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CHLORPYRIFOS INDUCED TOXIC IMPACTS ON GLYCOGEN CONTENT IN GILL, LIVER, KIDNEY, BRAIN AND MUSCLE OF FISH,*CHANNA PUNCTATUS*

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ABSTRACT

The *Channa punctatus* was exposed to various concentrations of chlorpyrifos 1.7, 1.8 and 1.9 ppm with contact time of 15, 30 and 45 days. After stipulated time, glycogen content in gill, liver, kidney, brain and muscle of treated fish found to be dropped after pesticide introduction. The efficiency of chlorpyrifos in the destruction of total glycogen content was evaluated by Langmuir and Freundlich isotherm models in relations of Q_m and K_f. The spontaneity of the reaction in between chlorpyrifos and fish glycogen was determined by Gibb's free energy equation. The present analysis reports the metabolic dysfunction in response to pesticide toxicity in the fish. The glycogen level was estimated by method of Kemp and Kitsvan Heijhinger and the data obtained were analyzed by applying Langmuir and Freundlich isotherms and thermodynamic analyses. The result shows deteriorated levels of glycogen during all the exposure periods when compared with control. The results of the current study show the toxic nature of the chlorpyrifos on the glycogen content in gill, liver, kidney, brain and muscle of treated fish.

Keywords: *Channa Punctatus*, Chlorpyrifos, Glycogen, Langmuir & Freundlich isotherms, Gibb's free energy.

1. INTRODUCTION

The aquatic environmental corruption by various pesticides is one of the major causes of pollution. Insecticides are frequently used to control insects in agricultural and domestic fields, but they also enter in to an aquatic system through on purpose application, runoff from farms, accidental or illegal discharge and affect to non-target organism like fish. The mortal effect of the pesticides on fish, particularly on its physiology and biochemical parameters was viewing in the toxicological studies [1-3]. Chlorpyrifos is an organophosphate pesticide widely used in food production and fishery insects' resistor and its disproportionate use creates several biochemical problems in fishes [4, 5]. Hence, biochemical parameters are the best physiological indicators of the fish health. Therefore they are important to be focused while studying the toxic effects of various pesticides and pollutants on fish [6].

The present study has been done on the toxic effects of chlorpyrifos concentration as low (1.7 ppm), medium (1.8 ppm) and high (1.9 ppm) on glycogen content in Gill, Liver, Kidney, Brain and Muscle of the fish *Channa punctatus*.

2. MATERIAL AND METHODS

2.1. Procurement of test fish *ChannaPunctatus*

The fresh water fish *Channa punctatus*, were collected from Poompuhar and Mayiladuthurai area local ponds with the help of fisherman. The fish *Channa punctatus* having average length 13-16 cm and weight about 20-25 gm were brought to the laboratory and transferred to aerated aquarium for acclimatization. The fish *Channa punctatus* were fed daily on commercial fish feed.

2.2. Pesticide

Commercially available organophosphorus pesticide, chlorpyrifos brought from local Agro- industry was used for the present research work.

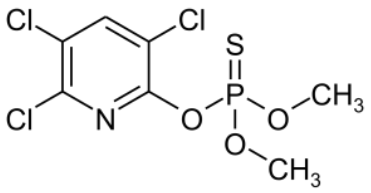

Fig.1: *Chlorpyrifos* Structure

2.3. Design of the experiment

A total of 40 *Channa punctatus* fish (10 *Channa punctatus* fish per aquarium) were separated as four groups. The following experimental groups were conducted in the period of 45 days.

Group 1 *C. punctatus*, without any pesticide exposure (control)

Group 2 *C. punctatus*, on exposure to 1.7 ppm chlorpyrifos for a period of 15, 30 and 45 days

Group 3 *C. punctatus*, on exposure to 1.8 ppm chlorpyrifos for a period of 15, 30 and 45 days

Group 4 *C. punctatus*, on exposure to 1.9 ppm chlorpyrifos for a period of 15, 30 and 45 days

2.4. Biochemical methods

The Glycogen content in gill, liver, kidney, brain and muscle of fish *Channa punctatus* were estimated and the data obtained were analyzed by applying Langmuir and Freundlich isotherms and thermodynamic analyses [7-9].

2.4.1. Estimation of glycogen

Glycogen contents in the tissues were estimated by the method of Kemp and Kitsvan Heijhinger [10]. One mL of respective sample was taken in a separate test tube and 3 mL of concentrated sulphuric acid was added to it. The mixture was heated in a boiling water bath for 6.5 min, cooled and developed colour was measured in a grating spectrophotometer (Cecil, Model CE 3373) against the reagent blank (3.0 mL concentrated sulphuric acid) at 520 nm. The quantities of glycogen present in the respective samples were read form the standard graph drawn previously from known quantities of glycogen. The glycogen values were expressed as mg/g wet weight of tissue.

2.4.2. Freundlich model

The Freundlich isotherm [11-13] was introduced on the basis of equilibrium destruction of fish glycogen in gill, liver, kidney, brain and muscle by chlorpyrifos. This isotherm was derived from the assumption that the chlorpyrifos reactive sites are distributed exponentially with respect to heat of reaction. The adsorption isotherm are expressed by the following equation

$$q_e = K_F C_e^{\frac{1}{nF}} \quad (1)$$

Which, can be linearized as

$$log q_e = log K_F + \frac{1}{n_F} log C_e \quad (2)$$

Where, q_e is the amount of glycogen destruction by chlorpyrifos at equilibrium (mg/g) and C_e is the concentration of glycogen in Gill, Liver, Kidney, Brain and Muscle of the fish *Channa punctatus* at equilibrium concentration of chlorpyrifos (ppm). K_F (L/g) and $1/n_F$ are the Freundlich constants related to chlorpyrifos glycogen destruction reaction capacity and glycogen destruction intensity, respectively. The Freundlich constants K_F and $1/n_F$ were calculated from the slope and intercept of the $logq_e$Vs$logC_e$ plot and the model parameters.

2.4.3. Langmuir isotherm

The Langmuir isotherm [14, 15] is based on the assumption that all chlorpyrifos reaction sites possess equal affinity to the glycogen ingill, liver, kidney, brain and muscle of the fish *Channa punctatus*. The Langmuir isotherm in a linear form can be represented as:

$$\frac{C_e}{q_e} = \frac{1}{Q_m K_L} + \frac{C_e}{Q_m} \quad (3)$$

Where, q_e is the amount of glycogen destruction at equilibrium (mg/g), C_e is the concentration of glycogen in the fish *Channa punctatus* at equilibrium (ppm), q_m is the maximum efficiency of chlorpyrifos in the destruction of glycogen (mg/g), and K_L is the Langmuir constant related to reaction energy provided by the chlorpyrifos in g/mg. A linear plot of C_e/q_eVsC_e was employed to determine the value of q_m and K_L, parameters.

2.4.4. Free energy change (ΔG°) analysis

The free energy change parameter [16] was evaluated to confirm the feasibility of the present study. The parameter ΔG° determined from following equation:

$$\Delta G^0 = -RTlnK_c \quad (4)$$

Where, ΔG° is the free energy change (kJ/mol), R is the universal gas constant (8.314 J/ mol/K), K_c the thermodynamic equilibrium constant and T is the absolute temperature (K).

3. RESULTS

The lethal concentration (LC50) is the commonly accepted basis for acute and chronic toxicity test and it is the concentration of a test pesticide which kill 50% of the test organisms after a particular time of exposure; usually 96 hrs and the mortality rate of fishes at various concentrations like 1.7, 1.8, and 1.9 ppm. The glycogen content obtained in different tissues of control fish and chlorpyrifos treated fish at different time intervals is shown in Table 1. The Freundlich constants K_F and $1/n_F$ were calculated from the slope and intercept of the $logq_e$ Vs $logC_e$ plots, as shown in Fig. 2 (a-e), and the model

parameters are shown in Table 2. A linear plot of C_e/q_e Vs C_e was employed to determine the value of q_m and K_L, as shown in Fig. 3(a-e), and the data so obtained were also presented in Table 3. The efficiency of chlorpyrifos (q_m) on the destruction glycogen in gill, liver, kidney, brain and muscle in the fish *Channa punctatus* shows the increasing trend when the concentration of chlorpyrifos increases. The Gibb's free energy change values tabulated in Table 4 and these values obtained from equation 4.

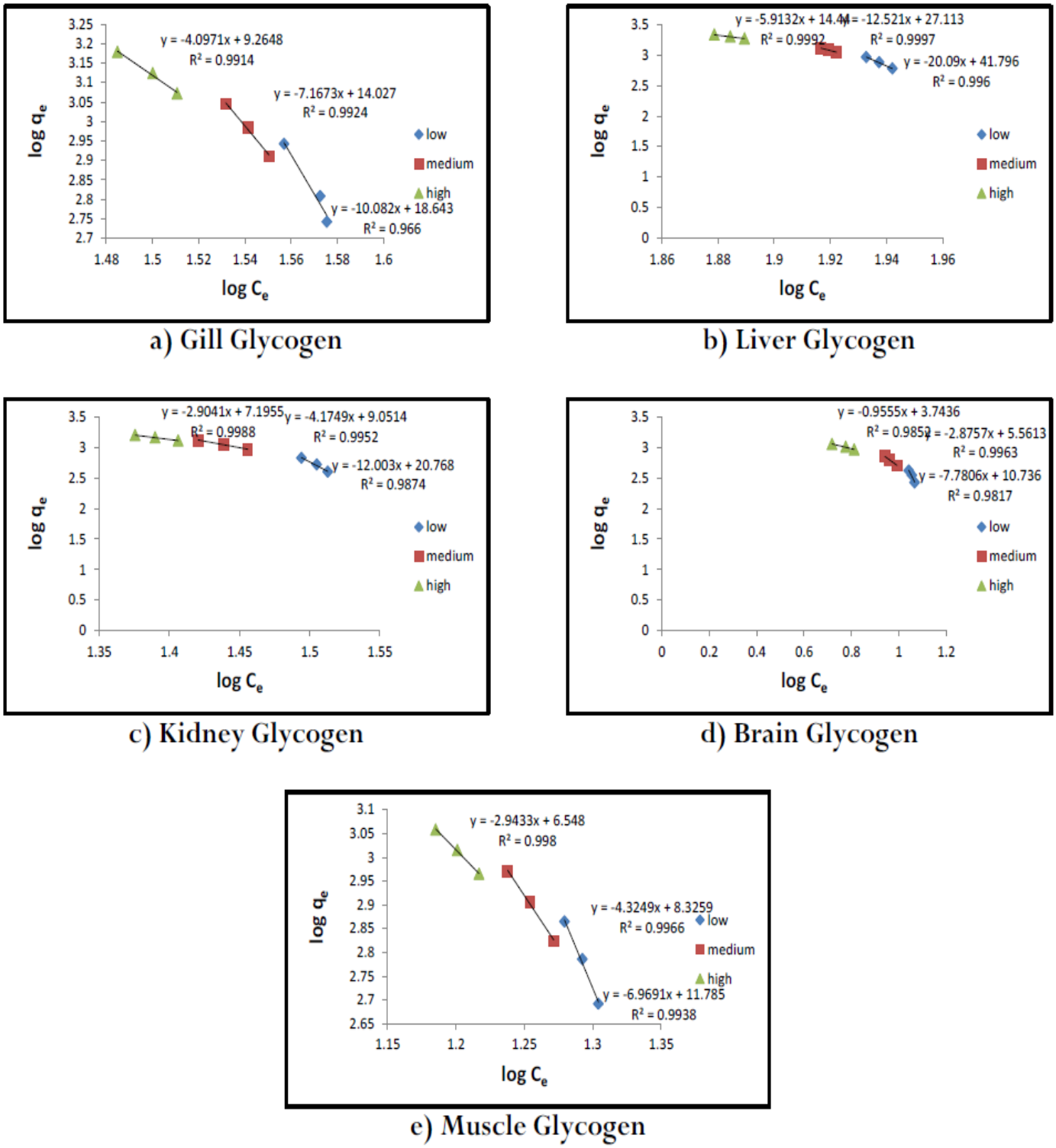

Fig. 2: (a-e) Freundlichisotherm for the Destruction of Fish Glycogen.

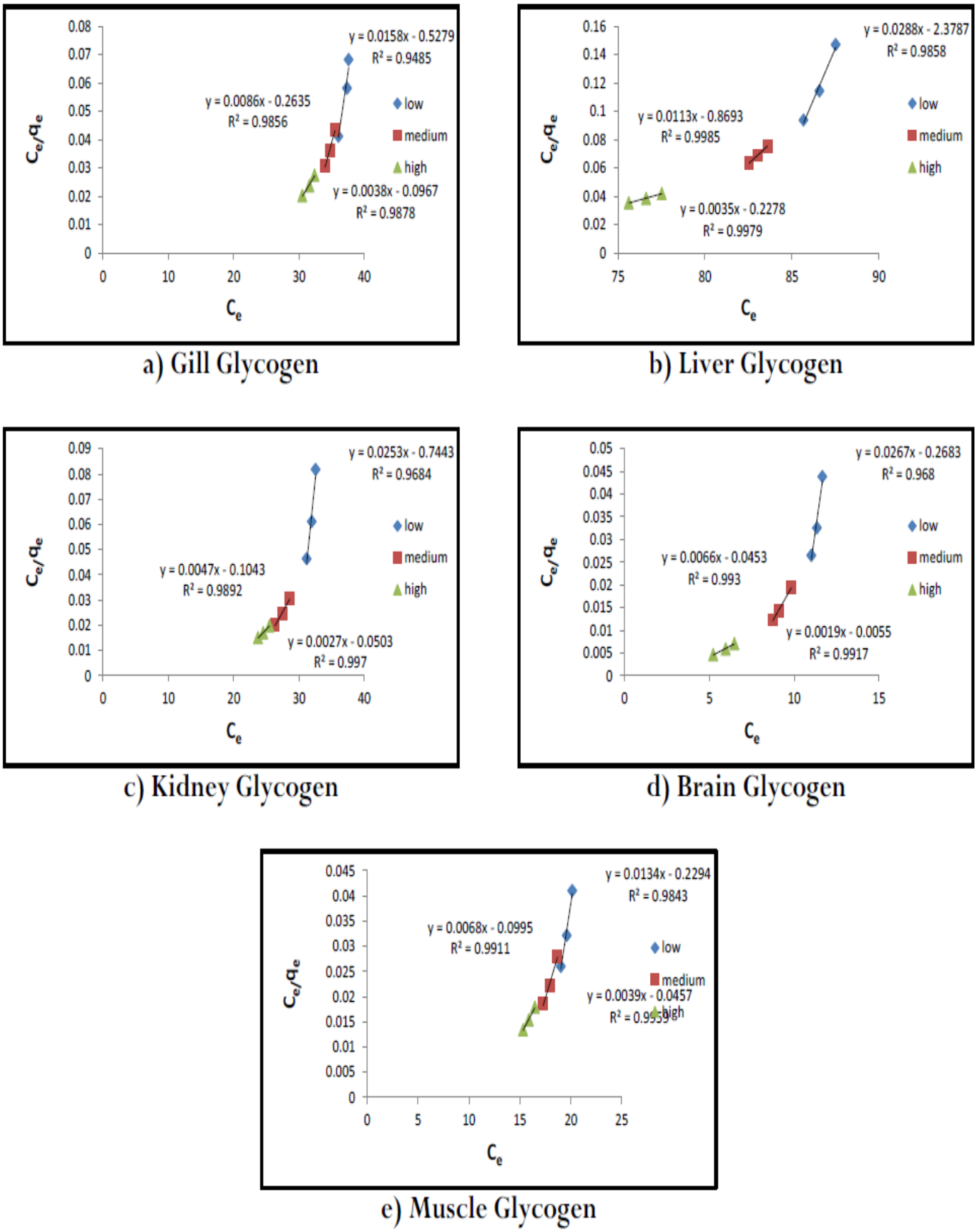

Fig. 3: (a-e) Langmuir isotherm for the Destruction of Fish Glycogen.

Table 1: Glycogen Destruction at Equilibrium (mg/g) in Gill, Liver, Kidney, Brain and Muscle of Fish *ChannaPunctatus*.

Tissues	Gill			Liver			Kidney			Brain			Muscle		
Exposure Periods (days)	15	30	45	15	30	45	15	30	45	15	30	45	15	30	45
Control	41.38	41.75	42.01	91.57	91.73	91.89	35.29	35.55	35.78	13.48	13.72	13.85	23.49	23.76	24.01
Low concentration	37.63	37.38	36.06	87.52	86.58	85.67	32.58	31.99	31.20	11.67	11.35	11.02	20.15	19.61	19.03
Medium Concentration	35.52	34.80	34.02	83.60	83.04	82.54	28.57	27.48	26.34	9.84	9.13	8.73	18.69	17.96	17.29
High concentration	32.41	31.63	30.54	77.52	76.62	75.63	25.49	24.54	23.75	6.48	5.97	5.23	16.48	15.89	15.32

Table 2: Freundlich isotherm Parameters for the Destruction of Fish Glycogen by Chlorpyrifos.

Concentration of chlorpyrifos in ppm	Gill Glycogen		Liver Glycogen		Kidney Glycogen		Brain Glycogen		Muscle Glycogen	
	K_f (mg/g)	n_f	K_f (mg/g)	n_f	K_f (mg/g)	n_f	K_f (mg/g)	n_f	K_f (mg/g)	n_f
Low (1.7)	4.39E+18	0.0992	6.25E+41	0.0498	5.87E+20	0.0833	5.44E+10	0.1285	6.10E+11	0.1435
Medium (1.8)	1.06E+14	0.1395	1.30E+27	0.0799	1.13E+09	0.2395	3.64E+05	0.3477	2.12E+08	0.2312
High (1.9)	1.84E+09	0.2441	2.75E+14	0.1691	1.57E+07	0.3443	5.54E+03	1.0466	3.53E+06	0.3398

Table 3: Langmuir isotherm Parameters for the Destruction of Fish Glycogen by Chlorpyrifos.

Concentration of chlorpyrifos in ppm	Gill Glycogen		Liver Glycogen		Kidney Glycogen		Brain Glycogen		Muscle Glycogen	
	Q_m (mg/g)	K_L (L/mg)	Q_m (mg/g)	K_L (L/mg)	Q_m (mg/g)	K_L (L/mg)	Q_m (mg/g)	K_L (L/mg)	Q_m (mg/g)	K_L (L/mg)
Low (1.7)	12276.76	9.13E-06	5982.66	4.09E-05	7822.98	1.68E-05	8582.41	8.66E-06	16049.21	4.43E-06
Medium (1.8)	22755.03	3.48E-06	16003.03	9.03E-06	35922.00	1.57E-06	28217.83	1.32E-06	27976.41	1.84E-06
High (1.9)	45662.75	1.16E-06	44217.93	1.89E-06	61418.60	6.62E-07	88030.77	1.99E-07	49426.15	7.38E-07

Table 4: Free Energy Change Involved in the Destruction of Fish Glycogen (ΔG° in J/K/mol)

Concentration of chlorpyrifos	Gill Glycogen (Days)			Liver Glycogen (Days)			Kidney Glycogen (Days)			Brain Glycogen (Days)			Muscle Glycogen (Days)		
in ppm	15	30	45	15	30	45	15	30	45	15	30	45	15	30	45
Low (1.7)	-5809.26	-5407.02	-4538.97	-7741.7	-7109.2	-6607.04	-6264.48	-5531.19	-4833.53	-4694.91	-3945.8	-3424.61	-4527.49	-3912.05	-3377.13
Medium (1.8)	-4539.36	-4058.02	-3649.63	-5920.89	-5686.08	-5486.46	-3645.88	-3086.72	-2584.97	-2505.22	-1732.38	-1344.24	-3424.45	-2847.36	-2380.69
High (1.9)	-3236.04	-2870.8	-2467	-4302.48	-4089.83	-3872.29	-2408.06	-2019.09	-1713.47	-194.4517	-657.3598	-1258.749	-2153.39	-1770.03	-1428.32

4. DISCUSSION

The acute and chronic toxicity studies are generally employed to compare the sensitivities of different species to different effectiveness of the chemicals using LC_{50} values. High concentrations of chlorpyrifos increase the mortality rates of fishes as well as glycogen level destructed and these data were shown in Table 1. The results of glycogen have shown a notable dose and contact time depending on decrease in gill, liver, kidney, brain and muscle in the treated fish. It has led a suggestion that there may be an increased glycogenolysis process and thus the carbohydrate catabolism plays on important role to compensate the increased energy demand under environmental stress induced by chlorpyrifos. The concentration of the glycogen depletion of the treated fish *Channa punctatus* exposed to chlorpyrifos in sublethal concentrations for 15, 30 and 45 days are given in Table 1, evidently denotes the decrease in the concentration of fish glycogen in gill, liver, kidney, brain and muscle glycogen was compared to control fish glycogen level. The Freundlich isotherm, magnitude of K_F showed that chlorpyrifos had a high capacity for destruction of fish glycogen in gill, liver, kidney, brain and muscle in the fish *Channa punctatus* and exponent, n_F, shows the value within the range of 0 and 10 indicates that chlorpyrifos was favorably reacts with fish glycogen to make destruction reaction more effectively. The Langmuir model was recognition the efficiency of chlorpyrifos (q_m) on the destruction glycogen in gill, liver, kidney, brain and muscle in the fish *Channa punctatus* shows the increasing trend when the concentration of chlorpyrifos increases. The value of K_L decreased with an increase in the concentration of Chlorpyrifos, were K_L value indicates the reaction affinity of chlorpyrifos on fish glycogen in gill, liver, kidney, brain and muscle in the fish *Channa punctatus*.The Langmuir isotherm showed a better fit to the destruction of glycogen data than the Freundlich isotherm as shown in Fig. 3 and Fig. 2. The fact that the Langmuir isotherm fits the experimental data well may be due to uniformity of reactive sites on the chlorpyrifos surface, since the Langmuir equation assumes that the chlorpyrifos surface is energetically homogeneous. The table 4, clearly shows that the reaction in between fish glycogen such as gill, liver, kidney, brain and muscle in the fish *Channa punctatus* with the pesticide chlorpyrifos is spontaneous in nature as ΔG° values are negative at all the concentrations studied.

5. CONCLUSION

In the observation of above results and discussion in the present study the glycogen content in different tissues of treated fish *Channa punctatus* was reduced when exposed to sub lethal concentration of chlorpyrifos. The combination of chloroyrifos exhibited effectiveness in the destruction of glycogen in gill, liver, kidney, brain and muscle of the treated fish *Channa punctatus*. The destruction efficiency was controlled by pesticide concentration and contact time. Destruction reaction data are fitted well with the Langmuir and Freundlich models. Isotherm study indicates that the higher concentration of chlorpyrifos can be more effective towards the destruction of fish glycogenas a result, from the present work was concluded that insecticide intemperance disturbs the functional activity of cells with consequential changes in biochemical configuration of fish, which affect the nutritive value of fish. It may be hazardous to human beings due to the intake of those fish in which insecticides have accumulated in their tissues.

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