Assessment of biochemical changes due to sub-lethal chronic toxicity in Labeo rohita exposed to Chloropyrifos and Neem oil.

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Abstract

Chloropyrifos, an organophosphate pesticide which is wide used across the state, while on the other hand use of Neem oil as biopesticide is a restricted affair. A detailed comparative toxicological implication of Chloropyrifos and Neem oil is lacking. Thus, this study is conceptualized to determine the LC50 values of Chloropyrifos (Commercial grade named as Dursban) and Neem oil on Labeo rohita (a well-known adequately consumed Indian major carp) and to further investigate the biochemical effects of two sub-lethal doses 1/2 of LC50 (0.021 mg/l) and 1/4th of LC50 (0.0105mg/l) of Chloropyrifos along with ½ of LC50 (22.32 mg/l) and ¼ th of LC50 (11.16mg/l) Neem oil. Among the biochemical parameters tested Serum glucose, cholesterol, triglycerides increased significantly ($p < 0.0001$) for both the sub-lethal doses of Chloropyrifos and Neem oil for a chronic exposure of 30 days in a dose and exposure dependent relationship but the level of increase is more in case of Chloropyrifos than Neem oil. Total serum protein and Liver glycogen showed a marked decline ($p < 0.0001$) for both the sub-lethal doses of Chloropyrifos and Neem oil, but the decline is more prominent in Chloropyrifos than Neem oil when exposed for 30 days of chronic toxicity. Overall result of the study indicates that Chloropyrifos is more harmful than Neem oil for non-target aquatic organism like fishes, hence care should be taken to manage contaminated agricultural run-off into the nearby water bodies serving as culture hub of fishes like labeo rohita.

Keywords: Chloropyrifos, Neem oil, biochemical parameters, labeo rohita.

1. Introduction

Aquatic ecosystem that passes through the agricultural land areas always has a higher probability of being contaminated with agricultural run-off containing a variety of pesticides. Hence pesticides accumulation and persistence in the aquatic environment affects behaviour, physiology of aquatic life thereby bringing selective pressure on aquatic ecosystem. (Ogueji,2020., V.C. Renick et al 2016, Kuo J. et al 2010, P. Nicolopoulou-Stamati 2016). One of the most pestering ecological concerns being bioaccumulation of pesticide residues in non-target organism like fishes, hence fishes serve as sensitive biomarker of aquatic ecosystem. Application of broad spectrum organophosphorus pesticide like Chloropyrifos is extremely challenging on fish population. (Kadam and Patil 2016). On the other hand, Neem is traditional highly esteemed medicinal plant as considered by the people of the Indian subcontinent. Azadirachtin (a tetranotriterpenoid) is one of the major components (Kraus et al., 1981; Broughton et al., 1986) of neem, which have pesticide property. Indiscriminate use of chemical pesticide is already posing a huge problem both for environmental and agricultural sector. Hence Neem based pesticides are now being popularised as a promising natural compound much safer for the environment. Hence this study intends to find out the comparative account of biochemical changes induced in non-target organism like Labeo rohita when treated separately with Chloropyrifos and neem oil pesticides.

2. Materials and Method:

2.1 Determination of LC 50 value of Chloropyrifos and Neem oil

In order to find out the acute toxicity of Chloropyrifos on the fingerlings of Labeo sp, initially a range finding study was conducted with less number of exposed fishes for 24 hours duration which was then followed by dose determining study for 96 hours duration.20 + 2 glass aquaria is used for the study (20 for treatment and 2 for control; Each experimental set -up required 10 aquaria and one for control, as the experiment is set in duplicate). During this 96 hour of study, water quality parameters were checked and recorded, observed behavioural changes indicated as stress responses was recorded, respiratory distress was noted after an interval of 24 hours (Observations were done after every 12 hours – morning at 6 am and afternoon 6 pm, and then an average is made). 40 % of water was changed every day and proportionate amount of toxicant added, so that the dose of toxicant remains constant and fecal matters can be removed. Fishes are not fed during these 96 hours of study to eliminate any metabolic effect apart from toxicant on the treated fishes. Dead fishes are removed at regular interval to prevent contamination of water. In the range finding study it was observed that there was no death of fishes below 0.02 ppm and all fishes died above 0.2 ppm. Hence, of dose finding study, the concentration of Chloropyrifos chosen was from .02mg/ l to .2 mg/l dissolved initially in acetone before being added to the aquarium

water. The doses given were as follows: 0.02, 0.04, 0.06, 0.08, 0.10, 0.12,0.14,0.16, 0.18 and 0.2 mg/l. Total number of fishes exposed was 10 for each concentration. For Neem oil In the range finding study it was observed that there was no death of fishes below 40 ppm and all fishes died above 65 ppm. Hence, of dose finding study, the concentration of Chloropyrifos chosen was from 40 mg/ l to 60 mg/l dissolved initially in Dimethyl sulfoxide before being added to the aquarium water. The doses given were as follows: 40, 42, 44, 46, 48,50,52, 54, 56, 58, 60 mg/l. Total number of fishes exposed was 10 for each concentration.

2.2 Determination of the sub-lethal dose for chronic toxicity study

Chronic toxicity studies were conducted for a period of 30 days with Chloropyrifos and Neem oil Treatment with two sublethal doses of each toxicant to understand the biochemical changes in Labeo sp in comparison to control condition. Control condition in exhibited in two forms- dechlorinated water with acetone for Chloropyrifos while dechlorinated water with DMSO in case of Neem oil. For each sublethal dose of each toxicant, studies were conducted in triplicates to avoid statistical errors and reinforce authenticity of results. The sublethal doses for both Chloropyrifos and Neem oil were ½ of 96 hours LC50 value [CPF_T1(0.021 mg/l) and NO_T1(22.32 mg/l)] and 1/4th of 96 hours LC50 value [CPF_T2 (0.0105mg/l)and NO_T2 (11.16mg/l)]. For chronic toxicity studies, observations were taken at an interval of 10 days in the study period of 30 days. There were 6 groups – 4 treated and 2 control group. Acclimatised 10 fishes (n=10) were introduced in each replicate of each group. In order to maintain water quality and concentration of test media, the medium of all test groups and control were replaced in every 5 days with freshly made Chloropyrifos and Neem oil solution. For 30 days the exposed fishes were fed 3 times a day, until they looked visually satiated. Fishes were kept in 12 hours photoperiod (12 hours of light and 12 hours of darkness) and water quality parameters like dissolved oxygen, temperature, hardness, alkalinity, conductivity and free carbondioxide was maintained and measured at an interval of 10 days in a 30 day period , so that any change in control or test fishes due to change in physico-chemical properties of the aquaria water could be avoided. The bioassays and chemical analysis of the water were carried out following the American Public Health Association's guidelines (APHA)

2.3 Determination of Biochemical endpoint

For biochemical analysis, fishes in control and treated groups were stopped feeding 24 hours prior to blood collection to avoid any metabolic disparity among the groups. From each group (i.e Control_CPF, Control_NO, CPF_T1, CPF_T2, NO_T1, NO_T2), 3 fishes were sampled (one from each replicate). Fishes were anesthesised prior to blood collection with tricaine methane sulfonate (MS- 222; 0.3 g/L) to avoid any stress condition. Blood was drawn from caudal vein with 24 gauge needle in 2.5 ml sterile syringe (the needle and syringe being washed with EDTA solution prior to use).Following biochemical parameters are analysed for the treated and control groups: Plasma Glucose (Glucose oxidase–peroxidase (GOD–POD) [Trinder, P. (1969)], Liver Glycogen (Anthrone method) [Caroll et.al 1956], Total Protein (Lowry's method), Cholesterol (Zlatkis–Zak method) Zlatkis,1953] and Triglycerides (Enzymatic GPO–PAP (Bucolo–David method))

2.4 Statistical calculation

The Shapiro-Wilk test examined the normality distribution of data and data transformation was applied in case of asymmetry to conform to normality while Levene's test was utilized to check homogeneity. The Graph Pad Prism 8.1.2 computer program (Prism, USA) was employed for data analysis. Comparisons between control and exposed fishes were performed by two-way ANOVA followed by Tukey's Comparison Test to determine significant differences among the means ($p < 0.05$ – Gomez and Gomez, 1984). Results are summarized as mean ± standard deviation (SD). For all analyses, statistical significance was ascertained at no less than $p < 0.05$.

3. Result and discussion:

3.1 Serum Glucose and liver glycogen:

It was observed that the serum glucose level increased significantly in CPF_T1 and CPF_T2 and reached their respective peak in 30 days chronic exposure. There is dose and time dependent increase observed both for Chloropyrifos and Neem Oil treatment. Although serum glucose level of the Neem oil exposed fishes showed significant variation from control but the difference was less than Chloropyrifos. It was observed that the liver glycogen level decreased significantly in CPF_T1 and CPF_T2 and reached their respective peak in 30 days chronic exposure. There is dose and time dependent increase observed both for Chloropyrifos and Neem Oil treatment. Although liver glycogen level of the Neem oil exposed fishes showed significant variation from control but the difference was less than Chloropyrifos.

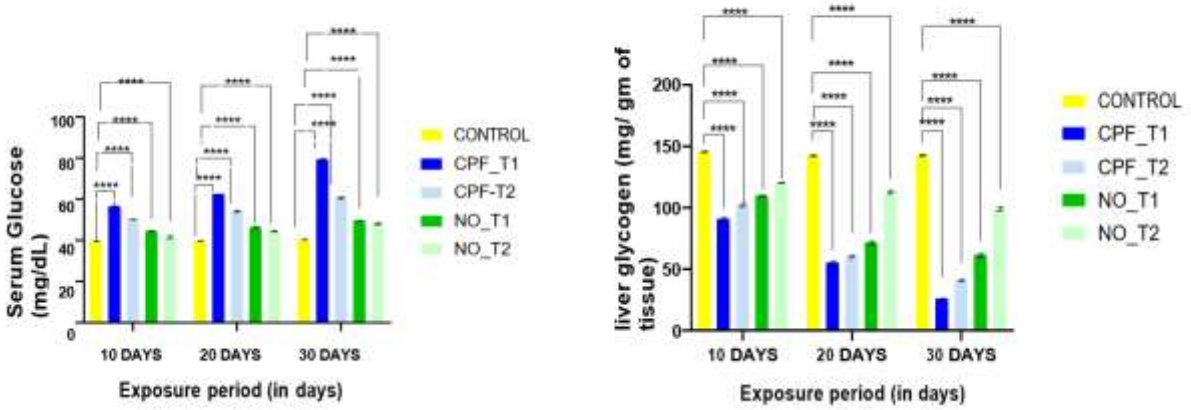

Fig1. Differences in the mean values ± SD of Serum Glucose and liver glycogen in Labeo rohita exposed to Chloropyrifos at CPF_T1 (0.021 mg/L) and CPF_T2 (0.0105 mg/L) as well as Neem oil NO_T1 (22.32 mg/l) and NO_T2 (11.16 mg/l) for 10, 20 and 30 days. Where error bars = SD, * denotes significant differences to control within the same exposure time ($p < 0.0001$).

3.2. Total Protein:

It was observed that the total serum protein level decreased significantly in CPF_T1 and CPF_T2 and reached their respective lowest values in 30 days chronic exposure. There is dose and time dependent increase observed both for Chloropyrifos and Neem Oil treatment. Although total serum protein of the Neem oil exposed fishes showed significant variation from control but the difference was less than Chloropyrifos.

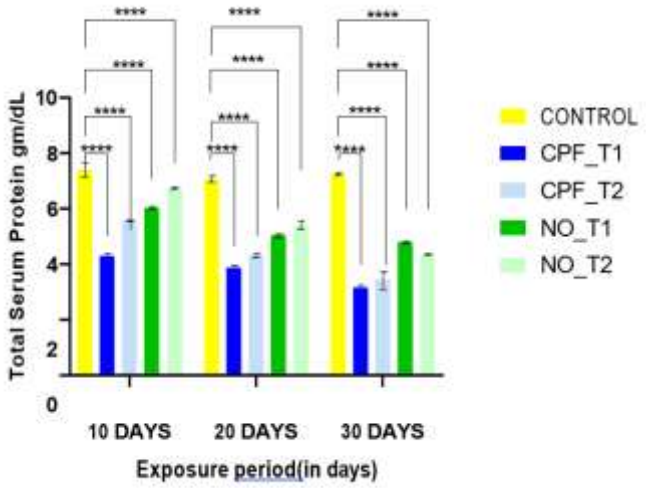

Fig2. Differences in the mean values of ± SD of total serum protein content in Labeo rohita exposed to Chloropyrifos at CPF_T1 (0.021 mg/L) and CPF_T2 (0.0105 mg/L) as well as Neem oil NO_T1(22.32 mg/l) and NO_T2 (11.16 mg/l) for 10, 20 and 30 days in comparison to control. Where error bars = SD, * denotes significant differences to control within the same exposure time ($p < 0.0001$).

3.3 Total Cholesterol and Triglycerides:

It was observed that the serum cholesterol content increased significantly in CPF_T1 and CPF_T2 in chronic exposure to pesticide. There is dose and time dependent increase observed both for Chloropyrifos and Neem Oil treatment. Changes in Cholesterol level for Neem oil treated fishes were insignificant (ns at $p < 0.05$) during 10, 20and 30 days interval, while on the other hand changes in cholesterol level for Chloropyrifos treated fishes were significantly ($p < 0.0001$) different from the control fishes for the same time of exposure.It was observed that the serum triglyceride level increased significantly in CPF_T1 and CPF_T2 in chronic exposure to pesticide. There is dose and time dependent increase observed both for Chloropyrifos and Neem Oil treatment. Changes in Cholesterol level for Neem oil treated fishes were insignificant (ns at $p < 0.05$) during 10, days interval for NO_T2, but significant ($p < 0.001$) at 20 days and 30 days interval. Changes in triglycerides level for Chloropyrifos treated fishes were significantly ($p < 0.0001$) different from the

control fishes for the same time of exposure.

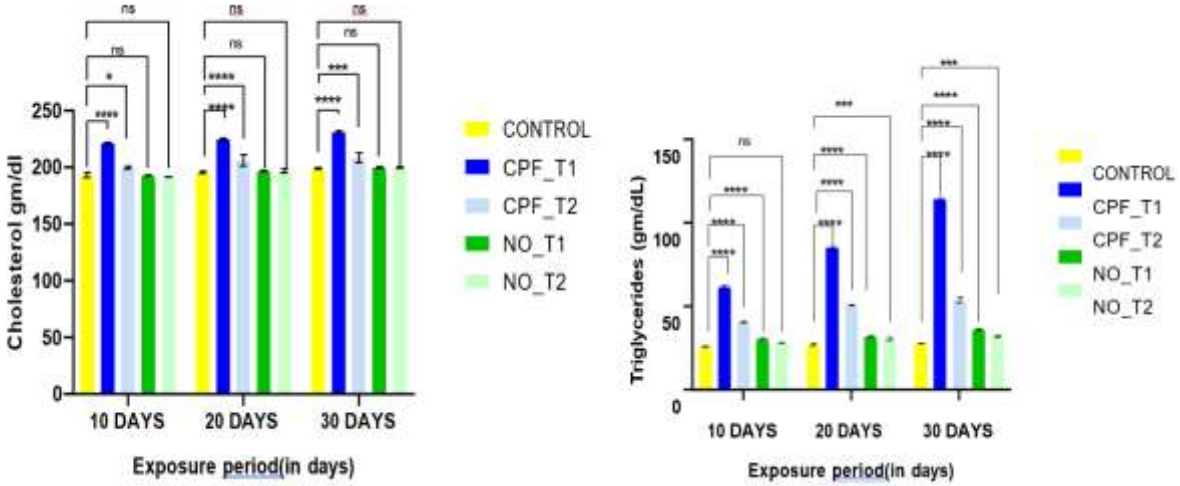

Fig3. Differences in the mean values of ± SD of total serum cholesterol and triglyceride content (gm/dL)in Labeo rohita exposed to Chloropyrifos at CPF_T1 (0.021 mg/L) and CPF_T2 (0.0105 mg/L) as well as Neem oil NO_T1 (22.32 mg/l) and NO_T2 (11.16 mg/l)for 10, 20 and 30 days in comparison to control. Where error bars = SD, * denotes significant differences to control within the same exposure time ($p < 0.0001$).

Our result showed two-fold higher in blood glucose level which was dose dependent may be considered as stress induced by chlorpyrifos exposure. Sadguru Prakash (2020) reported blood glucose level has long been used as indicators of toxicant chlorpyrifos in fish Heteropneustes fossils blood glucose level may be due to enhanced conversion of liver and muscle-glycogen in to glucose to meet an increased energy needs under stress conditions. Pesticide produce adverse effect on the carbohydrate metabolism by enhancing the blood glucose level directly interfering with the glucose regulatory mechanism of blood. Similar findings reported by Neeraja et al.,(2014) in fresh water fish Labeo rohita exposed to Deltamethrin. Bhoi., (2019) explained blood glucose level increased due to severe metabolic stress exposure of Cypermethrin and Fenvalerate. In the present investigation there was the total protein level in blood of labeo rohita declined in all exposure periods in the sub lethal concentrations of chlorpyrifos. Rajeshwari et al., (2017) explained plasma proteins were declined significantly with exposure period of pesticides. This could be attributed to renal excretion or impaired protein synthesis or due to liver disorders,the observed decrease of protein could also result from the breakdown of protein in to amino acids first and possibly in to nitrogen and other elementary molecules. Shubhajit Saha (2021) investigated the depletion of total serum protein levels in TG level in blood exposed C.batraches compared to the control group. Similar patterns of lowered serum protein levels have been investigated on Oreochromis niloticus exposed to diazinon by Soyingbe. (2012). Triglycerides and cholesterol showed gradually increased with control level. Natizish Iftiskar (2021) reported triglycerides was a very important source of energy during stress and an increase in TG level in blood may be due to lipid metabolism to cope with increased energy demand. Gaber et al., (2013) noticed high content of triglycerides in the blood may be transfer from the synthesis site for consequent use by process of oxidation or steady instauration of these molecules.

4. Conclusion

The results of the study indicates that Chloropyrifos is far more harmful with respect to biochemical endpoint determination than neem oil in Labeo sp when exposed to chronic sub-lethal dose of both the pesticide. Hence choice of pesticide, its pattern of usage is the key factor in keep up the growth and survival of the non-target organisms like fishes

5. Acknowledgments:

Author is thankful to Department of Higher Education, Science & Technology and Biotechnology, (Science & Technology and Biotechnology Branch) Government of West Bengal, Vigyan Chetana Bhavan (2nd floor) West Bengal Department of Science and Technology for providing the grant (vide Memo No : 436 (Sanc) /ST/P/S&T/17G-18/2018 dated 11.03.2019 and 1157(Sanc.)/STBT-11012(27)/20/2021-ST SEC dated 15.02.2022) for successfully conducting the work

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