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Research Article
Impact of Acetamiprid Toxicity on Electrophoretic Patterns in Liver, Brain and Gill Tissues of the Fish *Oreochromis mossambicus*

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Abstract

Background and Objective: Acetamiprid is a neonicotinoid pesticide and it is used by the farmers to protect their crops. This pesticide residue reaches to the aquatic ecosystem by many ways and affects the aquatic fauna. The aim of the study was to analyze the effect of pesticide-acetamiprid on electrophoretic protein patterns of liver, brain and gill tissues of *Oreochromis mossambicus* (*O. mossambicus*). **Materials and Methods:** Lethal concentration (LC_{50}) of acetamiprid for *O. mossambicus* has been calculated by probit analysis. Sublethal or safe level concentrations were derived from 96 h LC_{50}. In the present study 1/15 of the 96 h LC_{50} were selected as sublethal concentration and the fishes were exposed to this concentration for a period of 7 and 14 days. **Results:** The electrophoretogram of 7th and 14th day exposure represents the decrease in the intensity of liver, brain and gill protein subunits and increase in protein bands due to acetamiprid toxicity, when compared to control. Studies revealed that due to the toxic effect of the pesticides the protein bands get denatured, as a result there was a decrease in the intensity of protein bands and there was an increase in new protein bands due to the enhancement of stress proteins. **Conclusion:** The alterations in electrophoretic protein patterns observed in various tissues of the experimental fish could serve as sensitive biochemical indicators of acetamiprid pollution, which might help in aquatic management. It may be concluded that long term exposure to acetamiprid creates a continuous health hazard for *Oreochromis mossambicus*.

Key words: Neonicotinoid, stress proteins, LC_{50}, pesticides, *Oreochromis mossambicus*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pesticides have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases. At the same time use of these pollutants has posed potential health hazards to the life of fishes[1]. Pesticides are major cause of concern for aquatic environment because of their toxicity, persistency and tendency to accumulate in the organisms[2]. Pesticides become part of the water column and fish ingest the pesticides, usually through their gills, although sometimes through their scales[3]. The pesticides diffuse into their organs and fat tissues and sequestered there causes severe alterations in the tissue biochemistry and histology of fishes[4,5]. Fish is considered as the most important and vital link in the food chain of ecosystem and the inland fisheries are important sources of protein in a nation's diet, a thorough understanding of pesticide effects on fishes would be really vital for fish conservation and fisheries development[6]. Many pesticides have been reported to produce a number of biochemical changes in fish both at lethal and more often, at sublethal level changes in ion concentrations, organic constituents, enzyme activity, endocrinal activity and osmoregulation in fish has been attributed to pesticides[7]. *Oreochromis mossambicus* showed a declining trend of protein, carbohydrate and lipid content in the tissues like brain, gill, kidney, liver and muscles upon exposure to organochlorine and carbamate pesticides[5,7] and there is an increase in chromatid break and chromosomal exchange due to carbamate pesticides[8]. Proteins are the primary effector molecules of all living systems and any adaptive responses to environmental, physiological or pathological conditions will be reflected by alterations in protein activity or content[9-11]. Global techniques such as proteomics, therefore provide effective strategies for toxicological studies and are regarded as a powerful tool to investigate the cellular responses to environmental pollutants[12], such as pesticides. The electrophoresis of proteins is an effective technique for generating systematic data from macromolecules. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility. Reduction in total serum protein content induces proteinaemia and may be correlated with reduced protein synthesis by liver[13]. Protein synthesis can be disturbed either by affecting the nucleic acid metabolism or structure, or in the protein forming system itself. Proteomic analysis of *Sparus latus* liver treated with methyl parathion and identified 16 differentially expressed proteins that are involved in cell redox homeostasis, metabolic processes and cytoskeleton system. Proteomics, or the study of the proteins expressed by a genome, is gaining application as a measure of environmental stress, providing more meaningful data and a more accurate reflection of functional status compared with mRNA expression[14]. The underlying principle is that a proteome differs from cell to cell and constantly changes via biochemical interactions with the genome and environment. Accordingly, environmental conditions drive the expression of a unique set of proteins in the exposed organism, tissue, or cell type[15]. Alterations in the cytoplasmic protein fractions of the liver and the skeletal muscle of fish *Clarias batrachus* exposed to endosulfan and methyl parathion was demonstrated for 1-28 days[16]. Sublethal concentration of cadmium and diazinon has showed slight reduction or decrease in intensity of proteins in the muscle and gills of *O. mossambicus.* These protein fractions could be stress proteins ,which indicates that these proteins were highly affected by the stress caused by the pesticides[17-19]. This study aimed to determine the effect of pesticide-acetamiprid on electrophoretic protein patterns of liver, brain and gill tissues of *Oreochromis mossambicus.*

MATERIALS AND METHODS

Specimens of *O. mossambicus* were obtained from local vicinity and introduced into large glass tank (30×30×60 cm) disinfected with potassium permanganate and washed thoroughly prior to introduction of fish (to prevent fungal infection). Fishes with same weight were acclimatized for about 20 days before the commencement of the experiment. They were fed on commercial fish food which was given daily at morning hours. The LC_{50} of acetamiprid was calculated by the log-dose/probit regression line method[20] and was recorded. Sublethal or safe level concentrations were derived from 96 h LC_{50} as per the procedure given by APHA[21] to observe various responses of the test fishes on prolonged exposure to acetamiprid. In the present study 1/15 of the 96 h LC_{50} were selected as sublethal concentration and the fishes were exposed to this concentration for a period of 7 and 14 days. A control batch corresponding to each test group was simultaneously experimented to compare the toxicated effect of acetamiprid in various tissues. The electrophoretic analysis (SDS-PAGE) was done by the method of Laemmli[22]. Fresh concentrations were supplied daily to maintain a constant toxic media. At the end of each exposure period, fishes were sacrificed and tissues such as liver, brain and gill were dissected and were used for the analysis.

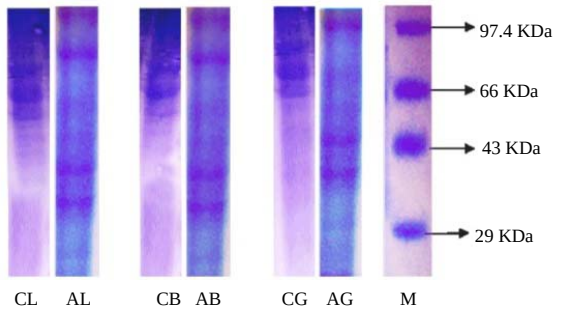

Fig. 1: Protein profile showing difference in protein fractions in comparison with standard, control and acetamiprid treated tissues of *Oreochromis mossambicus* at 7th day exposure
(CL, CB, CG: Control liver, brain and gill, AL, AB, AG: Acetamiprid treated liver, brain and gill and M: Protein marker)

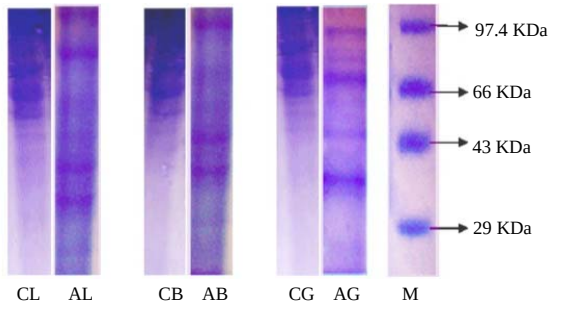

Fig. 2: Protein profile showing difference in protein fractions in comparison with standard, control and acetamiprid treated tissues of *Oreochromis mossambicus* at 14th day exposure
(CL, CB, CG: Control liver, brain and gill, AL, AB, AG: Acetamiprid treated liver, brain and gill and M: Protein marker)

RESULTS

The fishes were exposed to lethal (96 h LC_{50}) and sublethal concentrations of acetamiprid for 7 and 14 days. Liver, brain and gill tissues were dissected out and subjected to SDS PAGE electrophoretic analysis. Result indicates the presence of protein bands in control and treated liver, brain and gill tissues. In electrophoretic analysis, several changes were observed in protein banding patterns of the pesticide treated fish in comparison with those of controls. The electrophoretogram (Fig. 1 and 2) of 7th and 14th day exposure represents the decrease in the intensity of liver, brain and gill protein subunits and increase in protein bands due to acetamiprid toxicity, when compared to control.

In the pesticide exposure tissue samples, the gill tissue protein sub units showed more decreased intensity in banding pattern compared to the acetamiprid treated liver and brain tissue sample. The protein bands get denatured due to pesticidal stress and there was an increase in new protein bands due to the enhancement of stress proteins, which indicates the deleterious effect of acetamiprid. The decrease in the intensity of protein bands was due to the toxic effect of the pesticides. The pesticides in turn cause protein degradation in all the treated tissues.

DISCUSSION

The pesticides may inhibit the expression of some genes (or) activate the others to produce specific mRNAs which may subsequently be translated into specific proteins called stress induced proteins[23-25]. An alteration of protein metabolism was observed in fish exposed to various types of environmental stresses like metals and pesticides[26]. Qualitative assessment of electrophoretic pattern of tissue proteins revealed the reduced intensity of some protein bands after prolonged exposure of 7-14 days to the insecticide acetamiprid. In the present study SDS polyacrylamide gel electrophoresis was performed for the tissues of liver, brain and gill of *Oreochromis mossambicus* exposed to acetamiprid. When compared to control the protein subunits of pesticide exposed tissues showed decrease in intensity and some protein sub units were disappeared. The proteins showed decreased intensity (or) significant fading in liver, brain and gill tissue samples in the initial exposure. The variations in protein sub unit band patterns may be due to change in the turn over (synthesis/degradation) of various proteins. The appearance of new protein bands at different time intervals after the exposure of the pesticide demonstrated clearly the alterations in the cytoplasmic protein pattern. This might be due to the inhibitory effect of the insecticide on the protein anabolic actions. 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 but a few studies have been carried out to reveal the effect of pesticide on piscine serum electrophoretic pattern. Similar trend was reported in the serum proteins of *Channa punctatus* under chronic exposure to organophosphorus and organochlorine insecticides in study by Sahai[27] and Ravinder *et al.*[28] in the catfish *Clarias batrachus* exposed to Desis 2.8 E C. In a study by Rita *et al.*[29] the fishes (O. *mossambicus)* were exposed to various concentrations of the carbamate pesticide methomyl for different durations revealed a definite pattern of variation in protein fractions. A number of authors have reported similar

observations. Studies on *Clarius batrachus* under sublethal malathion exposure revealed variations in serum proteins, this may be due to the alterations of protein mobility by malathion binding[30]. Kumar and Devi[31] demonstrated, that malathion, showed profound effect on the protein pattern of *Heteropneustes fossilis* and found new electrophoretic protein bands and some others disappeared after the treatment.

CONCLUSION

Measurement of biochemical parameters can be especially useful to help identify target organs of toxicity as well as the general health status of animals. The significant modulation of insecticide stress suggests that these compounds might have direct or indirect interactions with corresponding genes in *Oreochromis mossambicus.* The alterations of protein bands observed in different tissues of the insecticide treated fish could serve as sensitive biochemical indicators of acetamiprid pollution in the aquatic environment, which might help in water quality control and management. From the present study it may be concluded that long term exposure to acetamiprid means 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.

SIGNIFICANCE STATEMENTS

This study discovers the toxic effect of acetamiprid on electrophoretic protein pattern and that can be beneficial for monitoring the aquatic system ,especially fish population. This study will help the researcher to uncover the critical areas of aquatic toxicology that many researchers were not able to explore. Thus a new theory on the toxic effect of pesticide, acetamiprid on fish may be arrived at.

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