (Anamika Singh and Ajay Singh, 2017). Fish poisoned by the pesticide showed uncoordinated movements, restlessness, and exhibiting erratic swimming movements. Many workers have found this type of observation when fish exposed to pesticide medium (Bharti, and Rasool, 2021; Zahan *et al.*, 2019; Kalaimani and Kandeepan, 2017).

The amount of carbohydrate, protein, lipid, and phosphatase enzyme activity in liver and muscle of treated and control fish *M.vittatus* were presented in tables 2,3,4,5,6 & 7. The carbohydrate, protein, lipid content of muscle and liver were decreased in treated fish and increased in control fish *M.vittatus* as the experimental period increased. The magnitude of depletion was very high when compared to the control. Kandeepan (2014) reported that the pesticide exposed air-breathing loach *Lepidocephalus thermalis* body composition such as carbohydrate, protein, and fat content was also reduced when compared to the control fish. So that, the body components may be diminished due to the effects of pesticide stress.

Acid and alkaline phosphatases are known to be involved in a number of cellular functions such as synthesis, transport, and metabolic regulations. In the present study, the homogenates of muscle and liver of *M.vittatus* reveal that acid phosphatase activity was higher than the alkaline phosphatase activity (Table 8 & 9). The phosphatase activity increased with increasing days of exposure in endohyper treated fish. There are some reports that the activity of phosphatase of fishes was increased when they were exposed to chronic pollutant stress (Bharti and Rasool, 2021; Kalaimani and Kandeepan, 2017; Sharma *et al.*, 2016). Activation of an enzyme may be due to inducing denovo synthesis of enzyme or activating the inactive enzyme precursors (Al-Ghanim *et al*, 2020; Ahmed Hossam Mahmoud *et al*, 2020). Enhancement in the activity of phosphatases leads to the breakdown of body components and these results in the decrease of biochemical body components.

Table – 1. LC50 value of Endohyper for the fish *Mystus vittatus* at 96hr exposure

Period of exposure	LC ₅₀	95% C.I	99% C.I	Probit equation	Chi- square calculated	Chi square D.F 0.05	Chi square D.F 0.01
96 hours	0.7782	0.7268 to 0.8296	0.7106 to 0.8458	$Y=(-1.144)+8.008x$	-0.1091	7.815	11.341

p<0.05, p<0.01 – significant

Table-2 : Sublethal effects of Endohyper (0.002ppm) on protein content in muscle of *M.vittatus*.
Each value represents the (mean \pm SD) of three observations.
The results were expressed as mg/100mg wet tissue.

Period of exposure (days)	Control	Treated	% Change
1	15.3 \pm 0.06	14.5 \pm 0.12	-5.229
2	15.4 \pm 0.12	13.8 \pm 0.19	-10.323
3	15.5 \pm 0.13	13.3 \pm 0.10	-4.194
4	15.7 \pm 0.19	12.7 \pm 0.12	-19.355
5	15.6 \pm 0.18	11.6 \pm 0.18	-25.641
6	15.7 \pm 0.21	10.2 \pm 0.21	-35.031
7	15.8 \pm 0.07	8.8 \pm 0.17	-44.303
8	15.9 \pm 0.08	7.9 \pm 0.15	-50.314
9	16.1 \pm 0.21	7.2 \pm 0.11	-55.279
10	16.3 \pm 0.19	6.4 \pm 0.13	-60.736

Table-3. Sublethal effects of Endohyper (00.2ppm) on protein content in liver of *M.vittatus*.
Each value represents the (mean \pm SD) of three observations.
The results were expressed as mg/100mg wet tissue.

Period of Exposure(days)	Control	Treated	Percentage change (%)
1	8.5 \pm 0.17	7.4 \pm 0.18	-12.941
2	8.3 \pm 0.13	7.2 \pm 0.09	-13.253
3	8.6 \pm 0.09	7.1 \pm 0.13	-17.442
4	9.0 \pm 0.13	6.8 \pm 0.15	-24.444
5	9.2 \pm 0.17	5.1 \pm 0.18	-44.565
6	9.4 \pm 0.23	4.5 \pm 0.15	-52.128
7	9.6 \pm 0.12	4.1 \pm 0.17	-57.292
8	9.6 \pm 0.17	3.9 \pm 0.21	-59.375
9	9.7 \pm 0.19	3.2 \pm 0.11	-67.010
10	9.8 \pm 0.12	2.6 \pm 0.13	-73.469

Table- 4: Sublethal effects of Endohyper(0.002ppm) Carbohydrate content in muscle of *M.vittatus*. Each value represents the (mean \pm SD) of three observations. The results were expressed as mg/100 mg wet tissue.

Period of Exposure(days)	Control	Treated	Percentage Change (%)
1	2.04 \pm 0.027	1.82 \pm 0.018	-10.784
2	2.05 \pm 0.007	1.73 \pm 0.028	-15.609
3	2.14 \pm 0.013	1.61 \pm 0.013	-24.766
4	2.16 \pm 0.014	1.47 \pm 0.017	-31.944
5	2.14 \pm 0.017	1.32 \pm 0.019	-38.318
6	2.13 \pm 0.009	1.20 \pm 0.012	-43.662
7	2.15 \pm 0.018	1.05 \pm 0.015	-51.163
8	2.11 \pm 0.019	0.85 \pm 0.008	-59.716
9	2.10 \pm 0.018	0.64 \pm 0.015	-69.524
10	2.12 \pm 0.028	0.57 \pm 0.012	-73.113

Table 5. Sublethal effects of Endohyper (0.002 ppm) on Carbohydrate content in liver of *M.vittatus*. Each value represents the (mean \pm SD) of three observations. The results were expressed as mg/100mg wet tissue.

Period of Exposure(days)	Control	Treated	Percentage Change(%)
1	8.34 \pm 0.021	7.87 \pm 0.029	-5.635
2	8.40 \pm 0.027	7.37 \pm 0.025	-12.262
3	8.45 \pm 0.021	7.12 \pm 0.027	- 15.739
4	8.50 \pm 0.020	6.68 \pm 0.023	- 21.411
5	8.51 \pm 0.015	5.75 \pm 0.015	-32.432
6	8.50 \pm 0.012	4.92 \pm 0.018	-42.118
7	8.55 \pm 0.025	3.72 \pm 0.015	-56.491
8	8.58 \pm 0.029	3.15 \pm 0.016	-63.287
9	8.60 \pm 0.017	2.87 \pm 0.014	-66.628
10	8.62 \pm 0.013	2.29 \pm 0.017	-73.434

Table- 6 : Sublethal effects of Endohyper (0.002ppm) on Lipid content in muscle of *M.vittatus*.
Each value represents the (mean \pm SD) of three observations.
The results were expressed as mg/100mg wet tissue.

Period of Exposure (days)	Control	Treated	Percentage change(%)
1	4.11 \pm 0.023	3.81 \pm 0.017	-7.299
2	4.19 \pm 0.021	3.70 \pm 0.023	-11.695
3	4.25 \pm 0.031	3.31 \pm 0.021	- 22.118
4	4.30 \pm 0.022	3.15 \pm 0.013	-26.744
5	4.45 \pm 0.026	2.85 \pm 0.018	-35.955
6	4.55 \pm 0.018	2.72 \pm 0.015	-40.219
7	4.60 \pm 0.016	2.65 \pm 0.019	-42.391
8	4.70 \pm 0.023	2.50 \pm 0.020	-46.809
9	4.85 \pm 0.019	2.41 \pm 0.017	-50.309
10	4.95 \pm 0.020	2.19 \pm 0.022	-55.758

Table-7 : Sublethal effects of Endohyper(0.002ppm) on Lipid content in liver of *M. vittatus*.
Each value represents the (mean \pm SD) of three observations.
The results were expressed as mg/100mg wet tissue.

Period of Exposure (days)	Control	Treated	Percentage change (%)
1	4.11 \pm 0.023	3.81 \pm 0.017	-7.299
2	4.19 \pm 0.021	3.70 \pm 0.023	-11.695
3	4.25 \pm 0.031	3.31 \pm 0.021	- 22.118
4	4.30 \pm 0.022	3.15 \pm 0.013	-26.744
5	4.45 \pm 0.026	2.85 \pm 0.018	-35.955
6	4.55 \pm 0.018	2.72 \pm 0.015	-40.219
7	4.60 \pm 0.016	2.65 \pm 0.019	-42.391
8	4.70 \pm 0.023	2.50 \pm 0.020	-46.809
9	4.85 \pm 0.019	2.41 \pm 0.017	-50.309
10	4.95 \pm 0.020	2.19 \pm 0.022	-55.758

Table-8 : Sublethal effects of Endohyper (0.002ppm) on Acid phosphatase in muscle and liver of *M.vittatus*.Each value represents the (mean \pm SD) of three observations. The results are expressed as μ mole p-nitrophenol mg protein⁻¹ h⁻¹.Each value represents the (mean \pm SD) of three observations.

Period of Exposure(days)	Muscle		Liver	
	Control	Treated	Control	Treated
1	1.35 \pm 0.180	1.93 \pm 0.274	3.67 \pm 0.181	3.95 \pm 0.320
2	1.46 \pm 0.172	2.05 \pm 0.270	3.72 \pm 0.262	4.37 \pm 0.331
3	1.49 \pm 0.346	2.70 \pm 0.280	3.85 \pm 0.220	4.68 \pm 0.339
4	1.52 \pm 0.346	3.12 \pm 0.203	4.02 \pm 0.113	4.95 \pm 0.320
5	1.63 \pm 0.212	3.33 \pm 0.306	4.12 \pm 0.146	5.42 \pm 0.426
6	1.72 \pm 0.236	3.75 \pm 0.260	4.37 \pm 0.321	6.02 \pm 0.426
7	1.83 \pm 0.342	3.92 \pm 0.266	4.43 \pm 0.476	6.61 \pm 0.471
8	1.95 \pm 0.240	4.41 \pm 0.243	4.71 \pm 0.193	7.02 \pm 0.246
9	1.93 \pm 0.279	4.93 \pm 0.199	4.78 \pm 0.286	7.33 \pm 0.239
10	2.10 \pm 0.180	5.83 \pm 0.242	4.95 \pm 0.240	7.56 \pm 0.426

Table-9: Sublethal effects of Endohyper (0.002ppm) on Alkaline Phosphatase in muscle and liver of *M.vittatus*.Each value represents the (mean \pm SD) of three observations. The results are expressed as μ mole p-nitrophenol mg protein⁻¹h⁻¹.Each value represents the (mean \pm SD) of three observations.

Period of Exposure(days)	Muscle		Liver	
	Control	Treated	Control	Treated
1	2.90 \pm 0.280	3.54 \pm 0.268	4.92 \pm 0.486	5.14 \pm 0.315
2	2.93 \pm 0.329	3.89 \pm 0.257	5.01 \pm 0.283	5.80 \pm 0.412
3	3.01 \pm 0.313	4.10 \pm 0.250	5.07 \pm 0.374	6.15 \pm 0.312
4	3.27 \pm 0.321	4.62 \pm 0.194	5.12 \pm 0.386	7.12 \pm 0.275
5	3.40 \pm 0.450	5.12 \pm 0.242	5.27 \pm 0.371	7.83 \pm 0.319
6	3.82 \pm 0.416	5.72 \pm 0.276	5.40 \pm 0.420	8.15 \pm 0.420
7	3.91 \pm 0.379	6.15 \pm 0.325	5.38 \pm 0.379	9.70 \pm 0.370
8	3.99 \pm 0.458	7.01 \pm 0.311	5.40 \pm 0.420	10.42 \pm 0.366
9	4.08 \pm 0.324	8.21 \pm 0.323	5.45 \pm 0.435	12.61 \pm 0.323
10	4.11 \pm 0.371	10.15 \pm 0.425	5.47 \pm 0.321	13.56 \pm 0.658

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