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Research Paper

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 efeected 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^{0}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^{0}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_2SO_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:

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

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:

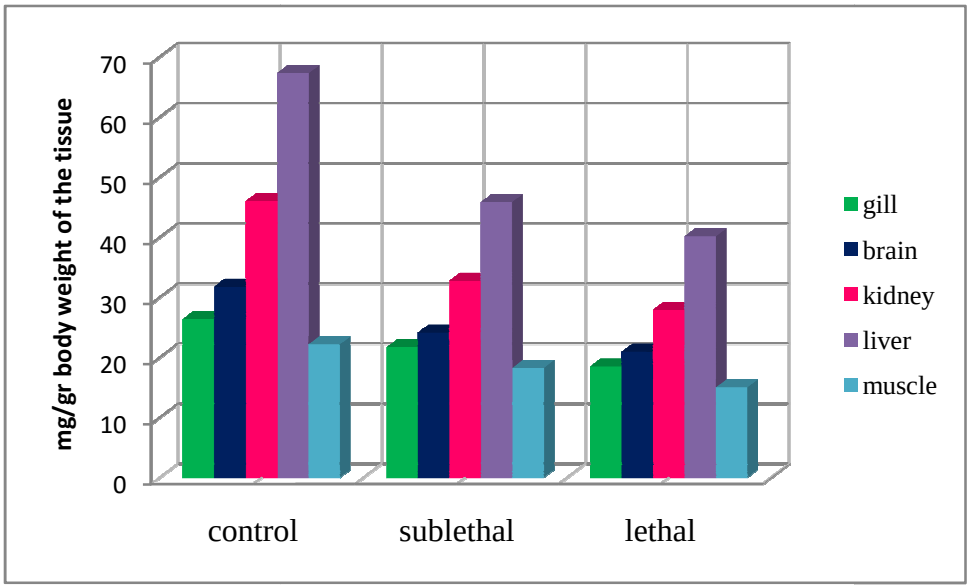
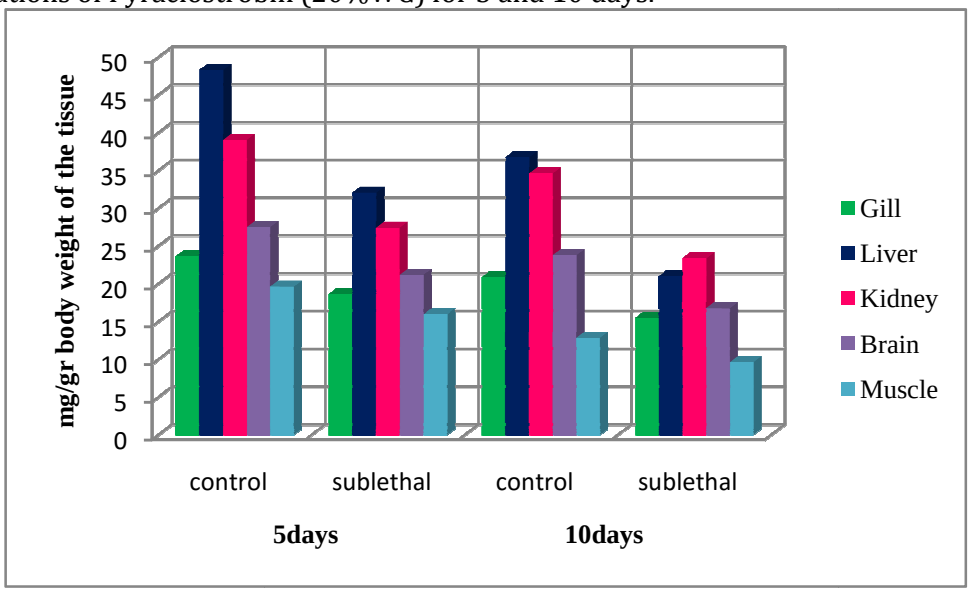


Table:2 Changes in the glycogen content (mg/g wet weight of the tissue) and % change over the control, in different tissue of the freshwater fish *Labeo rohita,* exposed to sublethal concentrations of Pyraclostrobin (20%WG) for 5 and 10 days.

Tissues	5days			10days		
	Control (mg/g)	Sub-lethal (mg/g)	% Change	Control (mg/g)	Sub-lethal (mg/g)	% Change
Gill	23.65 ±0.04	18.68 ±0.03	21.00	20.92 ±0.09	15.48 ±0.04	26.00
Brain	27.54 ±0.03	21.19 ±0.02	23.05	23.81 ±0.01	16.76 ±0.04	29.60
Kidney	39.06 ±0.19	27.42 ±0.04	29.80	34.62 ±0.05	23.39 ±0.04	32.43
Liver	48.32 ±0.03	32.08 ±0.03	33.60	36.78 ±0.03	21.05 ±0.03	42.76
Muscle	19.63 ±0.05	15.97 ±0.02	18.64	12.84 ±0.11	9.64 ±0.03	24.92

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 glycolisis 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), decresed the glycogen content [12]. [13]reported that muscular glycogen was reduced in *Clarius Batracus* exposed to the organophosphate pesticide Rogor, and in muscle of *Cyprinus carpio* exposed to herbicide 2,4 Diamine [14]. Freshwater cry fish *Cherax Quandricarinatus* exposed to glyposate acid and polyoxyethylenamine reduced glycogen level in different tissues [15]. Muscle glycogen decreased in organophosphorus toxicity on *Tilapia Zilli* [1] and same trend was observed in *Esomus 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 mrigala* [20]. Cypermethrin induced depletion of glycogen in *tilapia mossamibica* [21]; Indian major carp *Labeo rohita* [22][23].*Oreochromis nilotion* treated with malathion showed decreased glycogen[24].

[25], observed a significance depletion in glycogen levels in various tissues of freshwater fiah *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 glycogenolysis 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:

[1] Abdul –Salam Mohamed Ibraik Ohaida, Nuha Mahmoud A.Akrawee(2010). The effects of exposures to organophosphorus compounds on some aspects of carbohydrate metabolism in Tilapia zillii. The Egyptian journal of hospital medicine, 39:223-228.

[2] Roex, E.W.M., Keijzer, R.,Gestel, C.A.M (2003). Acetylcholinesterase inhibition and increased food consumption rate in the zebra fish, *Danio rerio,* after chronic exposure to parathion.Aquat.Toxicol,64:451-460

[3]Hernandez-moreno., Soler, M.P., Perez-lopez M(2010). Brain acetylcholinesterase, malondialdehyde and reduced glutathione as Biomarkers of continuous exposure of tech,Tinca tinica, to carbofuran or deltrametrhtin.Science of the total Environment, 408:4976-4983.

[4]Olivera, M., Ahemad, I., Maria, V.L., Pacheco, M.,Santos, M.A(2010). Monitoring pollution of coastal lagoon using Liza aurata kidney oxidative stress and genetic endpoints: an integrated biomarker approach.Ecology,19:643-653.

[5]Ray,A.Kand Ghosh, M.C (2006). Aquatic toxicity of carbamates and organophosphates.In: GuptaRR(Ed), Toxicology of organophophate and carbamate compound.Sction VII.Aquatic life & wildlife Academic press, USA, pp.657-672,chapter 45.

[6]Wang C.,Lu,Gui, J., Wang,P(2009). Sublethal effect of pesticide mixtures on selected biomarkers of *Carassius suratus*. Environ.toxicol. Pharmacol.28(3),414-419.

[7]Harpers Illustrated Biochemistry,(2012) (Large Medical Book); by Robert K.Murray, David A. Bender, Kathleen M.Botham, PeterJ.Kennelly, Victor W.Rodwell, P.Anthony Weil., ISBN-13: 978-0071765763, ISBN-10:007176576X, Edition:29th (2012), McGraw-Hill Medical Publishing.

[8]Lehninger principles of biochemistry.3td edition macmillan worth Publishers, Nelson, D.L, Cox,M.M(2005).

[9]Anita Susan,T.,Sobha, K., Veeraiah., and Tilak,K.S(2010). Studies on biochemical changes in the tissues of *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate technical grade.journal of toxicology and Environmental Health Science, 2(5):53-62.

[10]Ananth ,S.,Mathivan, V.,Aravinth, S., and Sangeetha,V(2014). Impact of arsenic metal toxicant on biochemical changes in the grass carp, *Ctenopharyngodon idella. I*nternational journal of modern research and Reviews, 2(2):74-78.

[11]Suneetha,K(2012). Effect of edosulfan and fenvalerate on carbohydrate metabolism of the freshwater fish, *Labeo rohita* (Hamilton). International Journal of pharmacy and pharmaceutical Science, 4(1):262-268.

[12]Uddhav Suryavanshi, R.A., Sreepada, Z.A., Ansari, Subhanchi Nigam, shahin Badesab (2009). A study of biochemical changes in the penaeid shrimp, *Metapenaeus monoceros* (fabricius) following exposure to sublethal doses of organochloride pesticide (endosulfan). Chemosphere,77(11):1540-1550.

[13]Begum,G.,and vijayaraghavan,S(1999). Effect of acute exposure of organophosphate insecticide rogor on some biochemical aspects of *Clarius batrachus* (Linn.).Environ.Res.A,80:80-83.

[14]Oruc ,E.O.,Uner, N.,(1999). Effects of 2,4 Diamin on some parameters of protein and carbohydrate metabolism in the serum, muscle and liver of *Cyprinus carpio.*Pollut.1055:267-272.

[15]Jimena L.Frontera, Anouk chaulet, Itzick Vatnick, Enrique M. Rodriguez(2011). Effects of Glyposate and polyox yethylenamine on growth and energetic Reserves in the Fresh Water Crayfish Cherax Quadricarinatus (Decapoda, Parastacidae).Arch Environ Contam toxicol,61:590-598.

[16]Vutukuru , S.S,(2005). Aqute Effects of Hexavalent Chromium on Survival, Oxygen consumption, Hematological Parameters and some biochemical Profiles of the Indian Major Carp, *Labeo rohita.*Int.Environ.Res. Public Health, 2(3):456-462.

[17]Kumara, B and Ahsan, J(2011). Study of muscle glycogen content in both sexes of Indian teleost Clarias batrachus(Linn.) exposed to different concentrations of arsenic. Fish Physiology and biochemistry,37:161-7.

[18]Sobha rani,A., sudharsan,R.,Reddy,T.N.Reddy,P.V.M and Raju,T.N(2000). Effect of sodium arsenite on glucose and glycogen levels in freshwater teleost fish, *Tilapia mossambica.*poll.res,19(1):129-13.

[19]Kumari, B and Ahsan,J(2011). Study of muscle glycogen content in both sexes of Indian teleost *clarias batrachus*(Linn.) exposed to different concentrations of arsenic Fish Physiology and Biochemistry,37:161-7.

[20]Tilak, K.S,P Wilson Raju, M S Butchiram,(2009) Effects of alachlor on biochemical parameters of the freshwater fish, Channa punctatus (Bloch). *Journal of Environmental Biology* 30(3):420-6.

[21]Reddy, A. T. V. and Yellamma, K: Perturbations in carbohydrate metabolism during cypermethrin toxicity in fish, *Tilapia mossambica* (Peters). *J. Biochem*. 23; 633-638, 1991.

[22]Veeraiah, K. and Durga Prasad, M. K: Study on the toxic effects of cypermethrin (technical) on organic constituents of freshwater fish, *Labeo rohita* (Ham). Proc. *Acad. Environ. Bio*. 7; 143-148, 1998.

[23]Veeraiah,K., Srinivas Rao,P.,symyuktha Rani,A.,and Dhilleswarao, H.,(2013). Changes in biochemical parameters of freshwater fish *Labeo rohita* exposed to lethal and sub-lethal concentrations of indoxacarb.Int.J.Bioassays,2010:1382-1387.

[24]Al-Ghanim, K, and Mahboob, S(2012). Effect of sodium cyanide on the activities of some oxidative enzymes and metabolites in *clarias gariepinus*. African Journal of Agricultural research,7(4):9849-9854.

[25]Muley,D.V.,Karanjkar, D.M.,Maske,S.V(2007). Impact of industrial effluents on the biochemical composition of freshwater fish *Labeo rohita.*Journal of Envi.biol.,28(2):245-249.

[26]Cicik, B., and Engin, K (2005). The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cuprinus carpio*(L.,1758). Turk.J.Vet.Anim. Sci.,29:113-117.

[28]Mistra ,V.,Kumar,V.,Pandey, S.D., Vishwanathan, P.N(1990). Carbohydrate metabolism chages in fish fingerlings and yearling exposed to linear alkylbenzene sulphonates. Water, air soil pollute, 50(3-4), 233-339.

[27]Ezemonye,L.I.N.,Ogeleka,D.F., and Okieimen,F.E(2007). Biological alterations fish fingerlings(Tilapia guineensis) exposed to industrial and corrosion inhibitors, Chemistry and Ecology, 23(5):373-382.

[29]Saxsena, K.K and Gupta, P (2005). Pyrethroid induced toxicity to muscle glycogen content in *Channa punctatus*(Bl) Proc. Nat Acd.Sci. India.75(B) (2):103-106.

[30]Ahemad, L., Khan,A, Khan, M.Z(2012). Pyrethroid- induced reproductive toxicopathology in non-torget species. Pakistan .Vet.J,32:1-9.

[31]Arockia –Rita, J.J., and Milton, J.M.C(2006). Effect of carbamate pesticide lannate(methomyl) on the biochemical components of the freshwater fish cichlid *Oreochromis mossambicus* (Peters). Indian journal of environmental and Ecoplan, 12:263-8.

[32]Ramakrishna,R.,and Sivakumar, A.A(1993). Effect of quinolphos on the fish *Oreochromis mossambicus*. Journal of Ecobiology 5:45-50.

[33]Venkaeswarlu , P., shobha, R.V.J., Janaiah, C., Prasad, M.S.K (1990). Effect of Endosulfan and Kelthane on haematology and serum biochemical parameters of the teleost, *Clarias atuachus*(Linn.). Indian J. comp.Anim.Physiol.,8(1):8-13.

[34]Neeraja, S.R.K and Giridhar,P(2014). Impact of deltamethrin on some aspects of Carbohydratemetabolism in fresh water fish *Labeo rohita* (Hamilton). International Journal of Advanced Research, 2(6):361-366.