

Pesticides impact on protein in fish (*Oreochromis mossambicus*) tissues

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Indiscriminate use of agrochemicals to control pests in agriculture to increase the yield of crop causes chemical pollution. The pesticides are extremely toxic to non-target organisms, like fish and affect fish health through impairment of metabolism, sometimes leading to mortality, adversely affecting the complex food-web and population dynamics. In the present study, an attempt has been made to investigate the acute toxicity of organophosphate pesticides (chlorpyrifos and malathion), synthetic pyrethroid pesticides (cypermethrin, lambda-cyhalothrin) and herbicide (bucril) on total protein content of the fish (*Oreochromis mossambicus*). For this regard fish were treated for 24 and 48 hrs with different concentrations of pesticides. Total protein content in fish tissues were determined by Biuret method. The present study show that total protein content was inhibited in *Oreochromis mossambicus* after exposure to organophosphate pesticides (chlorpyrifos, malathion), synthetic pyrethroids (lambda-cyhalothrin, malathion) and herbicide (bucril). The levels of total protein content showed decrease for pesticide treated fish in the order of cypermethrin, malathion, chlorpyrifos, lambda-cyhalothrin and bucril respectively at 48 hrs. The present study reports metabolic dysfunction in response to pesticide toxicity in the fish. Pesticide acts as stress inducing agents which affect the functional state of tissues of the exposed organisms.

[**Key words:** *Oreochromis mossambicus*, cypermethrin, chlorpyrifos, malathion, protein].

Introduction

Pesticides are extensively used to enhance the agriculture production by controlling different kind of pests¹. But at the same time severe environmental problems arise due to pesticides which influence structure and function of ecosystem². Pesticides enter the aquatic environment through rivers, by direct application, spray, drift, aerial spraying, and washing, from the atmosphere by precipitation, erosion and runoff from agricultural land, factory effluents and sewage³. Marine organisms are prone to xenobiotic substances and their bioaccumulations are serious risk to life. Such toxic substances enter the human body through food chain, as fishes constitute an important part of animal protein. Pesticides affect non-target animals such as fish^{4,5,6}. Pesticides are highly toxic, not only to fishes but also to other organisms, which form food of the fishes^{7,8}. The intake of pesticides affects the biochemical composition of fishes^{9,5,6}. Organophosphates pesticides are used in agriculture in different parts of the world due to their biodegradability, high effectiveness and low

persistence in the environment¹⁰. Although other groups of insecticides with a shorter life and comparatively very low mammalian toxicity are available (e.g. pyrethroids). In Pakistan both pyrethroids and organophosphates are extensively used in agricultural land¹¹.

Fish are susceptible to aquatic pollutants as fishes are in direct contact with the surrounding water through their gills¹². Gills surface comprises over half the body surface area of fish and only a few microns of gill epithelium separate the internal environment of the fish from external environment¹³. Proteins are macromolecules considered as the architecture of cell. Proteins are involved in physical and chemical activities to maintain the homeostasis of the cell. Amino acids are regarded as building blocks of proteins. Amino acids are essential intermediates in protein synthesis and its degradation products appear in the form of different nitrogenous substances. Therefore, the assessment of the total protein content can be considered as a diagnostic tool to determine the physiological process of the cell¹⁴.

Oreochromis mossambicus is found in tropical and subtropical habitats, live in rivers, lagoons, creeks and streams. *Oreochromis mossambicus* are euryhaline fish that can live in a wide range of salinity from fresh water to full seawater¹⁵. This sturdy fish tolerates wide temperatures, has a broad, omnivorous diet, its firm texture and mild taste make it very popular for eating. It is used for aquaculture as it readily adapts to new situations, breeds quickly. In the present study, an attempt has been made to investigate the acute toxic effect of organophosphate pesticides (chlorpyrifos and malathion), synthetic pyrethroid pesticides (cypermethrin, lambda-cyhalothrin) and herbicide (buctril) on total protein content of the fish (*Oreochromis mossambicus*).

Material and Methods

Preparation of Chemicals

Pesticides were purchased from the market, organophosphate (chlorpyrifos 40% EC, malathion 57% EC) and synthetic pyrethroid pesticide (cypermethrin 10% EC, lambda-cyhalothrin 2.5% EC) and herbicide (buctril 60% EC) were used.

Fish

The fishes *Oreochromis mossambicus* were collected from Chilya hatchery Thatta. The fish were transported in clean aerated water to the laboratory ensuring minimum stress. The fishes were acclimatized in the laboratory conditions for 48 hours prior to experiments. The fishes were kept in clean aerated seawater in glass aquaria (92 cm Length x 39cm width x 47 cm height) at temperature ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$), with photoperiod 16 hour of light and 8 hour of dark (16 L: 8 D) cycle. Seawater in each aquarium was replenished everyday in order to remove faeces and remaining food everyday and to maintain the water quality and oxygen saturation level above 60%. Fishes were fed ad libitum and commercial diet two times a day. Fishes ($2.7\text{cm} \pm 1\text{cm}$) length, ($5\text{gm} \pm 1$) weight were used in this experiment.

All the glassware was acid washed prior to the tests¹⁶ and natural seawater was used throughout the experiments. Fish were exposed to different concentrations of selected pesticides. Experiments were carried out in glass aquarium (30.5 cm length x 30.5cm width x 30.5 cm height). All pesticide concentrations were prepared with filtered seawater. The experiment was performed in triplicate. Three controls were also set up for each experiment. The control has only seawater.

The experiment was performed at temperature ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$), Salinity 30 ppt, pH 7.5, photoperiod 16 hour light and 8 hour dark.

Total Protein

Total protein content in tissues of fish exposed to five pesticides was analyzed to determine degree of impact according to procedures described earlier⁹. Known weight of fish (six replicates; both test and control) tissue was homogenized with distilled water (2ml) using mortar and pestle. Content was centrifuged at 15000 rpm for 15 min. Supernatant was immediately analyzed for total protein using analysis kits which employs Biuret method¹⁷ Radox kit (TP 245)). Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. Measure the absorbance at 546 nm of the sample and of the standard against the reagent blank.

Calculation

$$\text{Total Protein Conc} = \frac{\text{A Sample} \times \text{Standard conc}}{\text{A Standard}}$$

A Sample = Absorbance of sample

A Standard = Absorbance of standard

Result

The present study show that total protein content was inhibited in *Oreochromis mossambicus* after exposure to organophosphate pesticides (chlorpyrifos, malathion), synthetic pyrethroid (lambda-cyhalothrin, malathion) and herbicide (buctril) (Figure 1). Total Protein contents were reduced in fish (*Oreochromis mossambicus*) tissues with increase in exposure period as compare to control (Figure 1). The present study shows that at 24 hrs the order of toxicity to fish (*Oreochromis mossambicus*) is malathion, lambda, cypermethrin, buctril, chlorpyrifos (Figure 1). On the contrary at 48 hrs the order of toxicity to fish is cypermethrin, malathion, chlorpyrifos, lambda-cyhalothrin and buctril (Figure 1). It is noticed that the changes in the total protein content fluctuated during different intervals of time.

In case of lambda-cyhalothrin and malathion significant percent activities ($p < 0.05$) were noticed in all the concentration treated fish at 24h and 48h but on the contrary at concentration 0.0001ppm percent activity was not significant. In buctril treated fish only two concentrations 0.001ppm and 0.0003ppm showed the significant percent activity ($p < 0.05$) at 24 hours exposure.

No significant percent activities were noticed in chlorpyrifos treated fish. In case of cypermethrin, all five concentrations treated fish, percent activity was significant ($p < 0.05$) at 24 and 48 hours exposure except at 0.0001ppm concentration.

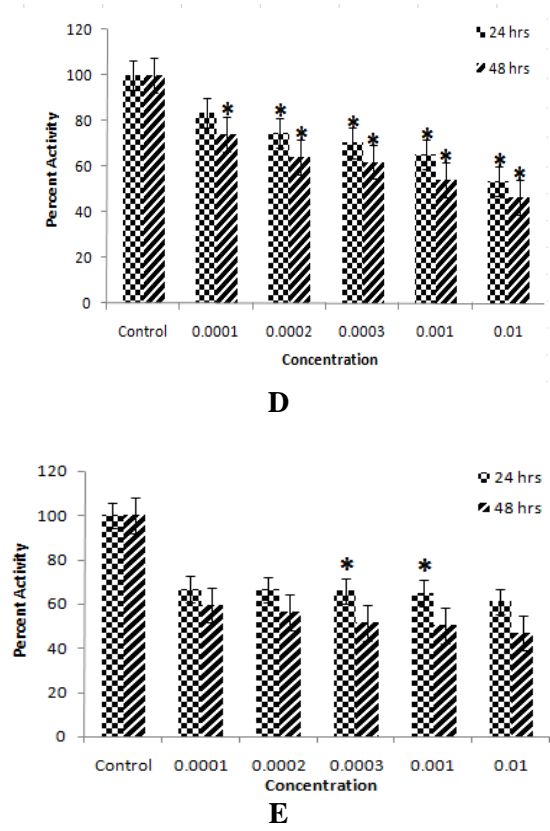
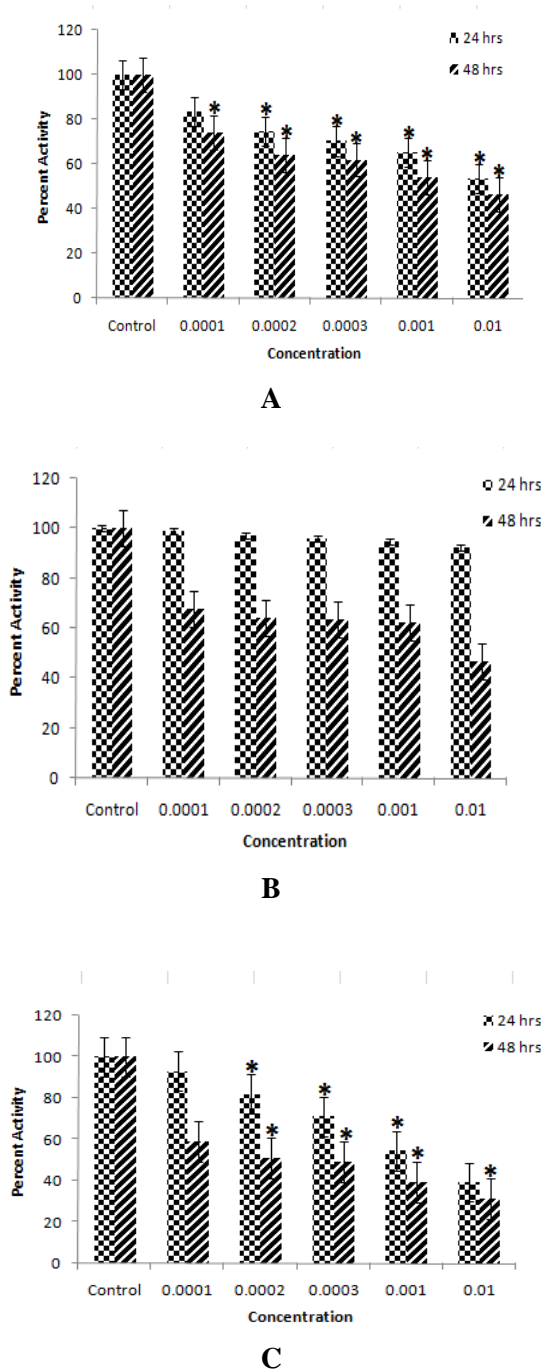


Figure 1. Effect of Pesticides on total protein content of Fish (*Oreochromis mossambicus*) at 24hr and 48hr. Bars with asterisk are significantly different from control ($p < 0.05$) A: Malathion B: Chlorpyrifos C: Cypermethrin D: Lambda-cyhalothrin E: Buctril

Discussion

Effect of pesticides on protein has been reported by different scientist on fish^{18,19,20,9,6,21}. Reduction in protein levels in tissues of fish exposed to pesticides, as indicated in the present study, is in conformity with observation made earlier on fish exposed to benthocarb²², fenvalerate²³, malathion²⁴, cypermethrin, permethrin and fenvalerate²⁵, fenitrothion²⁶, diazinon and endosulfan²⁷, endosulfan and thimet²⁸ and chlorpyrifos^{29,18}, methomyl¹⁹, cypermethrin²⁰, methyl parathion and chlorpyrifos⁹, fenvalerate and fenpropathrin⁶.

In the present study inhibition of protein increased in pesticides treated fish expose to 48h as compare to 24h. This may be attributed to the constantly increasing contact of the pesticide with the bio-system which ultimately resulted in protein breakdown.

Increased circulation of blood in gills may increase the possibility of greater uptake of contaminants from the surrounding medium and intensifying the stress Srivastava *et al.*³⁰.

The decreased protein content might also be due to tissue destruction, disturbance of cellular fraction and consequent impairment in protein synthetic machinery³¹. The present work indicates that pesticides caused alterations in the protein metabolism of fish treated tissues. The altered mobility and low content of proteins reflects a change in the rate of synthesis and degradation of protein. Proteins are mainly involved in the architecture of the cell. During stress conditions they are a source of energy as fish need more energy to detoxify the toxicant and to overcome stress. The amount of carbohydrates in fish is less so protein is the alternative source of energy to meet the increased energy demand. The depletion of total tissue protein may be due to the effect of pesticides on the following: i) the physiological adaptability of the fish to compensate for pesticide stress³², ii) increased proteolytic activity^{33,34}, iii) reduced protein synthesis³⁵ and utilization of their products for metabolic purposes and cause damage to tissues³⁴, iv) hormonal balance, which affect the tissue protein levels^{36,37}, v) DNA damage, destruction or necrosis of cells³¹, vi) altered enzymatic activities³⁸.

Fish is an integral part of the diet of a population and is one of the sources of protein for the people living in coastal areas. The nutritional value of different species of fish depends on their biochemical components such as protein. The alteration in the proximate component could disturb the metabolic system in fish, affecting the food value of fish, thus affecting the fishery industry. Furthermore, the data generated in the present study could be useful in the environmental risk assessment of marine organisms.

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