



## STUDIES ON THE IMPACT OF CHLORPYRIFOS AND CARBOFURAN IN THE INDIAN MAJOR CARP, *CATLA CATLA*

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### ABSTRACT

Fingerlings of Indian major carp, *Catla catlas* was exposed to two different pesticides chlorpyrifos and carbofuran for a period of 24hrs. Various parameters including biochemical and enzyme activity was envisaged to determine the effect of the pesticides. Total protein, carbohydrate and lipid were found to decrease in both pesticide treated groups in all the organs of the fish. Succinate dehydrogenase (SDH) increased in the liver (7.14 MIU/min/mg) of chlorpyrifos treated group and decreased in all the other tissues compared to control. Similar to SDH, Lactate dehydrogenase (LDH) activity also increased in the liver of both chlorpyrifos (7.66MIU/min/mg) and carbofuran (7.15MIU/min/mg) treated groups. However maximum LDH activity was observed in the muscle (8.58MIU/min/mg) of carbofuran treated groups. Acid phosphatase (ACP) increased in both the treated groups in brain, muscle and kidney whereas, alkaline phosphatase (ALP) was found to decrease in all the treated groups compared to control.

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### INTRODUCTION

Agriculture is the important need of the increasing population to meet their food demand. Pesticides are the major reason for large amount of crop yield. The high ability to control the harmful pest is the main reason for the increasing demand of the pesticide all over the world. Chemicals are the ubiquitous substances which have both negative and positive effect on the environment (Kyung-Taek Rim, 2017). Chlorpyrifos and carbofuran are the two pesticides used widely for their rapid environmental degradation (Eto, 1974). Due to their lack of target specificity they cause severe impact on the population of the non-target aquatic species (Schulz and Liess, 1999; Fulton and Key, 2001). These compounds enter through agriculturalrun- off, industrial effluent, and other sources in the aquatic media affects non-target organisms like fish which are of great economic importance to humans (Adhikari *et al.*, 2004). They cause biochemical changes by influencing the activities of several enzymes (Sancho *et al.*, 1998). Thus, determination of harmful and toxic substances in water, sediments and biota will give direct information on the significant of pollution in the aquatic environment (Subha *et al.*, 2016).

Biochemical and enzyme studies are the important tool to analyze the toxicity of the xenobiotics on fishes. It records good understanding about the pesticides and their impacts. Most of the toxicants react with the metabolites and bind with

all functional components of the cell. Such interaction may induce a sequence of structural and functional alternations which leads to the change in biochemical activity which in turn cause irreversible and detrimental disturbances of integrated functions (Singh *et al.*, 2000). Enzymes accelerate and control numerous chemical reactions and determine the metabolism and important activities of a cell of the organism. This physiological imbalance can be disturbed by the environmental pollutants. This changes in enzymes helps in the determination of impact of applied toxicants in the fish (Leena muralidharan *et al.*, 2014).

*Catla Catla* is a major cultured carp and an important staple freshwater fish generally found in rivers, ponds and reservoirs (Murty, 2010). Hence the objective of the present study was to evaluate the toxic effect of the pesticides chlorpyrifos and carbofuran in brain, muscle, liver and kidney tissues of the Indian major carp *Catla Catla*.

### MATERIALS AND METHODS

#### Animal collection

*Catla catla* weighing 12 to 15 gm were procured from the Poondi reservoir, Tiruvallur District, TamilNadu, India. The fishes were brought to the laboratories and acclimatized to the laboratory condition. About 30 fishes were introduced in two different tanks containing pesticides chlorpyrifos and carbofuran. The experiment was carried out for a period of 24 hours. Subsequently upon the exposure to pesticides tissues such as brain, muscle, liver and kidney were dissected to determine the biochemical and enzyme activity.

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**Biochemical analysis**

Protein content in various tissues (brain, muscle, liver and kidney) was determined according to the standard procedure of Bradford (1976). Similarly carbohydrate and lipid content were determined according to Carroll *et al.*, (1956) and Folchet *et al.*, (1957) respectively.

**Enzyme activity**

Succinate dehydrogenase and Lactate dehydrogenase activity was carried according to the standard protocol of Nachalaset *et al.*, 1960 and King, 1965. Acid and alkaline phosphate activity was determined according to the protocol of Tenniswood *et al.*, 1976.

**RESULTS**

**Biochemical variations**

**Protein**

Protein content in all the tissues showed variations in both control and treated groups (Fig. 1). Total protein in brain of chlorpyrifos (5.47±0.47 mg/g) and carbofuran (4.53±0.25 mg/g) treated fish was found to decrease compared to control (7.05±0.23 mg/g). Decrease in muscle was observed in both chlorpyrifos (4.18±0.04 mg/g) and carbofuran (6.23±0.30mg/g) compared to control (8.5±0.46mg/g) group. In liver maximum decrease was observed in Chlorpyrifos (2.23±0.11 mg/g) than the control (6.43±0.35mg/g). Minimum decrease was observed in carbofuran (3.06±0.05mg/g) treated group. In kidney control showed 7.37±0.10 mg/g where chlorpyrifos (2.53±0.25 mg/g) showed maximum decrease and carbofuran (4.43±0.43 mg/g) showed minimal.

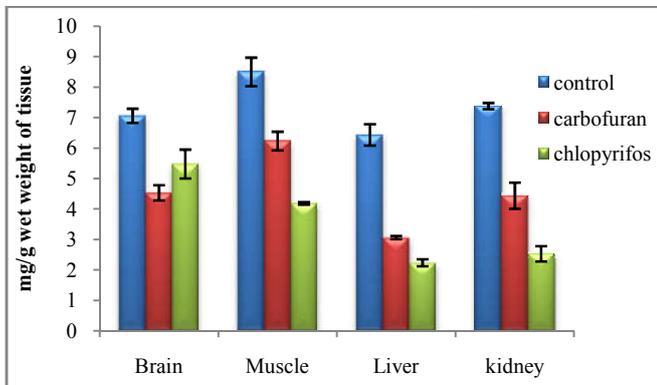


Fig 1 Impact of pesticides on total protein content in various tissues of *Catla Catla*

**Carbohydrate**

Carbohydrate content showed variations in all the tissues (Fig.2). In brain maximum decrease was found in chlorpyrifos (0.78±0.06 mg/g) treated group than the control (2.37±0.04mg/g). Minimum decrease was found in the carbofuran (1.86±0.11mg/g) and in muscle maximum decrease in chlorpyrifos (0.92±0.07mg/g) compared to the control (1.93±0.47mg/g). In liver and kidney maximum reduction was found in chlorpyrifos treated group i.e., in liver (4.6±0.36mg/g) and kidney (0.78±0.09mg/g) and minimum was found in carbofuran treated group ie., in liver(5.26±0.60mg/g) and kidney (1.02±0.13 mg/g) compared to control liver (8.6±0.3mg/g) and kidney (2.53±0.35mg/g).

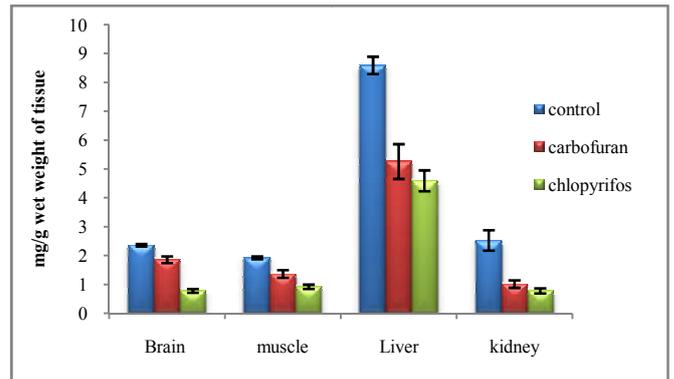


Fig 2 Impact of pesticides on carbohydrate content in various tissues of *Catla Catla*

**Lipid**

Similar to protein and carbohydrate, lipid content also showed variations in all the tissues (Fig.3). Maximum decrease of 4.5mg/g in brain was observed in chlorpyrifos treated group than the carbofuran (5.53±0.30 mg/g) compared to the control (7.4±0.55 mg/g). In muscle carbofuran (6.53±0.35 mg/g) showed maximum activity than the chlorpyrifos (4.18±0.11 mg/g). Control of liver recorded 8.13mg/g carbohydrate content. Reduced carbohydrate content was observed in chlorpyrifos (4.53±0.35 mg/g) and minimum reduction was observed in carbofuran (6.03±0.03 mg/g) treated fish. Overall the biochemical parameters clearly indicate that the protein, carbohydrate and lipid content decreased in all the pesticide treated groups compared to control.

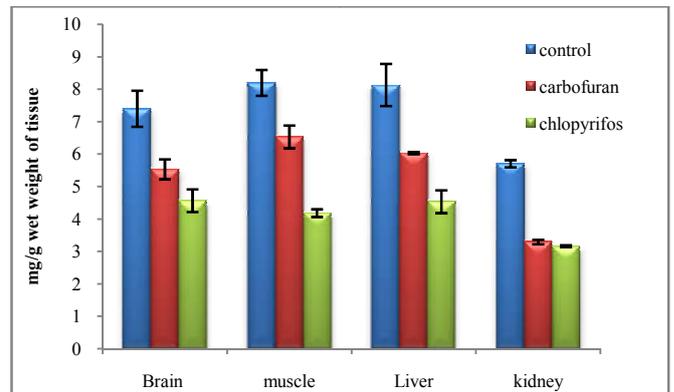


Fig 3 Impact of pesticides on lipid content in various tissues of *Catla Catla*

**Enzyme Activity**

**SDH**

Maximum increase was found in chlorpyrifos (7.4±0.04 MIU/min/mg) treated fish liver and maximum decrease was found in carbofuran (4.14±0.02 MIU/min/mg) treated fish liver (Fig.4). In brain chlorpyrifos (5.11±0.03 MIU/min/mg) showed increased activity whereas carbofuran (4.73 ±0.08MIU/min/mg) showed minimum activity but both showed decreased activity compared to control (6.91±0.04 MIU/min/mg). In muscle, control showed 8.36±0.21 MIU/min/mg whereas chlorpyrifos (4.24±0.07 MIU/min/mg) and carbofuran (5.3±0.5 MIU/min/mg) showed decreased SDH activity.

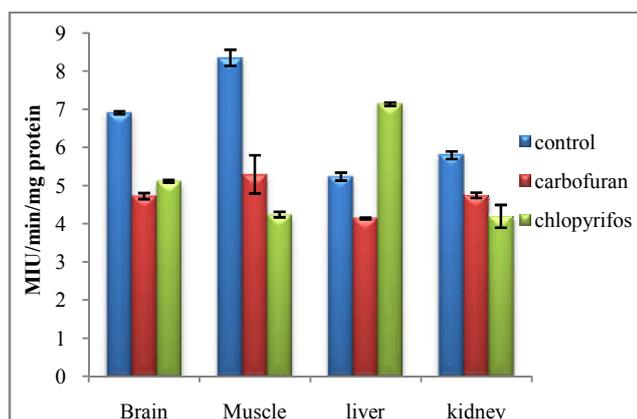


Fig 4 Impact of pesticides on succinate dehydrogenase (MIU/min/mg) in various tissues of *Catla Catla*

**LDH**

Carbofuran ( $4.17 \pm 0.05$  MIU/min/mg) treated fish showed increased activity in brain than chlorpyrifos ( $2.76 \pm 0.04$  MIU/min/mg) compared to control group ( $4.42 \pm 0.03$  MIU/min/mg). Carbofuran ( $8.52 \pm 0.07$  MIU/min/mg) in muscle showed increased activity than chlorpyrifos ( $5.4 \pm 0.25$  MIU/min/mg) and control ( $7.60 \pm 0.08$  MIU/min/mg). In liver chlorpyrifos ( $7.66 \pm 0.05$  MIU/min/mg) and carbofuran ( $7.15 \pm 0.05$  MIU/min/mg) showed increased activity than the control ( $6.83 \pm 0.07$  MIU/min/mg) (Fig.5). In kidney both chlorpyrifos ( $4.75 \pm 0.06$  MIU/min/mg) and carbofuran ( $4.65 \pm 0.03$  MIU/min/mg) recorded decreased activity than control ( $5.44 \pm 0.09$  MIU/min/mg).

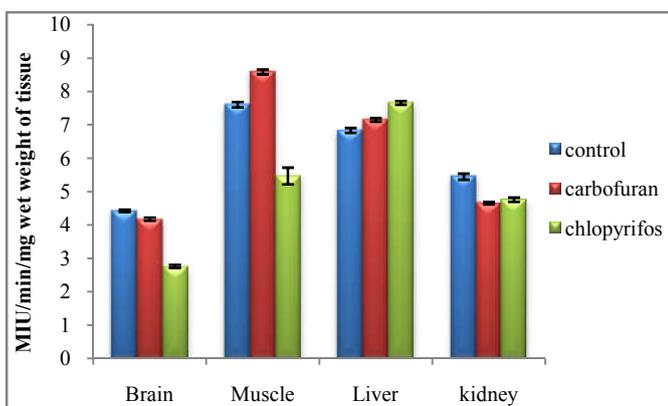


Fig 5 Impact of pesticides on lactate dehydrogenase (MIU/min/mg) in various tissues of *Catla Catla*

**ACP**

Chlorpyrifos ( $4.90 \pm 0.08$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) and carbofuran ( $4.56 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) showed a minimal rise in brain than the control ( $4.34 \pm 0.11$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) (Fig.6). Similar to the brain in muscle chlorpyrifos ( $6.4 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) and carbofuran ( $6.16 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) increased than the control group ( $5.70 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ). In liver carbofuran ( $5.64 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) and chlorpyrifos ( $5.15 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) showed minimum activity than control ( $6.41 \pm 0.05$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ). Carbofuran ( $7.06 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) in kidney showed maximum activity than control ( $6.56 \pm 0.35$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ )

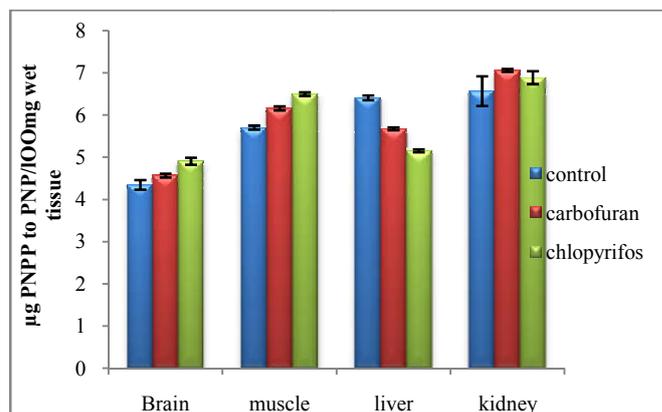


Fig 6 Impact of pesticides on Acid phosphatase ( $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) in various tissues of *Catla Catla*

**ALP**

Minimum ALP activity was observed in carbofuran ( $6.76 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) and chlorpyrifos ( $6.11 \pm 0.02$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) treated fish brain than the control ( $7.42 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) (Fig.7). In muscle, carbofuran ( $3.14 \pm 0.02$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) and chlorpyrifos ( $2.93 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) showed decreased activity than control ( $4.35 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$ ). Carbofuran recorded  $6.53 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$  and chlorpyrifos recorded  $6.05 \pm 0.04$   $\mu\text{g/PNPP to PNP}/100\text{mg}$  than the control  $7.54 \pm 0.03$   $\mu\text{g/PNPP to PNP}/100\text{mg}$  in liver.

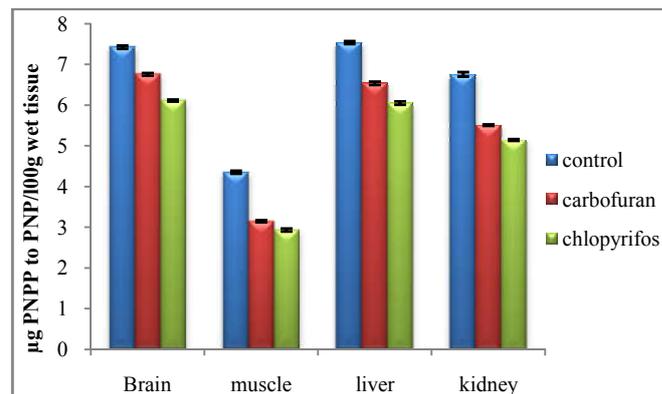


Fig 7 Impact of pesticides on Alkaline phosphatase ( $\mu\text{g/PNPP to PNP}/100\text{mg}$ ) in various tissues of *Catla Catla*

**DISCUSSION**

The quality of environment is assessed by studying the biochemical and enzymatic alteration in the fish. Diagnosing the biochemical parameters helps in the analyzing the extent of the damage caused by the toxicants in the exposed fish organs (Ahsan khan *et al.*, 2016). In the present study protein, carbohydrate and lipid was decreased in all the organs of the exposed group when compared to the control. Chlorpyrifos treated fishes showed the maximum decrease compared to carbofuran. The decrease in the biochemical parameters in the present study may be due to the stress produced by the treated toxicants. Protein content decreased in all the tissues in pesticides treated groups which may be attributed due to physical stress created by the toxicants which leads to structural and functional changes in the cellular proteins. The toxicant can stimulate the proteolysis in tissues by activating protease enzymes. The depletion of protein may create physiological mechanism and constitute compensatory

mechanism under stress and enhance osmolarity of the body fluids under stress(vimala k john and karthika ,2016).Carbohydrates serve as an energy reservoir for the aquatic organisms. In the present study carbohydrate content showed significant decrease in all the tissues examined the depletion in carbohydrate is observed to accomplish the energy demand caused by the stress (Suneetha, 2012). In the absence of carbohydrates lipid serves as the energy reserves to meet the energy demand during the toxic stress (Neelima *et al.*, 2016). Hence lipid content also found to show significant decrease in all the tissues. As mentioned earlier the decrease in lipid content is to meet the metabolic demand for the extra energy needed to manage with toxic stress (Senthilkumaar *et al.*, 2014).

Toxicants cause disturbance in physiological state of the organism and bring distortions in the organelles. It may cause increase or decrease in enzyme activity of the fish (Senthilkumaar *et al.*,2014) . Succinate dehydrogenase increased in the liver(7.19 MIU/min/mg) of the chlorpyrifos treated group and lactate dehydrogenase increased in muscle of carbofuran ( 8.57MIU/min/mg) treated group and in liver it increased in both the groups; carbofuran (7.13MIU/min/mg) and chlorpyrifos(7.72MIU/min/mg) but maximum was observed in chlorpyrifos. Similar to that of present study, Basanta Kumar Das and Subhas Chandra Mukherjee (2003) exposed cypermethrin to the fingerlings of *Labeorohita* where, succinate dehydrogenase activity decreased in brain, kidney and liver. Acid and alkaline phosphatases is the hydrolytic lysosomal enzymes, plays a role in detoxification function. The alteration in the ACP and ALP levels may be due to the direct impact of the toxicant on physiology of the fish. The present study thus, provides primary information on the impact of pesticides in various tissues in the Indian major carp *Catla Catla*.

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