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Research Article

FLUXES IN BIOCHEMICAL MOIETIES OF BODY MUCUS OF FISH *C. GACHUA* EXPOSED TO AN ORGANOPHOSPHATE PESTICIDE

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ABSTRACT

Water is an essential part of everyday life. But industrial revolution coupled with population explosion create stress on environment and introduce harmful chemicals and various pesticides in fresh water resources. Those pesticides and chemicals accumulates in the aquatic organisms and have detrimental effects on metabolism and physiology on non-target organisms in fresh water bodies. So it was decided to assess the toxicity and biochemical constituents in mucus of the experimental fish *Channa gachua* (F. Hamilton) exposed to dichlorvos. The fishes were exposed to LC0 (5 ppm) and LC50 (12.5 ppm) concentrations of dichlorvos for 96 hrs. (acute). After exposure to dichlorvos the glycogen was increased, lipid was decreased and fluctuations in protein was observed in the body mucus of fish. The pesticide intoxication might disturb the normal functioning of the cell with the alterations in the biochemical moieties in the exposed fish.

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INTRODUCTION

Water is an essential component of everyday life, it sustains life on earth. A community depends on the water for its domestic, agricultural and industrial needs. Availability of water has been a factor in the development of various civilizations near lakes, and rivers. India is blessed with an extensive wealth of water resources, consisting of numerous lakes and reservoirs (Bhilave, 2001). Rapid industrialization coupled with progressive urbanization and population explosion posed a major threat to environment. Environmental deterioration by man is attributed to major causative factors such as overpopulation, urbanization and industrialization. The increasing amount of wastes generated by these three ways deteriorates the quality of land, water, air and food. And now days human civilization is directly faced with pesticide pollution.

Aquatic toxicology is the study of the effects of environmental pollutants on aquatic organisms, such as pesticides especially the insecticides, on the health of fish or other aquatic organisms. Pesticides are substances used to control pests, including insects, aquatic weeds and plant diseases. Pesticides have been found to be highly toxic not only to fish but also to the other organisms, which constitute the food chain. Pesticides in general, are used very extensively in agriculture, forestry,

public health and in veterinary practices. Pesticides are categorized according to their target use. The three major pesticides are herbicides (weed control), insecticides (insect control), and fungicides (Mycotic control), but the more acute toxicity having insecticides.

Aquaculture is one of the fastest growing food producing sector. Fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is very important. Insecticides are the chemicals used to control insect pests by killing or preventing them from their destructive activities. The contamination of fresh water bodies by several insecticides is known to have deleterious effects on the growth, survival and reproduction of aquatic animals and subsequently on food web. Different concentrations of insecticides are present in many types of waste water and numerous studies have found them to be toxic to aquatic organisms, especially fish species. The major groups of insecticides based on their chemical nature are organophosphate, carbamates, organochlorine, pyrethroids, and neotenoides usually applied in agricultural, industrial and domestic fields to control pests. The insecticidal residues which contaminate the water bodies mainly due to the intensive agricultural runoff combined with domestic and industrial drainage, usually after few weeks of application. Insecticides may lead to decrease rate of growth, reproductive disorders, spinal deformities and histopathological

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changes in gills, liver, hematopoietic tissue such as spleen, head of the kidney and renal tubules, endocrine tissues as well as brain, neurological, behavioral disorder and genetic defect are other biological indicators of exposure to insecticides. Fishes are particularly sensitive to the environmental contamination of water. Hence, these pollutants such as insecticides may significantly damage certain physiological and biochemical processes which subsequently impair physiology and health status of fishes. So it is very essential to understand toxic nature of dichlorvos for public health and proper management of fish culture. However, the toxic effect of dichlorvos 76 % EC on biochemical constituents in body mucus of fish *C. gachua* has been not elucidated yet. Therefore, present research work aimed to assess the toxicity and biochemical fluctuations in mucus of the experimental fish *C. gachua* (F. Hamilton) after exposure to dichlorvos.

MATERIAL AND METHODS

Material

Experimental fish

The fish *C. Gachua* (F. Hamilton) was selected for experiment due to its easy availability and suitability for toxicity testing. Live specimen of *C. gachua* of size 15 ± 1 cm and weight 50 ± 5 gm. were obtained from Krishna River around Karad city with the help of fisherman. The collected fish were kept in 1% solution of $KMnO_4$ for some time to protect from dermal infection. Finally they were kept in glass aquarium and fed on commercial fish food. They were acclimatized in laboratory for 10 days at room temperature.

Pesticide

Commercially available organophosphorus pesticide Dichlorvos was used for present research work. The pesticide Dichlorvos 76% EC brought from Local agro chemist shop.

Methods

Determination of LC50

For determination of LC_{50} well acclimatized fishes were divided into six groups and exposed to different concentrations viz. 5, 7.5, 10, 12.5, 15, 17.5 and 20 ppm of dichlorvos for 96 hrs. During this period behaviour of fish was also observed.

Experimental set up

Under the experiment healthy fishes were divided into three groups each group contained ten fishes. Group 1st was considered as control and group 2nd and 3rd as experimental groups. Fishes in the experimental groups were exposed to LC0 and LC50 concentration dichlorvos 96 hrs. At the end of each exposure period mucus was collected by carefully scrapped from the dorsal body using a clean plastic spatula. Ventral skin mucus was not collected to avoid blood, urino-genital, intestinal debris for biochemical analysis.

Biochemical methods

i) Anthrone method was used to determine total glycogen content in mucus, (Siefert, 1951). ii) Total protein content was determined with Folin Ciocalteu reagent according to Lowry Method (Lowry, et. al., 1951). iii) Lipid were extracted by Flosch, et al.(1957), and estimated by sulpho phosphor vaniline method (Barnes and Black stock, 1973).

RESULTS

In the present work the amount of glycogen, protein and lipid in mucus was estimated in control fish and in fishes exposed to LC0 and LC50 concentration (acute) of dichlorvos. Exposure period and concentration of pesticides dependent alterations were observed in the levels of these biochemical constituents in the mucus under investigations over control fish which are shown in Tables 1 and are illustrated graphically by graph 1.

Total glycogen

Changes in the total glycogen content noticed in mucus of *C. gachua* exposed to dichlorvos after acute exposure for 96 hrs. In fishes exposed to 5 ppm and 12.5 ppm concentration of dichlorvos for 96 hrs. The glycogen content in mucus was found increased that was 2.0716 ± 0.048 and 2.9239 ± 0.041 as compared to control (1.1611 ± 0.019). The increase was highly significant ($P < 0.001$) in mucus.

Total Protein

Fluctuations in the total protein content noticed in mucus of *C. gachua* exposed to dichlorvos after acute exposure for 96 hrs. In fishes exposed to 5 ppm and 12.5 ppm concentration of dichlorvos for 96 hrs. The protein content in mucus was found increased that was 16.470 ± 1.233 and 13.647 ± 0.652 (which was decreased than 5 ppm Conc.) as compared to control (10.706 ± 0.955). The increase was highly significant ($P < 0.001$) in mucus.

Total lipid

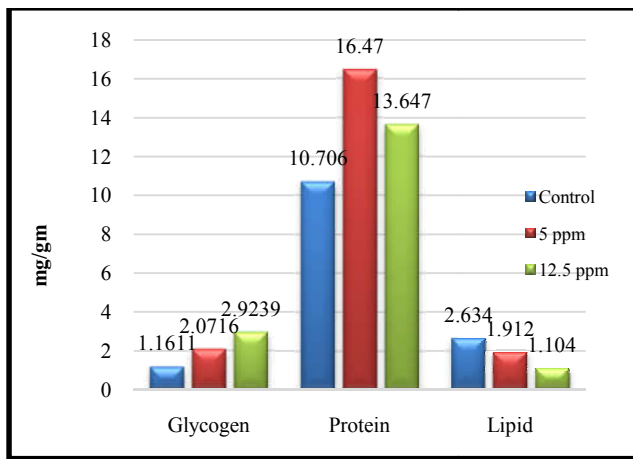
The lipid content in mucus was observed in control and experimental groups showed that, decreased lipid content in both (LC0 and LC50) groups when compared to the control group. Depletion in the total lipid in mucus was highly significant ($P < 0.001$). In fishes exposed to 5 ppm and 12.5 ppm concentration of dichlorvos for 96 hrs. The lipid content in mucus was found decreased that was 1.912 ± 0.048 and 1.104 ± 0.048 as compared to control (2.634 ± 0.037).

Though, the fluctuations in biochemical constituents observed in both groups of experimental fishes (5 ppm and 12.5 ppm) was highly significant when compared to control, it was more in the mucus of fishes exposed to 12.5 ppm concentration group than 5 ppm group. (Graph 1).

Table 1 Showing changes in the biochemical constituents (mg/gm. wet weight tissue) in mucus of control fish and in fishes exposed to LC0 and LC50 concentration of dichlorvos at 96 hrs. (acute).

Biochemical Constituents	Control	5 ppm (LC0) Concentration)	12.5 ppm (LC50) Concentration)
Glycogen	1.1611 ± 0.019	2.0716 ± 0.048 ***	2.9239 ± 0.041 ***
Protein	10.706 ± 0.955	16.470 ± 1.233 ***	13.647 ± 0.652 ***
Lipid	2.634 ± 0.037	1.912 ± 0.048 ***	1.104 ± 0.048 ***

Values significant at NS = $P > 0.05$ (Non-significant), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.



Graph 1 Changes in biochemical constituents in body mucus of *C. gachua* exposed to LC0 and LC 50 conc. of dichlorvos after acute exposure.

DISCUSSION

The contamination of water by widely utilized organophosphate pesticide such as dichlorvos is a potential problem for fishes and aquatic organism. In present study attempt has been made to study acute effect of dichlorvos on biochemical composition of body mucus of fresh water fish *C. gachua*.

The body mucus provides a stable physical or chemical barrier against the invading pathogens and harmful chemicals present in water. In fish, the epithelial surfaces are covered by a slimy, slippery layer called the mucus (Dash *et al.*, 2018). The 'slipperiness' of fish is because of the high water content and the presence of high-molecular-weight, gel-forming macromolecules (Shephard, 1994). Most of the constituents of mammalian mucus are also found in fish mucus where glycoproteins are the major macromolecular. In addition, a variety of the other materials has been identified where glycosaminoglycans, lysozyme, immunoglobulins, complement, carbonic anhydrase, a range of lectins and calmodulin have all been found in fish mucus (Shephard, 1994). Mucus from several species of fishes contained up to 20 times more lipid per unit area than human sebum and from previous study, its revealed free fatty acids which may provide antioxidant agents and protection against bacterial and fungal attack (Lewis, 1970).

In fish, mucus surfaces are dynamic layers that display important functions in fish, playing major roles in physiological functions such as osmoregulation and protection against infections and toxicants (Beklioglu, 2006). Mucus contains a wide variety of biologically active molecules that take part in numerous roles and biological interactions, some of which have drawn attention as potential for toxicant repellent. We have discussed the multiple roles of mucus in organism interactions in nature.

The biochemical constituents are source of energy in animal body is present in the form of stored energy which is used in starved and stressed condition. Glycogen is the prime and important biochemical constituents in animal. It acts as building blocks and reservoir of chemical energy in the cell which can increased or decreased according to organismal need (Kumar and Ali, 2013). Glycogen provides the energy for the animal which is essential for performing different process

(Lehninger, 2004). Carbohydrates are mainly stored in liver of animal in the form of glycogen and it is exported in the form of hexose units for maintenance of blood glucose and readily available for glycolysis (Herper, 2003). Protein serves as an alternative source of energy for animal when the insufficient energy is available from the other sources like carbohydrates and lipids. Protein is the building blocks of animal body. It is the most important constituents for growth, development and maintenance of life (Waghmare and Wani, 2014). Protein plays an important role in cellular metabolism and regulates intra and extra cellular interactions media as a part of cell membrane and enzyme (Anitha and VenkataRathnamma, 2016). All cells contain lipid in the form of globules scattered in the cytoplasm (Tamizhazhagan *et al.*, 2016). Lipid is an essential biochemical constituent in tissues of all animals, and plays vital role in the energy metabolism. (Kumar and Ali, 2013). Lipids are also the strong form of energy like glycogen. Lipid form an essential component of protoplasm and during extreme starvation considerable amount can be extracted from tissues.

Due to pesticidal stress, such prime and important energy source is affected significantly and alters various processes in the fish. The biochemical constituents like glycogen, proteins and lipids are important to indicate the susceptibility of organs system to pollutants (Verma and Tonk, 1983). The results obtained in the present work showed fluctuations in the glycogen, protein and lipid content in the body mucus of fish, *C. gachua* exposed to lethal concentrations of dichlorvos at acute exposure period.

The alterations were time of exposure and concentration of pesticides dependent. The biochemical changes occurring in the body of the organisms give first indication of stress. Here, in present investigation increase in glycogen content was might be due to the excessive secretion of mucus. Excessive mucus secretion by fish was the response of fish against the toxic medium that form the barrier between body and toxic medium which may reduce the contact between body of fish and toxic medium. Similar assumption was mentioned by Rao (2006) in *O. mossambicus* exposed to organophosphate insecticides (RPR-V). According to him mucus forms a barrier between the body and the toxic medium to minimize its irritating effects or to scavenge it through epidermal mucus. Mucus viscoelasticity determines its ability to block many types of toxicants, and several studies showed that fish tend to increase their mucus secretion and change their composition when exposed to toxic substances, which may contribute to the defense against toxic substances. Stress conditions (e.g., handling stress, confinement, food deprivation, exposure to toxic substances) can change the mucous production and composition (e.g., level of proteins and immune molecules), compromising fish health and increasing the fish susceptibility to toxicants.

However in fluctuations was also observed in the protein content. At LC0 concentration the protein content was increased may be due to excessive mucus secretion at low concentration. But at LC50 concentration the protein content was also increased but decreased than at LC0 concentration. It might be due to the accumulation of pesticide residues in the skin of mucus and the metabolites of pesticides degenerate the mucous cells in skin and inhibit the secretion of protein. The depletion in lipid level might be due to the lipolysis in different in mucus to overcome the high energy demand by the fish

under pesticidal stress, rate of lipolysis might be accelerated for the production of energy and subsequently used for glucose synthesis. Pesticide may affect on cellular structure of the skin and lipid might be used in cell repair and tissue organization due to that depletion in lipid content may occur. As stated earlier by (Lewis, 1970) the lipid provide antioxidant agents and protects fish skin from pathogenic and chemical attack. So here it is assumed that to protect the skin from chemicals or pesticide metabolites the lipids in mucus may be utilized and simultaneously for antioxidative activities due to that the lipid content may be decreased in mucus of the fish.

CONCLUSION

The widespread use of pesticides in agriculture raise toxicological and environmental problems resulting potential toxic effects in human and animals. The pesticide intoxication might disturb the normal functioning of the cell with the alterations in the biochemical mechanism in fish. This may results in the mortality of fish on chronic or acute exposure to the pesticide. The pesticide might be deposited in fish accidentally or by means of contaminated water bodies with pesticide and it might lead to harmful effects in human being on continuous consumption of those fish. Finally, Protection of wildlife and water quality is possible when rationalize the use of pesticides. Also, when Pesticides must choosing judiciously and are used in combination with other pest management tools, and applied safely, the surface water pollution and contamination of our aquatic life could be avoided. A constant monitoring program should be introduced by the Government of India to provide a hazard free environment to the aquatic biota and to ensure safe and healthy supply of fish for human consumption.

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