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Effect of Quinphos Toxicity on Electrophoretic Patterns of Proteins in Gill and Muscle of *Oreochromis niloticus*

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ABSTRACT

Quinalphos is an organophosphorous pesticide widely used in agriculture. This Pesticide entered into the aquatic system and create pollution, which pose a great threat to aquatic organisms.The aim of the study was carried out to determine the effect of Quinalphos on electrophoretic patterns of proteins in the gill and muscle of *Oreochromis niloticus*. Lethal concentration(LC50) of quinalphos for *Oreochromis niloticus*has been calculated by probit analysis.In the present study fish were exposed to the 1/2th,1/5th and 1/10th of 96h LC50 value for a period of 15 and 30 days. The changes in the tissue proteins of vital organs such as gill and muscle were examined on SDS –PAGE.The electrophoretogramof 15th and 30th day exposure represents the decrease in the intensity ofgill and muscle protein bands compared to control due toquinalphos toxicity. In the present study, the reason for the loss of protein bands are expected to be due to the damage caused by the binding of quinalphos with the proteins and the protein synthetic machinery, affecting adversely the anabolic reactions. The biochemical studies like electrophoreticestimation of protein are very important in assessing thepesticide-induced stress in fish. The marked reduction in the protein fractions,is a good indicator, which might help, in the earlydetectionof pollution by pesticides.

Keywords: Quinalphos, SDS –PAGE, *Oreochromis niloticus*, LC50

I. INTRODUCTION

Pesticides play an important role in modern agriculture on one hand by providing reliable, consistent and reasonably complete control against harmful pests with less cost and effort while on the other hand are considered as powerful aquatic pollutants. These chemicals can make their ways towards water reservoirs, rivers and streams, thus exerting adverse effects on fish and other aquatic organisms[1].Due to the rapid biodegradability and lesser persistency in the environment, the OP pesticides replaced the more persistent organochlorine compounds. Extensive use of OP compounds has resulted in a wide spread distribution of these chemicals in the environment. Quinalphos is one of the organophosphorous pesticide extensively used in agriculture, which hasbecome a

matter of concern today because of itspotentiality and hazardous effect.

Fish can act as a biological indictor of aquatic pollution due to direct contact to pesticide and play an important role in assessing prospective risk related with contaminated aquatic environment[2].Fishes are extremely delicate to defilement of water, in this manner; toxins may altogether adjust some biochemical and physiological systems when they go into the organs of fishes[3]. Nile tilapia,*Oreochromis niloticus* is one of the most important commercially cultured fish species. They are very good species for aquaculture especially in developing countries such as Asia and Africa where there are high levels of animal protein deficiencies and integrated paddy-cum-fish-culture system has gained considerable attention in earlier times[4,5]. But recently in India such integrated paddy-cum-fish-culture system is almost non-existent because of increasing use of inorganic fertilizers and pesticides in rice fields causing deleterious effects on fish[6].Exposure of pesticide showed decrease in protein content indicating physiological adaptability of the exposed fishes to compensate for toxic stress.

Global techniques such asproteomics, isregarded as powerful tool to investigate the cellular response to OP pesticides.SDS-PAGE, is a technique used to separate proteins according to their electrophoretic mobility. In the present investigation an attempt has been made to study the effects of quinalphos on electrphoretic patterns of proteins in gill and muscle of *Oreochromis niloticus*through SDS-PAGE.

II. MATERIALS & METHODS

The test fish, *O.niloticus* with an average weight of 40.20±3.51g were procured from a local farm and transported to laboratory in polythene bags containing aerated water. The fish were acclimated in fibre tank for 15days prior to experiment. The fishes were fed commercial feed throughout the experiment.About 25% of the water in all tanks were replenished at 24h intervals to maintain water quality and to prevent degradation of the pesticide. Stock solution of Quinalphos was prepared by adding a certain amount of QP in water. The semi-static acute toxicity bioassay was conducted as per standard method of APHA 2005[7], and Reish and Oshida, 1987[8], to determine the 96h LC50 of Quinalphos(CASNo13593-03-8)in *Oreochromis niloticus*.In the present study 1/2th,1/5th and 1/10th of96h LC50 were selected as sublethal concentration and the fishes were exposed to this concentration for a period of 15 and 30days.Upon completion of exposure, tissues like muscle and gills were used for further analysis.

Preparation of sample for analysis

Analysis was carried out in frozen whole-body tissues of fish *O.niloticus*. Samples were washed in physiological saline (0.9% Nacl), and kept at -20°C until analysis.The tissue samples were homogenized for5 min in ice-cold 0.1M Tris-HCl buffer solution pH 7.2 using mortar and pestle in an ice bath. After homogenization, the crude sample was clarified by centrifugation 8000rpm for 30min. Supernatant is used for further analysis.

SDS-PAGE ANALYSIS

The electrophoretic analysis was done by the method ofLaemmli.,1970[9].Supernatant was dissolved in sample buffer of 3.5 ml deionised water,1.25ml 0.5M Tris HCl (pH 6.8), 2.5ml glycerol, 2ml 10% SDS, 0.1 ml 2-mercaptoethanol ,0.2 ml 1% bromophenol blue in the ratio 1:2 and heated at 95^{0}c for 3min. An aliquot of 0.1ml of the tissue extract was loaded on to the separating gel directly and layered with running buffer in order to avoid disturbance to the sample. A constant voltage of 60volts was applied until the dye front crossed the stacking gel and it was increased to 140volts and electrophoresis was continued until the dye front reached the bottom of the gel.

StainingProcedure and standardization of protein bands

Immediately after the completion of electrophoresis, the gels were carefullyseparated from the trays into plasic trays and washed in tap water to remove excess SDS. After staining the gels for two hours in Coomassie Brilliant Blue R 250,in 40% methanol and 10% acetic acid. De stain thegels in 7% acetic acid solution to remove excess dye. The molecular weight standards used in comparing the variations noticed in the SDS-PAGE, were of wide range molecular weight protein standards (6,500 to 200,0000 Da) from the Sigma Aldrich.

III. RESULTS & DISCUSSION

The relative mobilities of the protein patterns in muscle and gill tissues of the fish *O.niloticus* exposed to quinalphos for 15 and 30days were presented in (fig. 1and 2). In electrophoretic analysis, several changes were observed in protein banding patterns of the pesticide treated fish in comparison with controls. Qualitative assessment of electrophoretic pattern of serum proteins revealed the reduced intensity of some protein bands after prolonged exposure of quinalphos for 30 days. The insecticide exposure for a period of 15 days did not make much difference in the electrophoretic protein pattern in gill for 1/5th and 1/10th of 96h LC50 treated fish, and at 1/2th of 96h LC50 treated fish the 66,000 Da protein band was found to be with reduced bandwidth. The intensity in thickness of the bands between 200,000Da and55,000Da appeared to be decreased in 1/2th ,1/5th and 1/10th of 96h LC50 pesticide treated fish when compared to the protein bands from control after 30day exposure to the pesticide quinalphos. The insecticide exposure for a period of 15days did not make much difference in the electrophoretic protein pattern of muscle tissues but the results are similar with gill of the exposure for 30days.This might be due to the protein degradation in the treated tissues that results in the reduced intensity of protein bands.

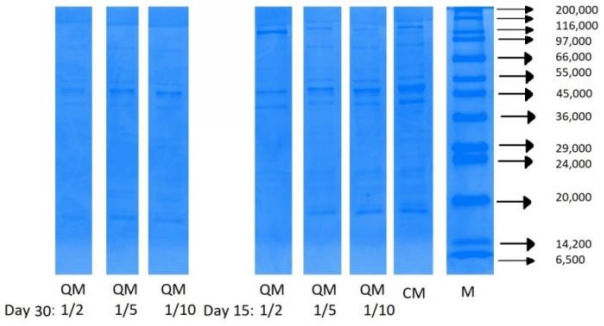

Fig.1 : Protein profile showing difference in protein fractions in comparison with standard, control and quinalphos treated muscle tissues of *O.niloticus* at 15th and 30th day exposure.

(QM: quinalphos treated muscle,CM: control muscle, M : Protein marker)

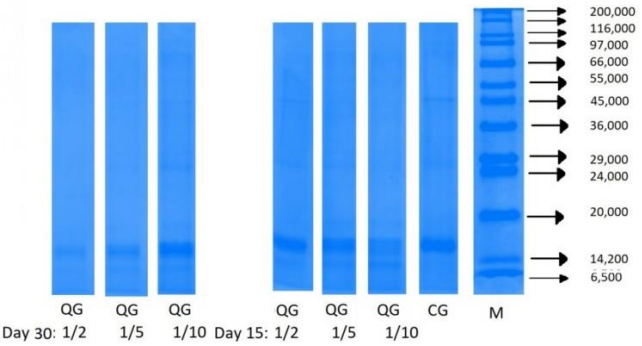

Fig.2 : Protein profile showing difference in protein fractions in comparison with standard, control and quinalphos treated gill tissues of *O.niloticus* at 15th and 30th day exposure.
(QG: quinalphos treated gill, CG: control gill, M : Protein marker)

In fish, proteins are the primary energy source and are involved in regulating physiological and metabolic processes in the body. It is the major source of energy during stress conditions apart from carbohydrate and

lipids[10].A significant difference was observed in the electrophoretic pattern of gill and muscle tissues in *Oreochromis niloticus* on different exposure concentration and periods. During stress, fish need more energy to detoxify toxicants and to overcome stress the protein is used to meet the increased energy demand. The decreased protein content might also be attributed to the destruction or necrosis of hepatocyte cells and consequent impairment in protein synthesis machinery.

The insecticide might have altered the functional conformations of the structural proteins in the cells and tissues. This is expected to result in the denaturation of these high molecular weight proteins. This might have impaired the normal metabolic processes. Similar studies have been carried out to reveal the effect of pesticide on electrophoretic pattern of tissues.

Sastry and Siddiqui[11], who reported decreased protein content in liver, muscle, kidney, intestine, brain and gill of quinalphos exposed *Channa punctatus.* According to Dhar and Chatterjee[12], on treatment with pesticides the number of protein fractionsdecreased and some completely disappeared and sometimes-new fractions alsoappeared. In the present investigation the reduction in the intensity and number ofprotein bands in tissue subjected to different sublethal concentrations of quinalphos was observed. This might be due to proteolysis of the serum proteins and thedamage to the protein synthetic machinery

The probable cause of decreased protein content observed in the present study may include the metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose or the maintenance of osmotic and ionic regulation[13]. A number of authors have reported similar observations on the elecetrophoretic pattern of tissues. Kumar and Devi[14], demonstrated that malathion showed profound effect on the protin pattern of *Heterneustes fossillis* and electrophoretic protein bands disappeared after treatment.The impact of acetamiprid toxicity on electrphoretic patterns in liver, brain and gill tissues of fresh water fish*Oreochromis massambicus* had observed the similar results by using electrphoretic studies[15].Suneetha K et al.,[16]found the decrease in the protein sub units induced by Endosulphon and Fenvalrate in fresh water fish *Labeo rohita* through SDS-PAGE.

In the present study we report that the effect of quinalphos on electrophoretic patterns of proteins in gill and muscle of fish *Oreochromis niloticus*, when exposed to quinalphos pesticide the protein subunits showed decrease in intensity and some protein subunits have completely disappeared.

IV. CONCLUSION

Measurement of biochemical parameters can be useful to help identify target organs of toxicity as well as the general status of animals. The changes of protein bands observed in different tissues of the fish could serve as biochemical indicators of quinalphos toxicity in aquatic environment. Thus, Present study has concluded that the long term exposure of quinalphos becomes a continuous health hazard for the fish population. Therefore it is required to monitor the aquatic system and predict the toxic effect of pesticides on fish.

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