Effect of Sublethal Concentration of Methomyl 40 % SP Carbamate Insecticide on Glycogen Content in the Gill of Freshwater Fish *Puntius sophore* (Hamilton 1822)

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Abstract : This study has been undertaken to investigate the impact of sub lethal concentration of Methomyl 40 % SP Carbamate insecticide on glycogen content in the gill of fresh water fish *Puntius sophore* (Hamilton 1822). During our present study test fishes were exposed to the sub lethal concentration of Methomyl 40 % SP a carbamate insecticide for 10, 20 and 30 days respectively. The glycogen contents in the gill tissues were determined by standard procedures from control fish and the fish exposed to Methomyl. The results showed a significant decline in the glycogen content in gill in response to the toxicant stress.

IndexTerms **– Sub lethal, Methomyl, Gill, Glycogen, Decline.**

I. INTRODUCTION

Pesticides are broadly used to in the agriculture field to control different kind of pests. Pesticides enter the surface water such as rivers, by direct application, spray, drift, aerial spraying, and runoff from agricultural land, industrial plant effluents and sewage [1]. Fishes are in direct contact with the surrounding water through their gills. Pesticides have an effect on non-target animals like fish. The intake of pesticides affects the biochemical composition of fishes [2].

Methomyl is a carbamate insecticide introduced in 1966. Trade names include DuPont 1179, Lannate, Lanox, Memilene, Methavin, Methomex, Nudrin, and NuBait. Methomyl is a highly toxic compound in EPA toxicity class I. Methomyl is the carbamate insecticide, Methomyl insecticide, is the most preferred control agent for control of pests in vegetable crops in India. It is registered in more than 70 countries worldwide and is effective on over 100 insect species. Methomyl is used for the control of lepidopteron insects; it works as an effective ovicide, larvicide and adulticide. Methomyl insecticide affects the nervous system of the pests in the vegetable crops resulting in uncontrolled transmission of nerve impulses. Once affected, pests are unable to behave normally or feed the crop. Methomyl is highly toxic to fish, humans, livestock, pets, and wildlife and other aquatic animals with highly effective, broad spectrum, low residual and safe using, It has the poisoning actions of contact, stomach and can kill the insect's ova. It is effective to aphids, thrips, citrus leaf miners, cotton bollworms and tobacco insects. It can prevent the Asiatic rice borers, plant hoppers and has certain effects to insects of fruit trees. [3] Srivastava (1982) [4] studied comparative effect of copper, cadmium and mercury on tissue glycogen of cat fish *Heteropneustes fossilis*. Lawe and Nivmi (1984) [5] studied effect of cadmium on glycogen reserves and liver size in rainbow trout *Salmo gairdneri*.

Considering the above facts, In the present study, an attempt has been made to investigate the impact of carbamate insecticide Methomyl 40% SP on glycogen content in the gill tissues of the freshwater fish *Puntius sophore* (Hamilton 1822).

II. RESEARCH METHODOLOGY

2.1Experimental Test Fish

For the present study, the live fresh water fish *Puntius sophore* were collected from river Bhīma of Pune district (M.S.) India in polythene bags and brought to the laboratory. Intensive care was taken to reduce hyperactivity and physical injuries to the fish. Then stocked and maintained in large Aquarium tank containing chlorine free water for 10 days under normal temperature for acclimatization. Before stocking, the tank was washed with 1 percentage KMnO4 to avoid the fungal infection. Water was changed in alternate days. The fishes were fed a commercial fish diet. The test fishes was chosen as test animal because of their availability throughout the year, survival capacity in the laboratory condition and their importance as edible fish especially by the poor section of people and also being used in Aquarium trade

2.2 Experimental Set Up

Well acclimatized freshwater test fish *Puntius sophore* of average length (7±2cm) and weight (8±1gm) were selected from the stock and exposed to sub lethal concentration of Methomyl 40% SP for 10, 20, 30 days respectively to investigate impact of sub lethal concentration of Methomyl on glycogen content in the gill. In the present study 1/10th of 96hr LC_{50} concentration were selected as sub lethal concentration. The experiments were carried out in glass aquarium with 10 fishes each. The experimental medium was renewed daily till the end of the experiment. The experiment was repeated five times and the mean values were recorded. Simultaneously 10 fishes were reared in pesticide-free medium and are treated as control for the experiment.

Figure No-1. Experimental Test Fish, *Puntius sophore* (Hamilton 1822).

2.3 Estimation of Glycogen content

Total Glycogen content in the gill tissue was estimated by using Anthron reagent method (Dezwaan and Zandee, 1972). [6] 100mg tissue was dissected out and homogenated in 3ml of 30 % KOH, then homogenate was kept to boiling water bath for 5-10 minutes which helps tissues to dissolve and then cooled. Then 0.5 ml of saturated Na2SO4 and 2 ml of 96% ethanol alcohol was added and mixture was refrigerated for next 24 hours. The mixture was centrifuged at 3000 rpm for 10 minutes. Supernatant was discarded and in residue free of lipid and protein were then dissolved in 2 ml of HCl (5 M) and neutralized with 0.5 M NaOH followed by 5ml distilled water was added and kept for the hot water bath (at 70^0c) for 5 minutes. After cooling 1 ml sample solution was taken, 1 ml distilled water and 5ml of Anthron reagent added in it. Along with control (2ml of distilled water and 5ml of Anthron reagent) optical density of sample solution was measured at 620 nm.

The total Glycogen content expressed as in mg / 100 mg wet weight of tissue.

III. RESULTS AND DISCUSSION

The glycogen content of the gill of the test fish *Puntius sophore* treated with the sub lethal concentration of Methomyl 40 % SP for different exposure period interval showed that declined trend. The changes in the level of glycogen contents in gill of the fish, *Puntius sophore*, were given in the Table No. 1 and Figure No. 2.

Table No-1. Effect of Sub lethal concentration of Methomyl (0.32 ppm) (1/10th of 96hr LC_{50} value) on glycogen content (mg/100mg) in gill tissue of *Puntius sophore* after chronic exposure.

Organ	Control	Exposure Period		
		10 Days (0.32 ppm)	20 Days (0.32 ppm)	30 Days (0.32 ppm)
Gill	03.80 ± 0.24	03.32 ± 0.22 (12.63)	02.98 ± 0.20 (21.57)	02.10 ± 0.18 (44.73)

Each value are expressed in mg /100mg wet wt. of tissue.
Each value indicates the mean (X ± SD) of five estimations.
Figures in bracket indicate difference in percentage over control.

In the present study, The Glycogen content in the gill tissue showed a declined trend. In the test fish, the glycogen content recorded were 03.32 ± 0.22, 02.98 ± 0.20and 02.10 ± 0.18mg/100mg wet tissue at 0.32 ppm sublethal concentration of Methomyl 40 % SP for 10, 20 and 30 days of exposure respectively. The percentage of changes in the gill glycogen content over the control (03.80 ± 0.24) was 12.63%, 21.57% and 44.73% after 10, 20 and 30 days of exposure respectively.

This result also coincides with the findings of Susan *et al* (1999) [7] they reported that drastic decreased glycogen content in liver of *Catla catla* under fenvalerate toxicity stress. Das and Mukherjee (2003) [8] observed that the sub-lethal exposure of cypermethrin up to 45 days alterated blood glucose level in *Labeo rohita*. Decrease in glycogen has also been suggested by Maruthi, Y.A. and M.V. Subba Rao (2000) [9] in the fish *Channa punctatus* treated with distillery effluent.

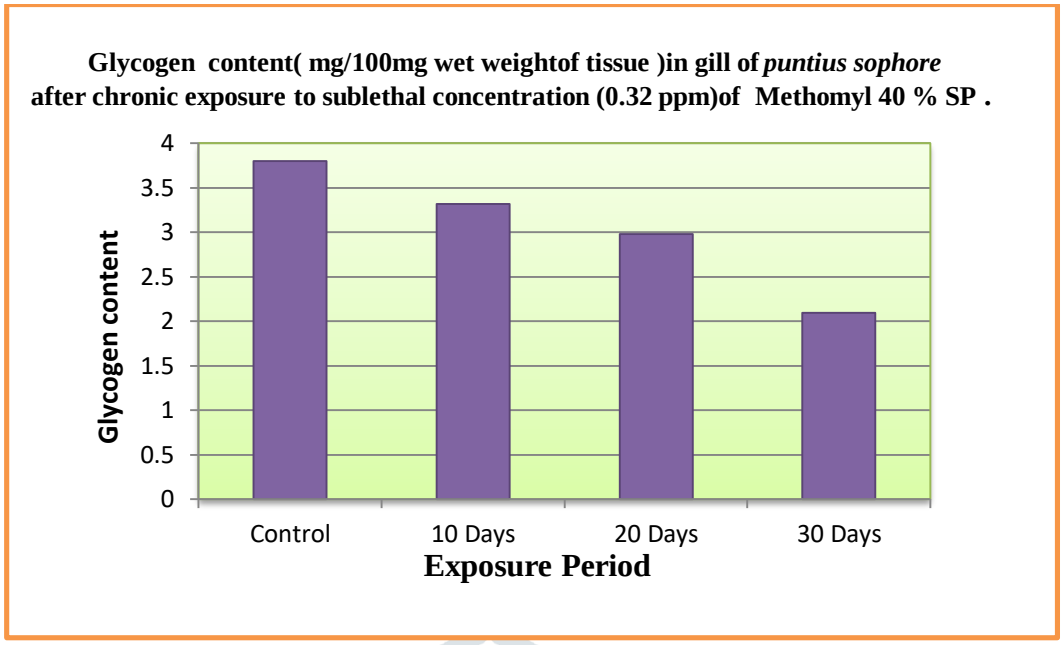

Figure No-2. Effect of Sub lethal concentration of Methomyl (0.32 ppm) (1/10th of 96hr LC_{50} value) on glycogen content (mg/100mg) in gill tissue of *Puntius sophore* after chronic exposure.

The present work indicates that insecticides caused alterations within the glycogen metabolism of fish. This decrease in tissue glycogen may be due to glycolysis for production of energy to overcome toxic effect of the toxicants. During stress conditions glycogen act as a source of energy as fish want a lot of energy to detoxify the toxicant and to beat stress. This could happen by rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase (Jha and Pandey, 1989). [10] The depletion of total tissue glycogen is also because of the impact of pesticides on the physiological adaptability of the fish to compensate for pesticide stress. (Mastan, S.A. and Rammayya, P.J. (2010), Khilare Y.K, Wagh SB. (1998)) [11, 12]

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