International Journal of Research in Engineering, Science and Management
Volume 4, Issue 7, July 2021
https://www.ijresm.com | ISSN (Online): 2581-5792

Stress Protein Profile of Pesticide Exposed Fish

L. K. Sreekala*
Assistant Professor, Department of Zoology, MES Mampad (Autonomous) College, Malappuram, India

***Abstract*: Response to stress is well documented in many different biological systems. A common feature of this response at the molecular level is the induction of a set of proteins, which were first named heat- shock proteins (HSPs). These proteins are involved in the protection, enhanced survival and restoration of normal activities of stressed cells. Present study evaluated stress protein response of *Etroplus suratensis* exposed to pesticide, endosulfan. Specimens were collected from Vembanadu Lake. Pesticide tested was endosulfan (OC). Protein profiles of the gills, liver and muscle of controlled and exposed fish were analysed by SDS-PAGE technique. The result revealed considerable differences in the protein profiles of the gills, liver and muscle of both controlled and exposed fish. In the exposed fish noticeable stress response was detected in all the seven groups. Most of the proteins in the HMWP, HSP80, HSP70, LMWP and VLMWP groups were supressed throughout exposure and recovery. Augmentation of a few proteins occurred in HMWP, HSP60, and LMWP groups. Four proteins were newly elicited in HMWP, HSP60 and LMWP group. Available information shows that HSP70 family is a popular choice for biomarker research. Induction of these proteins is vital to the protection of stressed cells. But the present result showed that HSP70 is not a sensitive indicator of pesticide stress. The response of HSP60 family was more regular under pesticide stress. Considering the chaperone function of these proteins, it would be reasonable to presume that these proteins would serve as better biomarker than HSP70 in pesticide induced stress in fish.**

***Keywords*: Fish, HSP, Pesticide, Stress response, Stressor specific, Tissue specific.**

1. Introduction

In an agricultural country like India, pesticides are the major aquatic chemical pollutant. They include herbicides, insecticides, fungicides, nematocides and rodenticides (Ongley,1996). There are mainly three groups of synthetic organic pesticides – organochlorines, organophosphates and carbamates. All the different groups of pesticides or even different pesticides of the same group do not have the same effect on fishes. The mode and site of action of different pesticides also differ greatly (Pandey. *et al*., 1984). DDT and its analogues, hexa-chlorocyclohexane (HCH), cyclodienes and similar compounds, toxaphene and related chemicals and caged structures, mirex and chlordecone, are the five major groups of organochlorine pesticides. Of these cyclodienes and related compounds include aldrin, isodrin, dieldrin, endrin, telodrin, hepatochlor, isobenzene, chlordane and Endosulfan (Smith, 1991). Endosulfan, a broad spectrum cyclodienes pesticide, widely used in agriculture is very toxic to fish (Capel, *et al*., 1988). Its principal breakdown product, endosulfan sulfate is persisted in the environment (Tomlin, 1994).

All organisms encounter both natural and anthropogenic stress. They adapt to it for the survival of the fittest. This adaptability at the molecular level became a marvellous mechanism to repair DNA, and evolve an epigenetic repair and recycling machinery to maintain the protein integrity. This epigenetic repair system is the classical cellular "stress response" or the "heat-shock response". It is involved in protecting organisms from damage as a result of exposure to a wide variety of environmental stressors including elevated temperature, ultraviolet light, trace metals, xenobiotics and even infectious agents. A major feature of the response is the rapid synthesis of a suite of proteins described originally as 'heat- shock protein'(HSPs) (Sanders, 1994).

The heat shock proteins were first discovered in cells from *Drosophila melanogaster* subjected to high temperature stress (Ritossa, 1962). HSPs contribute to stress tolerance by functioning as molecular chaperones. HSP-inducing stresses disrupt the native conformation of cellular proteins (Parsell, and Lindquist, 1993). Some HSPs are expressed constitutively; others increase expression with stress or are expressed only during or after stress (Morimoto *et al*., 1994). The mechanism of induction of stress protein involves binding of an activated heat shock factor protein to the respective heat shock element in the genome, which indicates the process of transcription of these proteins (Morimoto, 1997). Aminoacid analogues that create abnormal proteins induce synthesis of stress protein. Biochemical conditions that alter protein conformation also affect expression of the stress response in a predictable manner[6]. Based on the molecular weight, seven different types of stress proteins have been reported in eukaryotes. They are: high molecular weight proteins, stress protein 90, stress protein 70, stress protein 60 (chaperon), stress protein 40, low molecular weight proteins and very low molecular weight metal binding proteins (metallothioneins) (Schlesinger, 1988, Lindquist, 1986, Fowler *et al*.,1987). Stressors of various kinds induce stress response in different tissues of organisms.

Stress proteins exhibit fluctuating response with time and dose or both (Lewis *et al*.,2001). Variety of proteins synthesized makes possible the identification of the effects of specific contaminants or set of contaminants. Thus, the stress protein response becomes an integrated signal for environmental stress (Bradley *et al*.,1994). Most of these proteins are synthesized in high level in stressed cell and in very

*Corresponding author: skala375@gmail.com

low level in unstressed cell (Welch,1990).

From the foregoing it is clear that stress response of an organism is initiated at the molecular level by way of induction of stress proteins aimed at coping up with the stress and improving chances of survival by restoring homeostasis. It is also clear that this molecular stress response is ubiquitous among animals and can serve as molecular biomarkers to environmental contamination in the aquatic realm. Considering the high potential of stress proteins as biochemical markers of environmental stress, protein profiles of gill, liver and muscle of *Etroplus suratensis* exposed to sublethal concentrations of endosulfan was analyzed during the present study.

2. Materials and Methods

The test fish *Etroplus suratensis* were collected from Vembanadu Lake situated between longitude 76^0 23' E-76^0 30' E. Live and healthy fishes of 50-80 mm size were collected and disinfected with 5% potassium permanganate solution and were acclimatized to lab conditions for ten days. An organochlorine pesticide, endosulfan was selected for the present study. Preliminary tests were conducted to determine the range of concentration of the toxicant to be used for a definitive toxicity test. This test was conducted by using standard methods (USEPA 1975). From this method, sublethal concentration of endosulfan was determined as 0.7 ppb.

Six healthy fish of almost same size (50 mm to 80 mm each) were introduced in glass tank of 15-liter capacity having 0.7 ppb endosulfan in well water. Five experimental and one control tanks were set up. After 24h, 72h, 96h, 10 days and 30 days after exposure three fishes in each day were taken out for the study of stress protein profiles. After 30 days, the fishes were transferred into another tank having clear well water for the study of the recovery of the fish from pesticides. The recovery studies were carried out up to 96h. The study of stress protein profiles was carried out by using electrophoresis. Tissues such as gill, liver and muscle of pesticide exposed and control fish were used for the present study. SDS-PAGE method was adopted (Laemmli, 1970) for the separating gel or running gel and stacking gel preparation by using acrylamide (Merk,India) and bisacrylamide (BDH, India). Tissue samples were sonicated using cell disrupror and centrifuged in a cooling centrifuge at 4^0 C. samples were run in the vertical configuration at a current of 10 A for stacking and 20 A for separation. The gels were stained with Coomassive blue stain and destained with 5:1:5 methanol-acetic acid-water. The profile of wide range molecular weight standards was also run for comparison.

3. Result

Stress Protein Response in the Gill of Control and Endosulfan exposed Fish E. suratensis

Seventeen proteins with apparent molecular weights 205kDa, between 205 and 116, 116, 84, between 84 and 66, between 66 and 55, 55, between 55 and 45, 45, between 45 and 36, 36, between 36 and 29, 29, between 29 and 24, 24, 20, between 14.2 and 6.5 kDa and representing six protein groups (HMWP, HSP80, HSP70, HSP60, LMWP and VLMWP) were distinguishable in the protein profile of the gill of control fish. Of these, five protein bands (between 205 and 116kDa, 116, 84, 55 and 29 kDa) were very intensely expressed. Exposure to endosulfan elicited noticeable stress response in seven protein groups. Most of the proteins in the HMWP, HSP80, HSP70, HSP60, LMWP and VLMWP groups were suppressed throughout exposure and recovery. Augmentation of a few protein occurred in the HMWP, HSP60 and LMWP groups. Four proteins were newly elicited (97 kDa in HMWP, 66 kDa in HSP60, between 24 and 20 kDa and between 20 and 14.2 kDa in LMWP group). A new protein in the HSP90 group was found to be elicited during recovery phase (Plate 1 and Table 1).

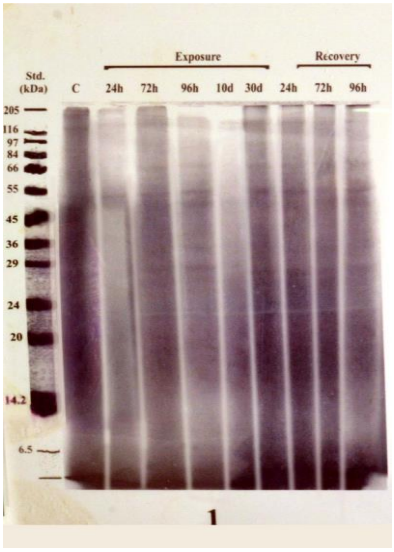

Fig. 1. Plate-1: Stress protein profiles in the gill of *E. suratensis* control fish, fish exposed to endosulfan and of fish recovering from exposure

Stress Protein Response in the Liver of Control and Endosulfan exposed Fish E. suratensis

Seventeen proteins with apparent molecular weights 205 kDa, between 205 and 116, 116, 97, 84, 66, between 66 and 55, 55, 45, between 45 and 36, 36, 29, 24, between 24 and 20, 20, between 20 and 14.2 and 14.2 kDa and representing five protein groups (HMWP, HSP80, HSP60, LMWP and VLMWP) were distinguishable in the protein profile of the liver of control fish. Of these eight protein bands (66, 45, between 45 and 36, 36, 29, 24, 20, between 20 and 14.2kDa) were intensely expressed. Exposure to endosulfan elicited noticeable stress response in the liver of *E. suratensis.* The response was detected in six protein groups. Exposure to endosulfan resulted in the strong elicitation of two new proteins in the liver of *E. suratensis*. One in HSP70 (mol. wt. between 84 and 66 kDa) and the other in HSP60 region (mol. wt. between 55 and 45 kDa) (Plate 2 and Table 2).

Stress Protein Response in the Muscle of Control and Endosulfan exposed Fish E. suratensis

Eleven proteins with apparent molecular weights 205, between 205 and 116, 116, between 116 and 97, between 55 and 45, between 45 and 36, 36, between 36 and 29, between 24

and 20, between 20 and 14.2 and between 14.2 and 6.5 kDa and representing four protein groups (HMWP, HSP60, LMWP and VLMWP) were distinguishable in the protein profile of the muscle of the control fish. Of these, five proteins (between 45 and 36, 36, between 24 and 20, between 20 and 14.2, between 14.2 and 6.5 kDa) were very intensely expressed. Exposure to endosulfan resulted in the induction of ten new proteins in the muscle of *E. suratensis*: HMWP (97 kDa), HSP80 (84 kDa), HSP60 (66, 55 and 45 kDa), LMWP (29, between 29 and 24, 24 and 20 kDa), VLMWP (14.2 kDa). It also resulted in the suppression of several resident proteins. Increased synthesis of resident proteins was, however, not marked in the muscle (Plate 3 and Table 3).

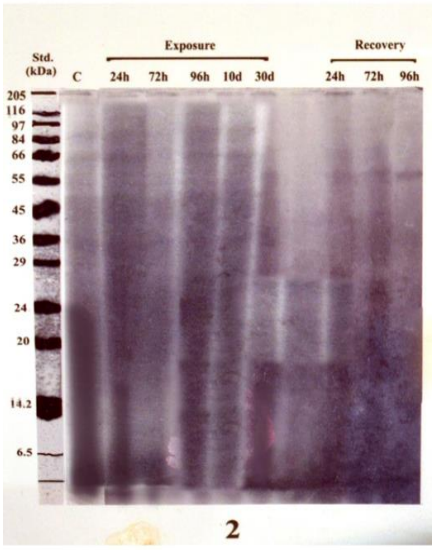

Fig. 2. Plate-2: Stress protein profiles in the liver of *E. suratensis* control fish, fish exposed to endosulfan and of fish recovering from exposure

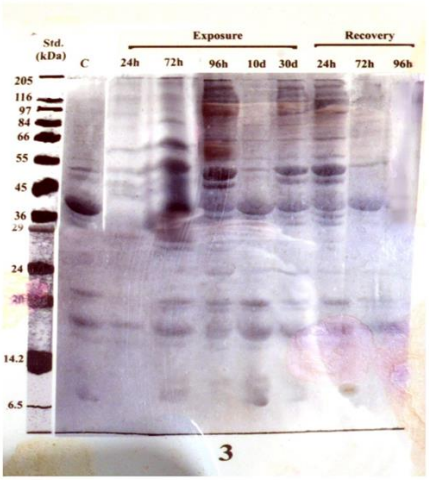

Fig. 3. Plate-3: Stress protein profiles in the muscle of *E. suratensis* control fish, fish exposed to endosulfan and of fish recovering from exposure

Tables: Stress protein response in different tissues of E. suratensis. Protein profiles of control fish, fish exposed to endosulfan and of fish recovering from exposure.

Table 1
Gill

S. No.	Mol. Wt. (kDa)	Protein group	Ctrl.	Exposure time					Recovery		
				24 h	72 h	96 h	10 d	30 d	24 h	72 h	96 h
1	205	HMWP	NrB	SU	AU	SU	SU	AU	AU	AU	AU
2	<->205-116		BrB	AU	AU	AU	AU	AU	AU	AU	SU
3	116		BrB	SU	SU	SU	SU	SU	SU	SU	SU
4	<->116-97		-	-	-	-	-	-	-	-	-
5	97		-	-	NI	NI	NI	NI	NI	NI	SU
6	<->97-84	HSP90	-	-	-	-	-	-	-	-	-
7	84	HSP80	BrB	SU	SU	SU	SU	SU	SU	SU	SU
8	<->84.66	HSP70	FeB	SU	SU	SU	SU	SU	SU	SU	SU
9	66	HSP60	-	-	NI	NI	NI	NI	NI	NI	NI
10	<->66-55		DiB	SU	SU	SU	SU	SU	SU	SU	SU
11	55		DiB	AU	AU	AU	AU	AU	AU	AU	AU
12	<->55-45		DiB	SU	SU	SU	SU	SU	SU	SU	SU
13	45		FeB	SU	SU	SU	SU	SU	SU	SU	SU
14	<->45-36	LMWP	FeB	SU	SU	SU	SU	SU	SU	SU	SU
15	36		FeB	SC	SC	SC	SC	SC	SC	SC	SC
16	<->36-29		FeB	SU	SU	SU	SU	SU	SU	SU	SU
17	29		BrB	AU	AU	AU	AU	AU	AU	AU	AU
18	<->29-24		FeB_s	SU	SU	SU	SU	SU	SU	SU	SU
19	24		DiB	SU	SU	SU	SU	SU	SU	SU	SU
20	<->24-20		-	NI	NI	NI	NI	NI	NI	NI	=
21	20		DiB	SU	SU	SU	SU	SU	SU	SU	SU
22	<->20-14.2		-	NI	NI	NI	NI	NI	NI	NI	NI
23	14.2	VLMWP	-	-	-	-	-	-	-	-	-
24	<->14.2-6.5		FeB	-	-	-	-	-	-	-	-
25	6.5		-	-	-	-	-	-	-	-	-
26	>6.5		-	-	-	-	-	-	-	-	-

Table 2
Liver

S. No.	Mol. Wt. (kDa)	Protein group	Ctrl.	Exposure time					Recovery		
				24 h	72 h	96 h	10 d	30 d	24 h	72 h	96 h
1	205	HMWP	NrB	SC	SC	SC	AU	AU	AU	AU	SU
2	<->205-116		FeB	SU	SU	SU	SU	SU	SU	SU	SU
3	116		FeB	SC	SC	SC	SC	SC	SC	SC	SC
4	<->116-97		-	-	-	-	-	-	-	-	-
5	97		FeB	SC	SC	SC	SC	SC	SC	SC	SC
6	<->97-84	HSP90	-	-	-	-	-	-	-	-	-
7	84	HSP80	FeB	AU	AU	AU	AU	AU	AU	AU	⇓
8	<->84.66	HSP70	-	NI	NI	NI	NI	NI	NI	SU	SU
9	66	HSP60	BrB	SC	SC	SC	SC	SC	SU	SU	SU
10	<->66-55		FeB	SU	SU	SU	SU	SU	SU	SU	SU
11	55		FeB	SC	SC	SC	SC	SC	SC	SC	SU
12	<->55-45		-	NI	NI	NI	NI	NI	NI	NI	NI
13	45		BrB	SC	SC	SC	SC	SC	SC	SC	SC
14	<->45-36	LMWP	BrB	SC	SC	SC	SC	SC	SU	SU	SU
15	36		BrB	SC	SC	SC	SC	SC	SC	SU	SU
16	<->36-29		-	-	-	-	-	-	-	*	-
17	29		BrB	SC	SC	SC	SC	SC	SC	SU	SU
18	<->29-24		-	-	-	-	-	-	-	-	-
19	24		BrB	SC	SC	SC	SC	SC	SC	SU	SU
20	<->24-20		DiB	SC	SC	SC	SC	SC	SU	SU	SU
21	20		BrB	SC	SC	SC	SC	SC	SC	SU	SU
22	<->20-14.2		BrB	SC	SC	SC	SC	SC	SC	SU	SU
23	14.2	VLMWP	DiB	SU	SU	SU	SU	SU	SU	SU	SU
24	<->14.2-6.5		-	-	-	-	-	-	-	-	-
25	6.5		-	-	-	-	-	-	-	-	-
26	>6.5		-	-	-	-	-	-	-	-	-

Table 3
Muscle

S. No.	Mol. Wt. (kDa)	Protein group	Ctrl.	Exposure time					Recovery		
				24 h	72 h	96 h	10 d	30 d	24 h	72 h	96 h
1	205	HMWP	NrB	SU	SU	SU	SU	SU	SU	SU	SU
2	<->205-116		NrB	SU	SU	SU	SU	SU	SU	SU	SU
3	116		NrB	SU	SU	SU	SU	SU	SU	SU	SU
4	<->116-97		FeB	SU	SU	SU	SU	SU	SU	SU	SU
5	97		-	-	NI	NI	NI	NI	NI	NI	NI
6	<->97-84	HSP90	-	-	-	-	-	-	-	-	-
7	84	HSP80	-	-	NI	NI	NI	NI	NI	NI	SU
8	<->84.66	HSP70	-	-	-	-	-	-	*	-	-
9	66	HSP60	-	-	NI	NI	NI	NI	NI	NI	SU
10	<->66-55		-	-	-	-	-	-	-	-	-
11	55		-	-	NI	NI	NI	NI	NI	NI	SU
12	<->55-45		NrB	⇓	+	+	↓	+	+	+	SU
13	45		-	-	NI	NI	NI	NI	NI	NI	SU
14	<->45-36	LMWP	BrB	=	=	=	+	+	+	+	=
15	36		BrB	SU	SU	SU	SU	SU	SU	SU	SU
16	<->36-29		FeB	=	SU	SU	SU	SU	SU	SU	SU
17	29		-	NI	NI	NI	NI	NI	NI	NI	SU
18	<->29-24		-	-	NI	NI	NI	NI	NI	SU	SU
19	24		-	-	NI	NI	NI	NI	NI	NI	SU
20	<->24-20		BrB	SU	SU	SU	SU	SU	SU	SU	SU
21	20		-	-	NI	NI	NI	NI	NI	NI	NI
22	<->20-14.2		BrB	SC	SC	SC	SC	SC	SC	SC	SU
23	14.2	VLMWP	-	NI	NI	NI	-	-	-	-	-
24	<->14.2-6.5		BrB	SU	SU	SU	SU	SU	SU	SU	SU
25	6.5		-	-	-	-	-	-	-	-	-
26	>6.5		-	-	-	-	-	-	-	-	-

NI- New induction,
AU- Augmentation of synthesis,
SU- Total suppression
SC- Same as control,
– No bands,
<-> Between
(Ctrl.) Control fish,
(BrB) Broad band,
(DiB) Diffused band, (FeB) Feeble band, (NrB) Narrow band

4. Discussion

All organisms, 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 total or partial suppression of the synthesis of a suite of proteins originally known as 'heat shock proteins' and also now as 'stress proteins' or 'stress related proteins' (Schlesinger et al.,1982, Sanders, 1990). Based on their apparent molecular weight, at least seven distinct groups of stress proteins have been recognized: high molecular weight proteins (HMWP), HSP90, HSP80, HSP70, HSP60, low molecular weight proteins (LMWP) and very low molecular weight proteins (VLMWP) (Schlesinger, 1988, Fowler *et al*., 1987). Stress protein 70 is a popular choice for biomarker research as this is the most highly conserved and widely studied of the HSPs (Ryan and Hightower, 1996). Stress protein 70 has been shown to have at least three functions: multi-meric protein assembly, unfolding for translocation and disaggregation of protein aggregates. These functions facilitate repair of proteins and protein complexes associated with common and critical metabolic processes, thereby protecting cells from stressors induced damage (Welch, 1990, Rothman, 1989). HSP70 interact with other proteins involved in cytoskeletal structure and function (Guildon.and Hightower, 1986). Structurally altered proteins serve as signal for the induction of HSP70 (Munro and Pelham, 1986, Ananthan, *et al*., 1986). In *M. edulis* four isoforms of stress proteins within the 70 kDa family were reported due to thermal stress (Smerdon *et al*.,1995). They proposed that this family is the most abundant and most conserved subset of eukaryotic stress proteins, acting as molecular chaperones that direct the folding, assembly and degradation of cellular proteins. Cytoplasmic HSP70 migrates to the nucleus and binds to pre-ribosomes and other protein complexes to protect them from denaturation and then return to the cytoplasm during recovery (Gething and Sambrook 1992).

In the present study, HSP70 was suppressed throughout endosulfan exposed gill tissue of *E. suratensis*. Where as in the liver tissue of endosulfan exposed fish HSP70 was induced throughout exposure. In the muscle tissue, both in the control and pesticide exposed fish, HSP70 was not expressed. The present results are in agreement with the findings of Vedel and Depledge 1995; Lewis *et al*. 2001. Vedel and Depledge (1995) reported that HSP70 in the common shore crab was not a sensitive indicator of copper exposure. Studies of HSP70 expression in the microalga, *E. intestinalis*, exposed to environmental stressors like copper and triazine herbicide, Irgarol 1051, reported that HSP70 is induced only by stressors that are strongly proteotoxic. Copper induced increase in HSP70 levels under nutrient replete condition; copper is known to bind to protein by interacting with the sulphydral group of intracellular proteins thereby disrupting their conformation. However, Irgarol1051 did not induce HSP70 suggesting that it may be only weakly proteotoxic (Lewis *et al*.,2001). Whereas the results of many studies advocate for the universality of HSP70 expression/ induction as a molecular response to environmental/ thermal/ chemical stress, in the light of the present observation as also of other similar ones, which have been primarily marked by the non-expression of HSP70 in the tissue of stressed organisms particularly fishes, suggest that caution must be exercised while considering HSP70 as a universal molecular biomarker of stress response.

When protein denaturation increases, as under stress, synthesis of the members of HSP60 family also increases; they bind to damaged proteins and help them to restore their original conformation. These proteins, according to Cheng *et al*., (1989) and Fayet *et al*., (1989) are, therefore essential for cell viability. The pattern of response of the proteins of this family in the tissues of *E. suratensis* exposed to the pesticide support the above condition. Induction of HSP60 family has been reported in the gill and mantle of *M. edulis* (Sanders *et al*.,1991) and in *Pimephales promelas* (Sanders *et al*.,1994), due to copper stress. Stress protein 60 is localized in mitochondria. Mitochondria are the sites of copper induced damage in tissues (George. 1982) and according to George (1982) and Totero *et al*, (1986) mitochondria are the major targets of copper toxicity in fish. Induction of different members of HSP60 family has also been noted in organisms subjected to heat-shock: in *P. promelas* (Dyer *et al*., 1991), in sea urchin (Sanders and Martin 1994) and in crayfish (Rochelle *et al*.,1991.

As early pointed out, the available information shows that HSP70 family is a popular choice for biomarker research as it is the most highly conserved proteins known in biology and induction of these proteins is vital in the protection of stressed cells (Sanders, 1993). But the present results show that HSP70 is not a sensitive indicator of pesticide stress. The response of proteins in the HSP60 family was more regular. For all three tissues studied, HSP60 as a group was newly induced very often or its synthesis was augmented. HSP60 proteins are essential for cell viability (Cheng *et al*., 1989, Fayet *et al*., 1989). Under stressful condition denaturation of many native proteins increases and consequently, synthesis of HSP60 family towards exposure to the pesticides and considering the chaperone function of these proteins, it would be responsible to presume that these proteins would serve as better biomarkers than HSP70 in pesticide induced stress in fish. Nevertheless, the varied response of different members of this group to the pesticide and the tissues studied warrants more detailed studies using different pesticides before labeling HSP60 as a reliable biomarker of pesticide induced stress.

5. Conclusion

Exposure to endosulfan elicited noticeable stress response in *E. suratensis*. In the current study HSP70 was absent in the muscle of the control, pesticide exposed and recovering fish and it was fully suppressed in the gill of endosulfan exposed fish. But its expression varied in liver tissue. In the liver tissue of fish exposed to endosulfan this protein was newly induced almost throughout exposure. Thus, HSP70 response to pesticide challenge was either erratic in *E. suratensis*, suggesting that at least in situations such as stress due to pesticides in fish, the trustworthiness of HSP70 as a biomarker is questionable. While the response of proteins in the HSP60 family was more regular. For all three tissues studied, through the response of the

different members of the family differed with tissue, HSP60 as a group was newly induced very often or its synthesis was augmented. In short, it seems responsible to conclude from the results of the current study on the molecular stress response of *E. suratensis* to pesticide, that HSP60 group of proteins might be better biomarker than HSP70, of stress response of fishes to pesticide challenge.

Acknowledgement

The author thankfully acknowledge Dr. Tresa Radhakrishnan Ph.D., Professor (Retd.) and Dr. S. Radhakrishnan Ph.D., Professor(Retd.), Department of Aquatic Biology and Fisheries, University of Kerala for their guidance, valuable suggestion and helpful advice throughout this study.

References

[1] Ananthan, J., Goldberg A.L. and Voellmy R. Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat-shock genes. *Science*, 232: 522-524, 1986.

[2] Bradley, B.P., Gonzales C.M., Bond J.A. and Tepper B.E. Complex mixture analysis using protein expression as a qualitative and quantitative tool. *Environ. Toxicol. Chem*., 13: 1043- 1050, 1994.

[3] Capel, P.D., Giger W., Reichert P. and Wanner O. Accidental input of pesti-cides into the Rhine River. *Environ. Sci. Technol*., 22: 992-997, 1988.

[4] Cheng, K., Davidson W.S., Hew C.L. and Fletcher G.L. Molecular cloning of metallothioneins cDNA and analysis of metallothionein expression in winter flounder tissue. *Can. J. Zool*., 67: 2520-2527, 1989.

[5] Dyer, S.D., Dickson K.L., Zimmerman E.G. and Sanders B.A. Tissue specific patterns of heat-shock protein synthesis and thermal tolerance of the fathead minnow, *Pimephales promelas*. *Can. J. Zool*., 69: 2021- 2027, 1991.

[6] Fayet, O., Ziegelhoffer T .and Georgopoulos C. The GroES and GroEL heat- shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. *J. Bact*., 171: 1379-1385, 1989.

[7] Fowler, B.A., Hilderbrand C.E., Kojima Y. and Webb W. Nomenclature of metallothioneins. *Experientia* (*Suppl.*), 52: 19-22, 1987.

[8] George. Subcellular accumulation and detoxification of metals in aquatic animals. In Physiological Mechanisms of zinc in the mussel, *Mytilus edulis*: A combined ultrastructural and biochemical study. *J. mar. boil. Ass.* U.K., 60: 575-590, 1982.

[9] Gething, M.J. and Sambrook J. Protein folding in the cell. *Nature (London)*, 355: 33-45, 1992.

[10] Guildon, P.T.and Hightower L.E. Purification and initial characterization of the 71 kDa rat heat-shock protein and its cognate as fatty acid binding proteins. *Biochemistry*, 25: 3231-3238, 1986.

[11] Laemmli, U.K. Most commonly used discontinuous buffer system for SDS electrophoresis, *Nature (Lond.)*, 227: 680-685, 1970.

[12] Lewis, S., Donkin M.E. and Depledge M.H. HSP 70 expression in *Enteromorpha intestinalis* exposed to environmental stressors. *Aquat. Toxicol*., 51(3): 277-291, 2001.

[13] Lewis, S., Donkin M.E. and Depledge M.H. HSP 70 expression in *Enteromorpha intestinalis* exposed to environmental stressors. *Aquat. Toxicol*., 51(3): 277-291, 2001.

[14] Lindquist, S. The heat-shock responses. *Annu. Rev. Biochem*., 55: 1151-1191, 1986.

[15] Morimoto, R. I. The heat-shock response: Regulation and function of heat-shock proteins and molecular chaperones. *Ess. Biochem.*, 32: 17-29, 1997.

[16] Morimoto, R.I., Jurivich D.A., Kroeger P.E., Mathur S.K., Murphy S.P., Nakai A., Sarge K., Abravaya K. and Sistonen L.T. Regulation of heat shock factors. *In*: The Biology of Heat-shock Proteins and Molecular Chaperones. (R.L Morimoto, A. Tissieres and C. Georgopoulos eds.), Coldspring Harbor Lab. Press, NY, 417-455, 1994.

[17] Munro, S. and Pelham P.J. An hsp 70-like protein in the ER: identity with the 78k Da glucose regulated protein and immunoglobulin heavy chain binding protein. *Cell*, 46: 291-300, 1986**.**

[18] Ongley, E.D. Pesticides as water pollutants. *In*: Control of water Pollution from Agriculture, FAO Irrigation and Drainage Paper 55, GEMS/Water Collaborating Centre, Canada, 1996.

[19] Pandey, B.N., Prasad S.S., Chanchal A.K. and Singh S.B. Effect of some pesticides on the oxidative metabolism and haematology of *Clarias batrachus.* Proc. Sem. Effect of Pesticides on Aquatic Fauna, June 1983, Mhow, India, 13-18, 1984.

[20] Parsell, D.A. and Lindquist S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet., 27: 437-496, 1993.

[21] Ritossa, F. A new puffing pattern induced by heat shock and DNP in *Drosophila. Experientia*., 18: 571-573, 1962.

[22] Rochelle, J.M., Grossfield R.M., Bunting D.L., Tyell M., Dwyer B.E. and Xue Z. Stress protein synthesis by crayfish CNS tissue *in vitro*. *Neurochem. Res*., 16: 530-538, 1991.

[23] Rothman, J.E. Polypeptide chain binding proteins: catalysts of protein folding and related processes in cells. *Cell*, 59: 591-601, 1989.

[24] Ryan, J.A., Hightower L.E. Stress proteins as molecular biomarkers for environmental toxicology. *In*: Stress-inducible Cellular Responses, (U. Feige, R.L. Morimoto, I. Yahara and B. Polla, eds.), Birkhauser, Basel, Switzerland, 1996.

[25] Sanders, B.M. Stress proteins: Potential as multitiered biomarkers. *In*: Bio-markers of Environmental Contamination, (J.F. McCarthy and L.R. Shugart, eds.), Lewis Publ., Boca Raton, FF, 165-191, 1990.

[26] Sanders, B.M. Stress proteins in aquatic organisms: An environmental perspective, *Crit. Rev. Toxicol*., 23: 49-51, 1993.

[27] Sanders, B.M. Stress proteins as molecular chaperones: Implications for toxicology. *Environ. Hlth. Persp.*, 102: 6-7, 1994.

[28] Sanders, B.M. and Martin L.S. Copper inhibits the induction of stress protein synthesis by elevated temperatures in embryos of the sea urchin, *Strongylocentrus purpuratus. Comp. Biochem. Physiol*., 109: 295-307, 1994.

[29] Sanders, B.M., Martin L.S., Nelson W.G., Phelps D.E. and Welch W. Relationship between accumulation of a 60 kDa stress protein and scope for growth in *Mytilus edulis* exposed to a range of copper concentrations. *Mar. Environ. Res*., 31: 79-88, 1991.

[30] Sanders, B.M., Martin M., Howe S.R., Nelson W.G., Hegre E.S. Phelps and D.K. Tissue-specific difference in accumulation of stress protein in *Mytilus edulis* exposed to a range of copper concentrations. *Toxicol. Appl. Pharmacol*., 125: 206-213, 1994.

[31] Schlesinger, M.J. Function of heat shock proteins. *Atlas Sci. Biochem*., 16: 161-164, 1988.

[32] Schlesinger, M.J., Ashburner M. and Tissieres A. Heat Shock from Bacteria to Man. Cold Spring Harbor Lab, Cold Spring Harbor, New York, 440 pp, 1982.

[33] Smerdon, G.R., Chapple J.P. and Hawkins J.S. The simultaneous immuno-logical detection of four stress 70 protein isoforms in *Mytilus edulis*. *Mar. Environ. Res.*, 40: 399-407, 1995.

[34] Smith, A.G. Chlorinated hydrocarbon insecticides. *In*: Handbook of Pesticide Toxicology, (W.J. Hayes Jr. and E.R. Laws Jr., eds.), Acad. Press, San Diego: 333- 346, 1991.

[35] Tomlin, C. The pesticide manual: Incorporating the agrochemical handbook. Crop Protection Publications, Farnham, U. K., 1341 pp. 1994

[36] Totero. E.A., Pisanti F.A., Glees P. and Continillo A. The effect of copper pollution on mitochondrial degradation. Mar. Environ. Res., 18: 245-253, 1986.

[37] USEPA Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Environmental Protection Agency, Washington, DC 3-8. 1975.

[38] Vedel, G.R. and Depledge M.H. Stress 70 levels in gills of *Carcinus maenus* exposed to copper. *Mar. Poll. Bull*., 31: 84-86, 1995.

[39] Welch, W.J. Mammalian stress response. *In*: Cell Physiology and Biochemistry of Stress Response in Biology and Disease, (R.I. Morimoto and A. Tissieres, eds.), Cold Spring Harbor Laboratory, 223-278, 1990.

[40] Whatley, S.A.., Leung T., Hall C. and Linn L. The brain 68kDa microtubule protein is a cognate form of the 70 kDa mammalian heat-shock protein and is present as a specific isoform in synaptosomal membranes. *J. Neurochem*., 47: 1576-1583, 1986.