Evaluation of toxicity of disyston and lindane on stress protein profiles of selected tissues in freshwater teleost, *Macrognathus aculeatum* (Lac.)

Ashok Kumar Singh

Department of Zoology, Magadh University, Bodh-Gaya (Bihar) - 824234

Abstract

Response to stress is well documented in many different biological system. A common feature of this response at the molecular level is the induction of suite of proteins, which were first named "heat - stock proteins (HSPs)" as they were first discovered in cells exposed to hyperthermia, or heat shock. The heat stock response to molecular stress response, as it is popular currently, is one of the most conserved stress responses of biological system; all organism ranging from bacteria to mammals as also higher plants, display molecular stress response by way of induction of a remarkably homologous set of proteins in response to different stressors. These proteins are involved in the protection, enhanced survival and restoration of the normal activities of the cells. HSPs are able to bind to denatured or unfolded proteins thereby providing cytoprotective benefits to the cells so that it may better cope up with stress. Stress proteins and their constitutively expressed congants play crucial roles in modulating protein to protein interactions . Many stress proteins seem to function as molecular chaperons by regulating protein folding. A persual of literature (Abidi, 1990; Bauman et al. 1993 and Burdon,1988 indicates that our information regarding the effect of disyston (organophoshphate) and lindane (organochorinee on changes in stress protein profiles of selected tissues (gills, liver and muscles) in fresh water teleost is very limited as such the present work has been taken into account in a freshwater mud dwelling teleostean eel fish, Macrognathus aculeatum Lac.

Key Words - Pesticide, Body Composition, *Macrognathus aculeatum*.

Introduction :

Modern agriculture and industrial activities have resulted in increased food production and improved economy. But at the same time they have adversely affected the aquatic environment. The aquatic environment is the ultimate sink for contaminants and pollutants. The adversity of any chemical compound manufactured on an industrial scale is sure to reach the aquatic realm sooner

or later (Zitto, 1975). Pollutants, individually and in combination, cause serious hazards to the aquatic ecosystem. They not only upset the physicochemical equilibrium but also modify the biotic communities the species composition and biodiversity in water. In polluted water, the susceptible species often vanish, whereas the tolerant ones tends to flourish and dominate causing disruption of the aquatic food web (Srivastava and Vidyarthi, 2002).

Materials and Methods

Live specimens of *Macrognathus aculeatum* (Bloch) were collected from local fish dealers at Gaya. Fishes were brought to the laboratory and special care was taken to avoid the mechanical injury while carrying to the laboratory. The fishes were acclimatized for 10 days under the laboratory conditions before the experimentation. In the laboratory the fishes were fed daily with pieces of goat liver.

The pesticides used were lindane (organochlorine) and disyston (organophosphate.) Preliminary tests were conducted to determine the range of concentration of the toxicants to be used for definite toxicity test. The LC_{50} values of lindane and disyston were determined by definitive toxicity testing. For lindane it was 0.60, 0.50, 0.44 and 0.40ppm respectively for 24hr., 48hr., 72hr. and 96hr. For the disyston for the same exposure period it was respectively 7.55, 7.40, 6.80 and 6.60ppm. One third of the LC_{50} (for 96h) was taken as the sub lethal concentration.

Health fishes ranging 55-60gm. were exposed to subleethal concentration of the pesticides in tap water.

Observations

Exposure to lindane elicited noticeable stress response in the gill of ***M. aculeatum***. The response was detected in all the seven protein groups. Most of the proteins in the HMWP, HSP80, HSP70, HSP60, LMWP and VLMWP groups were supposed throughout exposure and recovery Augmentation of a few proteins occurred in the HMWP, HSP60 and LMWP groups. Four proteins were newly elicited (97kDa in HMWP, 66 kDa in HSP60, between 24 and 20 kDa and 14.2kDa in LMWP group). A new protein in the HSP90 group was found to be elicited during recovery phase.

In the liver of lindane exposed fish stress response was detected in six protein groups. Exposure to lindane resulted in the strong elicitation of two new proteins in the liver : one in the HSP70 (mol. wt. between 84 and 66kDa) and the other in the HSP60 region (mol. wt. 55 and 45kDa).

Exposure to lindane resulted in the induction of ten new proteins in the muscle of ***M. aculeatum*** : HWMP (97 kDa), HSP80 (84kDa), HSP60 (66, 55 and 45kDa), LMWP (29, between 29 and 24, 24 and 20kDa), VLMWP (14.2kDa). It also resulted in the suppression of several resident proteins. Increased synthesis of resident proteins was, however, not marked in the muscle.

Exposure to disyston resulted in the elicitation in the gill of ***M. aculeatum*** six new proteins in five protein groups : two HMWP (between 116 and 97 kDa and at 97kDa), HSP90 (between 97 and 84kDa), HSP60 (66kDa), LMWP (between 24 and 20kDa) and VLMWP (6.5kDa).

In the liver of ***M. aculeatum*** exposed to disyston stress response was detected in five protein groups (HMWP, HSP80, HSP60, LMWP and VLMWP). Three new proteins were strongly elicited : HMWP (between 116 and 97 kDa), HSP60 (between 55 and 45kDa) and LMWP (between 29 and 24kDa.)

The molecular stress response in the muscle of disyston exposed fish was detectable as strong elicitation of six new proteins : HSP90, HSP80, HSP60 (between 66 and 55kDa, 55kDa and 45 kDa), LMWP (between 29 and 24kDa). Interestingly, HSP70, which was not expressed in the control or during exposure, was found to be elicited in fish recovering from disyston exposure at 24hr., as a narrow band.

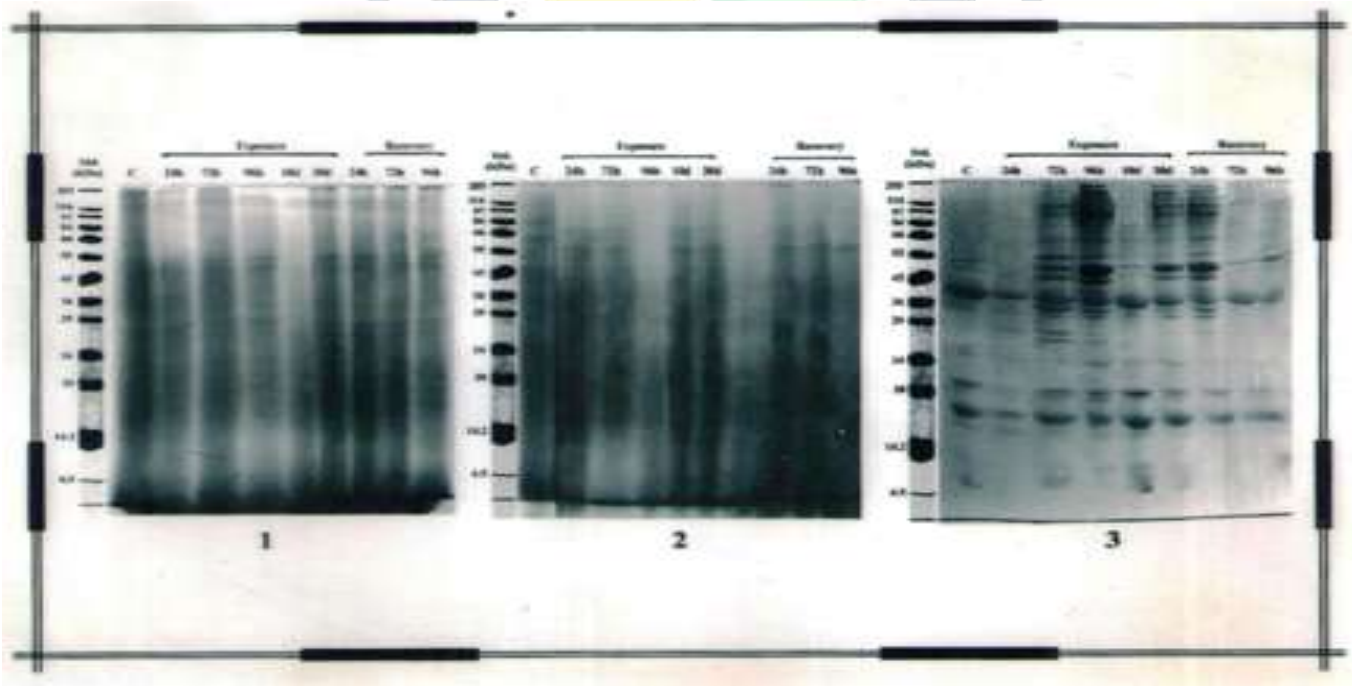

Fig. 1-3 SDS-PAGE-profile of the tissues of Macrognathus aculeatum during exposure to lindane and during recovery (C=control) (Fig. 1-Gill-Fig. 2 Liver, Fig. 3 Muscles)

Fig. 4-6 SDS-PAGE-profile of the tissues of Macrognathus aculeatum during exposure to disyston and during recovery (C=control) (Fig. 4-Gill-Fig. 5 Liver, Fig. 6 Muscles)

Discussion

All organisation, from bacteria to man alter their gene expression in response to stress for protecting themselves from damages induced by a variety of physical, chemical and biological stress, which, in turn, results in the induced synthesis, or increased synthesis, or total or partial suppression of the synthesis of a suite of proteins, originally known as 'heat-shock proteins' (Schlesinger, et al. 1982; Welch and Suhan, 1986 and Sanders, 1990). This response of an organism at the molecular level is well known as 'heat-shock response' or 'molecular stress response'.

Stress proteins play additional but related roles to molecular Chaperones, helping repair of denatured proteins and protecting others from damage, thus enabling the cells to endure stress.

The response of low and very low molecular weight stress proteins to stressors goes now, any explanation for the observed response of these proteins in ***M. aculeatum*** exposed to lindane and disyston would be too premature. Nonetheless, the earlier findings that HSP70 may not be a sensitive molecular indicator in instances where the stressor or toxicant is not strongly protetoxic (Vedel and Depledge, 1959; Lewis, et al. 2001) and the present results indicating a more consistent response by the HSP60 family proteins, strongly suggest the possibility of this group of stress proteins turning out to be a more reliable biomarker than HSP70 in pesticide pollution research.

The present findings clearly indicates that even sublethal concentration of lindane and disyston produce disfunction of many haematophysiological processes (including reduction in

serum protein) in fish and compensatory mechanisms are induced to meet the energy demand of the cell resulting from the pesticidal stress.

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