EFFECT OF GLYCOGEN CONTENT IN DIFFERENT TISSUE OF THE FRESHWATER FISH *Labeo rohita*, EXPOSED TO PYRACLOSTROBIN (20%WG)

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Abstract
Agriculture is the predominant occupation in rural India, accounting for 52% of employment and is therefore exposed to the pesticides used in agriculture. The environmental quality of aquatic systems has deteriorated marked by over last two decades, because of continuous growth in population, rapid industrialization and accompanying technologies involving waste disposal, the rate of discharge in pollutants into the environment is for higher than the rates of their purification. The water contamination cause damages to aquatic life especially to fishes which are very sensitive to wide range of toxicant in the water. Due to these reasons and maintains of our natural water resources is a very difficult task. The present investigation effect of the carbamate pesticide, Pyraclostrobin 20% WG on biochemical parameters like glycogen content was estimated in different tissues of freshwater fish *L.rohita* exposed to sublethal concentrations of 24hr, 5 days and 10 days. The glycogen content was in controls in order to expressed: liver>kidney>brain>gill>muscle where as in the case of lethal and sublethal concentrations liver is more effected and in muscle and gill tissues glycogen content was less effected when compared with controls.

Key words: *Labeo rohita*, Pyraclosrobin, sub lethal concentrations, different tissues.

INTRODUCTION
Pesticides are reported to reduce glycogen levels and increase phosphorylase activities; the boost of phosphatases activity reveals the increase the transportation of metabolites through cellular membrane [1]. In the event of decreased ATPase system, phosphorylation may be preceded by activated phosphates to catalyze the liberation of inorganic phosphates from phosphate esters.

Acetyl cholinesterase (Aceh) activity is a frequently used in environmental monitoring, usually in areas contaminated with pesticides, heavy metals and effluents. It is an enzyme that catalyses three hydrolysis of acetylcholine to choline and acetate in synaptic cleft. When inhibition occurs in AChE activity the neuro transmitter, acetylcholine is not hydrolyzed in the nerve synapse and neuromuscular junction, causing an abnormal amount of Ach in these areas, which leads to an over activation of the brain and muscular tissues [2], the inhibition of fish exposed to different type of toxicants [3]. In all cases, inhibition of AChE as a sentinel species...
will allow the detection of lower contamination levels of different environmental contaminates in cases of long term and sublethal exposures [4].

Inhibition of acetyl cholinesterase which plays a determinant role in neurofunction of vertebrates and invertebrates is by degrading the neurotransmitter acetylcholine in synapses. Therefore, the inhibition of the AChE has been widely used to detect the presence and adverse effects of carbamat, other group of pesticides[5][6].

MATERIALS AND METHODS
Fish *Labeo rohita* of size 6±7 cm and 6.5±7.5 g weight were brought from a local fish farm Kuchipudi, Gunture Distric of Andhra Pradesh, India and acclimatized at 28 ± 26°C in the laboratory for 15days. Such acclimatized fish were exposed to sublethal and lethal concentrations of Pyraclostrobin (20%WG) commercial grade for 24hr and 5daysand 10 days. The vital tissues like muscle, brain, liver, gill and kidney of the fish were taken for the estimation of glycogen with control exposures.

ESTIMATION OF GLYCOGEN:

The glycogen content was estimated by the method of Kemp et al., (1954). 5% homogenates of gill, brain, muscle and 2% homogenates of liver and kidney tissues were prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of trichloroacetic acid (TCA) and boiled for 15 minutes at 100°C and then cooled in running water. The solution was made up to 5 ml with TCA to compensate for evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of conc. H$_2$SO$_4$ was added and the mixture was boiled for 10 minutes. The mixture was cooled and the optical density was measured at 520 nm in a (ELICO Model SL207) against a blank. The standard graph was plotted with D-glucose (Analar supplied by B.D.H. Bombay) by the foresaid method. The glucose obtained was converted to glycogen by the multiplication factor 0.98 (Hawks, 1951) and is expressed as mg of glycogen/gm wet weight of the tissue.

RESULT AND DISCUSSION:

Under lethal and sublethal exposure to Pyraclostrobin for 24hr, the percentage of depletion was found in all the tissues of test fish, maximum percentage of depletion was in liver (40.35) and liver (31.87), minimum depletion in gill (29.84) and muscle (17.42).

Under sublethal exposure to Pyraclostrobin for 5th and 10th days, the glycogen levels was found to decrease in all the tissues of test fish *Labeo rohita* and maximum discretion was(33.60) and (42.76) in liver, minimum decrease was observed (18.64) and(24.92) in muscle.

Table:1 Changes in the glycogen content (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish *Labeo rohita*, exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24hr:

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Control (mg/g)</th>
<th>Sub-lethal (mg/g)</th>
<th>% Change</th>
<th>Lethal (mg/g)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>26.40±0.017</td>
<td>21.72±0.04</td>
<td>19.70</td>
<td>18.52±0.03</td>
<td>29.84</td>
</tr>
<tr>
<td>Brain</td>
<td>31.80±0.04</td>
<td>24.15±0.02</td>
<td>24.05</td>
<td>20.95±0.04</td>
<td>34.11</td>
</tr>
<tr>
<td>Kidney</td>
<td>46.02±0.04</td>
<td>32.76±0.03</td>
<td>28.81</td>
<td>27.92±0.03</td>
<td>39.33</td>
</tr>
<tr>
<td>Liver</td>
<td>67.29±0.03</td>
<td>45.84±0.07</td>
<td>31.87</td>
<td>40.15±0.03</td>
<td>40.35</td>
</tr>
<tr>
<td>Muscle</td>
<td>22.15±0.03</td>
<td>18.29±0.13</td>
<td>17.42</td>
<td>15.02±0.04</td>
<td>32.18</td>
</tr>
</tbody>
</table>

Values are the mean of five observations ;(±) indicates the standard deviation: Values are significantly at $P<0.05$
Figure 1 Changes in the glycogen content (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish *Labeo rohita*, exposed to sub-lethal and lethal concentrations of Pyraclostrobin (20%WG) for 24hr:

Table 2 Changes in the glycogen content (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish *Labeo rohita*, exposed to sublethal concentrations of Pyraclostrobin (20%WG) for 5 and 10 days.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>5 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Sub-lethal</td>
</tr>
<tr>
<td>Gill</td>
<td>23.65±0.04</td>
<td>18.68±0.03</td>
</tr>
<tr>
<td>Brain</td>
<td>27.54±0.03</td>
<td>21.19±0.02</td>
</tr>
<tr>
<td>Kidney</td>
<td>39.06±0.19</td>
<td>27.42±0.04</td>
</tr>
<tr>
<td>Liver</td>
<td>48.32±0.03</td>
<td>32.08±0.03</td>
</tr>
<tr>
<td>Muscle</td>
<td>19.63±0.05</td>
<td>15.97±0.02</td>
</tr>
</tbody>
</table>

Values are the mean of five observations; (±) indicates the standard deviation.

Values are significantly at P < 0.05
Figure 2: Changes in the glycogen content (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish *Labeo rohita*, exposed to sublethal concentrations of Pyraclostrobin (20%WG) for 5 and 10 days.

The result indicates that the liver is a vital organ of carbohydrate metabolism; it was drastically affected by Pyraclostrobin formulations. Among the test tissues, higher glycogen content was observed in liver of control and treated fish and is acceptable due to its involvement in glycogen synthesis and utilization. Glycogen is the major storage form of carbohydrate in animals which occurs mainly in liver and muscle. Liver glycogen is largely concerned with storage and export of hexose units for maintenance of blood glucose. Depletion of glycogen content in all the tissues such as muscle, brain, gill, liver and kidney of fish *Labeo rohita* under lethal and sublethal conditions of Pyraclostrobin.

The function of muscle glycogen is to act as a readily available source of hexose units for glycolysis with in the muscle itself [7]. Through brain tissue is metabolically active, lower glycogen content was observed, since it lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities [8].

A fall in glycogen levels indicates its rapid utilization to meet the enhanced energy demands in pesticide treated animals through glycolysis or hexose monophosphate pathway (HMP). Decreased glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthesis is also attributed to the inhibition of the enzyme glycogen synthesis, which mediates glycogen synthesis [9][10].

Suneetha, [11] reported that decreased glycogen content in freshwater fish *Labeo rohita* exposed to endosulfan and fevalerate sublethal concentrations. Organochlorine pesticide endosulfan sublethal exposure on penaeid shrimp, *Metapenaeus Monoceros* (Fabricius), decreased the glycogen content [12]. [13] reported that muscular glycogen was reduced in *Clarius Batracus* exposed to the organophosphate pesticide Rogor, and in muscle of *Cypinus carpio* exposed to herbicide 2,4 Diamine [14]. Freshwater cry fish *Cherax Quandricarinatus* exposed to glyposate acid and poloxyoxethlyenamine reduced glycogen level in different tissues [15]. Muscle glycogen decreased in organophosphorus toxicity on *Tilapia Zilli* [1] and same trend was observed in *Esomo danricusin* selected tissues exposed to copper [16].

Decreased glycogen content in liver and muscle tissue in *Clarius batrachus* was observed under sublethal exposure of arsenic [17]. Sodium arsenate on *tilapia mossamibica* [18]; arsenic to freshwater fish *Clarius batrachus* [19]; Fenthion and Avaunt on *Cirrhinus mirgala* [20]. Cypermethrin induced depletion of glycogen in *tilapia mossamibica* [21]; Indian major carp *Labeo rohita* [22][23].*Oreochromis nilotica* treated with malathion showed decreased glycogen [24].
observed a significance depletion in glycogen levels in various tissues of freshwater fish *Channa gachua* and *Labeo rohita* under sublethal concentration of chromium and industrial effluents had stated that these were tissue specific and time dependent[26] stated that toxic stress results in the disruption of enzymes associated with carbohydrate metabolism.

The decreased glycogen level is also attributed to the conversation of carbohydrates into amino acids [27]. [28] reported that stepped up glycolysis leads to a decrease in glycogen content. Similar changes were observed in *chenna punctatus* exposed to pyrithrod and industrial effluents[29], malathion [30], methomyl [31] and a time dependent depletion of glycogen levels in muscle and liver of fish *Oreochromis mossambicus* to quinolphos exposure[32], carbaryl on *cyprinus carpio* [33]; and deltamethrin on *Labeo rohita*[34].

In the present investigation, it was observed that exposure to sublethal and lethal concentrations of pyraclostrobin ,on the fish *Labeo rohita* caused changes in the total glycogen content, when compare with controls which may be attributed to toxic stress, resulting in the disruption of enzyme associated with carbohydrate metabolism.

REFERENCES:


