

PYRETHROID INSECTICIDE, LAMBDA-CYHALOTHRIN IMPACT ON TISSUE ACID PHOSPHATASE (AcP) ACTIVITY OF THE FISH, *ETROPLUS SURATENSIS*

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ABSTRACT

*Pyrethroid insecticides, lambda cyhalothrin are important tools used in agricultural fields, public health management where applications are made to control cockroaches, mosquitoes, ticks and flies, which may act as disease vectors. Specifically, lambda-cyhalothrin penetrates the insect cuticle, disrupting nerve conduction within minutes; this leads to cessation of feeding, loss of muscular control, paralysis, and eventually death. Fishes are excellent indicators of pesticide pollution in the aquatic environment, because they are masters of the aquatic environment and they dominate the trophic pyramid of almost all aquatic systems. In the present study, the acid phosphatase activity increased significantly in liver, muscle and brain tissues of *E.suratensis* exposed to lambda-cyhalothrin. From this investigation, enhanced activity of acid phosphatase (AcP) can be related to the lysosomal destabilization.*

Key words: *Acid Phosphatase, Brain, Impact, Lambda-cyhalothrin, Liver, Muscle.*

INTRODUCTION

India, being the main producer and consumer of pesticides in South Asia, the use of pesticides in agriculture has increased significantly during past three decades (Natarajan *et al.*, 1998). Pyrethroid insecticides, lambda cyhalothrin are important tools used in agricultural fields, public health management where applications are made to control cockroaches, mosquitoes, ticks and flies, which may act as disease vectors. Specifically, lambda-cyhalothrin penetrates the insect cuticle, disrupting nerve conduction within minutes; this leads to cessation of feeding, loss of muscular control, paralysis, and eventually death. Every year, the increase of human population accompanying with the growth of industrialization results in the increase of pollution in aquatic ecosystems (Caussy *et al.*, 2003). Biochemical indices of stress have been proposed to assess the health of non-target organisms exposed to toxic chemical in aquatic ecosystem (Nimmi, 1990). Insecticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides (Banaee *et al.*, 2008; Al-Kahtani, 2011) and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish.

Acid phosphatase is regarded as a key lysosomal enzyme, and it plays an important role in the autolytic degradation of tissues during metamorphosis (Nath and Butler, 1971). Phosphatase enzymes are considered as an important toxicological tool to study the pesticide effect that reflects the change in physiological and biochemical pathways (Ramano Rao *et al.*, 1996). Phosphatases are concerned with oxidative phosphorylation (Goodman and Rothstein, 1957), growth, differentiation (Barker and Alexander, 1958) and permeability (Seth *et al.*, 1969). Muthukrishnan and Senthamizhselvan (1985) have shown that acid phosphatase plays an important role in the utilization of yolk during embryonic development.

Altered phosphatase activities in the tissues of freshwater fish, *T. mossambica* were reported by Joshi and Desai (1981) with respect to increasing concentration of pesticides monocrotophos. Similar observations have also been reported in *S. mossambicus* under exposure to sumithion (Koundinya and Ramamurthi, 1981). Dubale and Awasthi (1982) demonstrated increase in the acid phosphatase activity in the liver and kidney of *H. fossilis* exposed to demethoate. Similar observation was also reported in the case of *M. vittatus* under the treatment of dichlorvos (Verma *et al.*, 1981). Evaluation of enzyme activities in the tissue and organs of aquatic organs in the diagnosis of the effects of pollutants is one of the emerging areas in toxicological monitoring and remediation programmes (Oluah *et al.*, 2005). Enzyme analysis is widely used for rapid detection to predict early warning of pesticide toxicity (Dutta and Areids, 2003). In view of the above facts, the present work was undertaken which attempts to evaluate the impact of the pyrethroid insecticide lambda-cyhalothrin, on the tissue acid phosphatase activity of the fish, *E. suratensis*.

MATERIALS AND METHODS

Well acclimated *E. suratensis* (6-7 g) of uniform size and weight were grouped into sets of 10, and each set was introduced into sub-lethal concentrations of 1/4, 1/8, 1/12, 1/16 and 1/20 LC₅₀ lambda-cyhalothrin respectively. The fish were fed with standard food pellets throughout the experimental period. The total period of exposure was 60 days. Suitable control was also maintained. After the stipulated exposure periods, 6 fishes were removed from each pesticide-treated group with the respective control were removed and sacrificed for tissue sampling. For acid phosphatase study, the tissues such as muscle, liver and the whole brain were removed and kept frozen until analysis was performed.

Acid phosphatase activity in the selected tissues was assayed spectrophotometrically using the method of Bergmeyer (1963) as modified by Butterworth and Probert (1970). Results were expressed as means \pm SD and two-way ANOVA were used to assess statistical significance. The activity of the AcP enzyme is expressed as μ mole α naphthal $p^{-1} h^{-1}$.

RESULTS

In control medium, the acid phosphatase activity in liver was $3.32 \pm 0.05 \mu$ mole α naphthal $p^{-1} h^{-1}$, and it increased to $3.72 \pm 0.07 \mu$ mole α naphthal $p^{-1} h^{-1}$ followed by 4.19 ± 0.05 , 5.78 ± 0.04 , 6.2 ± 0.39 and $6.73 \pm 0.18 \mu$ mole α naphthal $p^{-1} h^{-1}$ recorded in 0.005 ppm, 0.006 ppm, 0.008 ppm, 0.013 ppm and 0.026 ppm of lambda-cyhalothrin respectively (Table 1).

The acid phosphatase of muscle of control medium was $2.76 \pm 0.06 \mu \text{ mole } \alpha \text{ naphthal } p^{-1} h^{-1}$ and it increased to 3.42 ± 0.01 , 4.84 ± 0.02 , 5.21 ± 0.24 , 5.92 ± 0.08 and $6.14 \pm 0.04 \mu \text{ mole } \alpha \text{ naphthal } p^{-1} h^{-1}$ in those fishes exposed to 0.005 ppm, 0.006 ppm, 0.008 ppm, 0.013 ppm and 0.026 ppm lambda-cyhalothrin (Table 1).

The pesticide free medium, the brain acid phosphatase activity was $2.69 \pm 0.04 \mu \text{ mole } \alpha \text{ naphthal } p^{-1} h^{-1}$. This value gradually increased was noticed in fishes reared at 0.005 ppm ($3.32 \pm 0.15 \mu \text{ mole } \alpha \text{ naphthal } p^{-1} h^{-1}$), at 0.006 ppm (3.66 ± 0.06), 0.008 ppm (4.38 ± 0.23), 0.013 ppm (4.86 ± 0.09) and 0.026 ppm ($5.22 \pm 0.09 \mu \text{ mole } \alpha \text{ naphthal } p^{-1} h^{-1}$) concentration of lambda-cyhalothrin respectively (Table 1).

From the two-way ANOVA (Table 2) that there was significant variation ($P < 0.01$) between the tissues with respect to the acid phosphatase activity and deviation due to different concentrations was statistically highly significant ($P < 0.001$) compared with control group. The acid phosphatase activity in various tissues of *E. suratensis* recorded an increasing trend with increasing concentrations of lambda-cyhalothrin (Figure 1).

Table 1: Effect of lambda-cyhalothrin on acid phosphatase activity in different tissues of *E. suratensis* for 60 days

Tissues	Control	Concentrations of lambda-cyhalothrin (ppm)				
		0.005	0.006	0.008	0.013	0.026
Liver	3.32 ± 0.05	3.72 ± 0.07	4.19 ± 0.05	5.78 ± 0.04	6.2 ± 0.39	6.73 ± 0.18
Muscle	2.76 ± 0.06	3.42 ± 0.01	4.84 ± 0.02	5.21 ± 0.24	5.92 ± 0.08	6.14 ± 0.04
Brain	2.69 ± 0.04	3.32 ± 0.15	3.66 ± 0.06	4.38 ± 0.23	4.86 ± 0.09	5.22 ± 0.09

Values are expressed as mean \pm SD

Values are expressed as $\mu \text{ mole } \alpha \text{ naphthal } p^{-1} h^{-1}$

Table 2: Two-way ANOVA for AcP

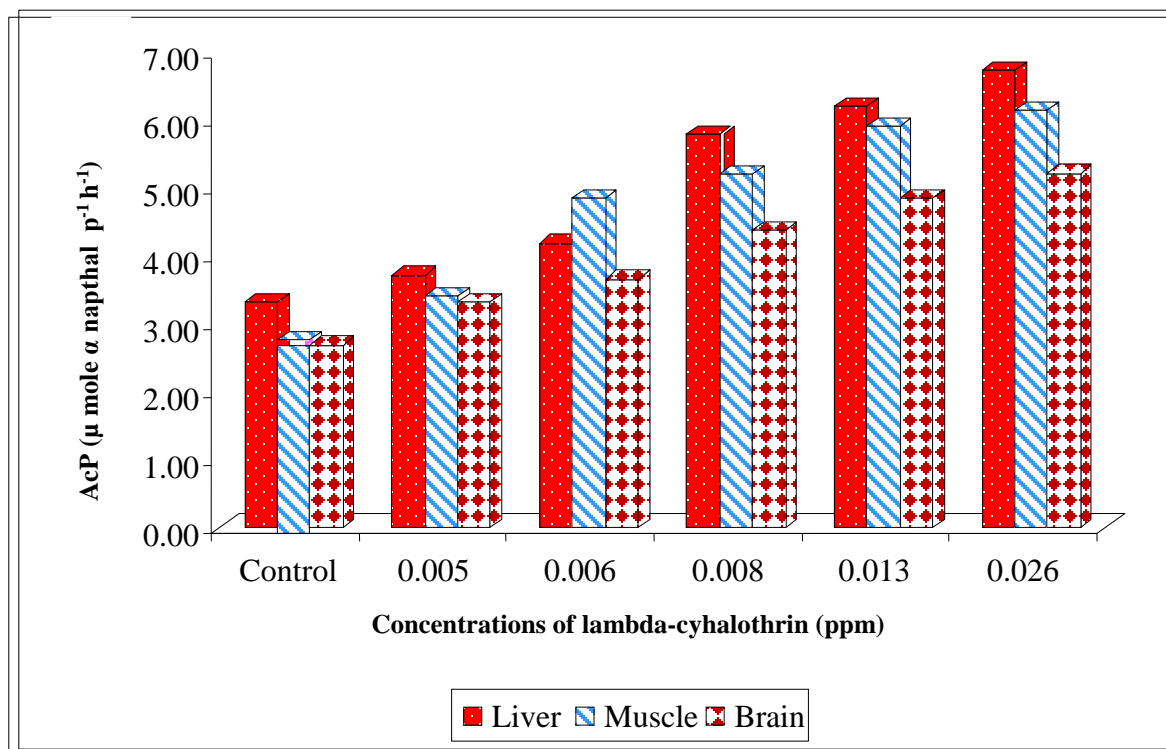
Source of Variation	SS	df	MS	F	P-value
Variation due to tissues	2.970503704	2	1.485251852	12.41895524	$P < 0.01$
Variation due to concentrations	22.91585185	5	4.58317037	38.32224658	$P < 0.001$
Error variance	1.195955556	10	0.119595556		
Total variance	27.08231111	17			

SS - sum of squares

df - degrees of freedom

MS - mean of squares

Figure 1: Effect of lambda-cyhalothrin on acid phosphatase activity in different tissues of *E. suratensis* exposed for 60 days



DISCUSSION

In the present study, the acid phosphatase activity increased significantly in liver, muscle and brain tissues of *E. suratensis* exposed to lambda-cyhalothrin. The acid phosphatase was enhanced during toxic exposure period and under stress condition. The elevated levels of phosphatase may indicate the increase in the rate of phosphorylation and transport of molecules across the cell membrane. The enhanced phosphatases activity reveals increase transportation of metabolites through cellular membrane. Abdul *et al.* (2004) and Venkateshwarlu *et al.* (1990) also reported that the pesticides cause significant increase in cellular damage which causes enhanced activity of phosphatases activity. The observed higher rates of acid phosphatase activity might be related to higher rate of enzyme synthesis. This observation, however has been reported earlier by several workers (Mukhopadhyay and Dehadrai, 1980; Mukhopadhyay *et al.*, 1982). The enhancement of AcP activity of lambda-cyhalothrin in the selected tissues in the present study was found to be closely associated with hepatic necrosis. This study was supported by Sherekar (1986) and Sherekar and Kulkarni (1987).

The present findings also support in the observations of Anastasi and Bonnister (1980) that have shown that the application of insecticides induces changes in the enzymatic equipment in different body organs. Radhakrishnan Nair (2002) reported elevation of acid and alkaline phosphatase activities in different tissues of *O. mossambicas* and *C. carpio* exposed to karate and curacron. Joshi and Desai (1981) have reported increase in acid phosphatase activities in the tissues of *T. mossambica* under sub-lethal concentrations of monocrotophos. Similar results were also reported by Sherekar and Kulkarni (1987) and Garg *et al.* (1987) on *C. orientalis* and *H. fossilis* exposed to methylparathion and hilbeech. Increased acid phosphatase activity in the liver and brain tissues of *C. punctatus* exposed to lihocin was reported by Abdul Naveed *et al.* (2010). Increased acid phosphatase activity in the selected tissues of *E. suratensis* may be due to cellular necrosis of tissues as suggested by Joshi and Desai (1981).

Increase in acid phosphatase in response to other toxicants has been reported earlier. Elevation of AcP activity in the liver, among other tissues has been reported in *H. fossilis* in response to different organic pesticides (Thomas and Murthy, 1976), in *O. punctatus* response to endrin (Sastry and Sharma, 1979), in *H. fossilis* response to mercuric chloride (Gupta and Sastry, 1981) and in *C. batrachus* response to lithium nitrate intoxication (Goel *et al.*, 1985). This result is in agreement with the significant increase in acid phosphatase in liver, brain and kidney of the fish, *C. punctatus* after intoxication with triazophos (Abdul Naveed *et al.*, 2010).

Acid phosphatase is a lysosomal marker enzyme (Kendall and Hawkins, 1975). Gupta *et al.* (1975) reported that acid phosphatase is a good indicator of stress condition in biological systems. Several mechanisms have been suggested for the release of acid phosphatase from the lysosomes. Verity and Reith (1967) observed that the lysosomes are structurally altered in response to toxic dosage of methyl mercury. Deung *et al.* (1978) reported an increase in the number of lysosomes in the liver cells of *C. carassius* exposed to mercuric chloride while Ferri and Macha (1980) observed a change in shape distribution and functional degree of lysosomes in the hepatic cells of *P. maculatus* exposed to cadmium. Assessment of this type of damage has been confirmed as an extremely sensitive general index of cellular conditions (Moore, 1980, 1982). Injury resulting in destabilization of the lysosomal membrane bears a quantitative relationship to the magnitude of stress response (Bayne *et al.*, 1979, 1982). Destabilization may involve increased lysosomal fusion with other intracellular vacuoles, leading to the formation of pathologically enlarged lysosomes. Since release of acid phosphatase from the lysosome is associated to membrane damage due to xenobiotics. Enhanced activity of acid phosphatase can be related to the lysosomal destabilization. When fishes are exposed to the fourth generation and current generation pesticide like lambda-cyhalothrin, they have various toxic effects which make the fishes less fit for survival. This in turn will affect the fecundity of the fish population and also other organisms including human beings through food chain. Biological methods could be used for controlling mosquito and flies instead of lambda-cyhalothrin in order to protect the natural environment.

REFERENCES

- Abdul Farah, M., B. Ateeg, M.N. Ali and W. Ahmad, 2004.** Studies on lethal concentrations and toxicity stress of some xenobiotics on aquatic organisms. *Chemosphere.*, **55**: 257-265.
- Abdul Naveed., P. Venkateswarlu and C. Janaiah, 2010.** Impact of sub-lethal concentration of triazophos on regulation of protein metabolism in the fish *Channa punctatus* (Bloch). *African Journal of Biotechnology.*, **9**(45): 7753-7758.
- Al-Kahtani, M.A., 2011.** Effect of an insecticide abamectin on some biochemical characteristics of Tilapia fish (*Oreochromis niloticus*). *American Journal of Agricultural and Biological Sciences.*, **6**(1): 62-68.
- Anastasi, A. and J.V. Bonnister, 1980.** Effect of pesticides on mediterranean fish muscle enzyme. *Acta adriat.*, **21**(1): 911-936.
- Banaee, M., A.R. Mirvagefi, G.R. Rafei and B. Majazi Amiri, 2008.** Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. *Int. J. Environ. Res.*, **2**(2): 189-198.
- Barker, R.J. and B.H. Alexander, 1958.** Acid and alkaline phosphatase in house fishes of different fishes of different ages. *Ann. Entamol. Soe. Am.*, **51** : 251-257.
- Bayne, B.L., M.N. Moore, J. Widdows, D.R. Livingstone and P. Salkeld, 1979.** Measurement of the responses of individuals to environmental stress and pollution. *Phil. Trans. R. Soc. Lond.* **286**: 563-581.
- Bayne, B.L., J. Widdows, M.N. Moore, P. Salkeld, C.M. Worall and P. Donkin, 1982.** Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. *Phil. Trans. R. Soc. Lond.*, **297**: 219-239.
- Bergmeyer, H.U., 1963.** *Methods of enzymatic analysis.* Academic Press, New York.
- Butterworth, P.J. and A.J. Probert, 1970.** Non-specific phosphomonoesterases of *Ascaris suum*. Effect of inhibitors, activators and chelators. *Exp. Parasitol.*, **28**: 555-565.
- Caussy, D., M. Gochfeld, E. Gurzau, C. Neagu and H. Rjede, 2003.** Lessons from case studies of metals: investigating exposure, bioavailability and risk. *Ecotoxicol. Environ. Safety.*, **56**: 45-51.
- Deung, Y.K., K.H. You, C.K. Choi and R.S. Choe, 1978.** Ultrastructural studies on mercury poisoning in the liver, kidney and gills of *Carassius carassius* L. *Korean. J. Zool.*, **21**: 87-102.
- Dubale, M.S. and H. Awasthi, 1982.** Biochemical changes in the liver and kidney of a catfish, *Heteropneustes fossilis* exposed to dimethoate. *Comp. Ecol. Physiol.*, **7**(2): 111-114.
- Dutta, H.M. and D.A. Areids, 2003.** Effects of endosulfan on brain acetyl cholinesterase activity in juvenile blue gill sunfish. *Environmental Research.*, **91**: 157-162.
- Ferri, S. and N. Macha, 1980.** Lysosomal enhancement in hepatic cells of a teleost fish induced by cadmium. *Cell. Biol. Int. Rep.*, **4**: 357- 363.
- Garg, V.K., S. Rani and S.K. Tyage, 1987.** Changes in alkaline phosphatase distribution in Indian catfish *Heteropneustes fossilis* exposed to sub-lethal concentration of hilbeech. *Uttar Pradesh. J. Zool.*, **7**(2): 160-165.
- Goel, K.A., S.D. Sharma and Maya, 1985.** Biochemical and enzymological changes in liver and kidney of *Clarias batrachus* following lithium intoxication. *Arch. Ind. Hyg. Toxicol.*, **36**: 249-253.
- Goodman, J. and A. Rothstein, 1957.** The active transport of phosphate into the yeast cell. *J. Genetic. Physiol.*, **40** : 915-925.

- Gupta, P.K., K. Dhar and S.R. Bawa, 1975.** Effect of malathion and radiation separately and jointly upon rat enzymes in vivo. *Environ. Physiol. Biochem.*, **5**: 49-53.
- Gupta, P.K. and K.V. Sastry, 1981.** Effect of mercuric chloride on enzyme activities in the digestive system and chemical composition of liver and muscles of the cat fish *Heteropneustes fossilis*. *Ecotoxicol. Environ. Saf.*, **5** : 389-400.
- Joshi, U.M. and A.K. Desai, 1981.** Effect of sub-lethal concentration of monocrotophos on acid and alkaline phosphatase activities in the tissue of freshwater fish, *Tilapia mossambica*. *J. Anim. Morphol. Physiol.*, **28** (1/2): 221-228.
- Kendall, M.W. and W.E. Hawkins, 1975.** Hepatic morphology and acid phosphatase localization in the channel catfish (*Ictalurus punctatus*). *J. Fish. Res. Bd. Can.*, **32**: 1459-1464.
- Koundinya, P.R. and R. Ramamurthi, 1981.** Effect of organophosphorus pesticide sumithion on alkaline phosphatase activity of freshwater teleost, *Sarotheredon mossambicus*. *Current science.*, **57**(4): 191.
- Moore, M.N., 1980.** Cytochemical determination of cellular responses to environmental stressors in marine organisms. In: *Biological effects of Marine pollution and the problem of monitoring organisms*. (Eds. McIntyre, A.D. and Pearce, J.B.). *Rapp. P.V. Reun. Con. Int. Explor. Mer.*, **179**: 7-15.
- Moore, M.N., 1982.** Lysosomes and environmental stress. *Mar. Pollute Bull.*, **13**: 42-43.
- Mukhopadhyay, P.K. and P.V. Dehadrai, 1980.** Biochemical changes in the airbreathing cat fish, *Clarius batrachus* (Linn.) exposed to malathion. *Environ. Pollut. (Ser.A.)*, **22**: 149-158.
- Muthukrishnan, J. and M. Senthamizhselvan, 1985.** Phosphomonestreases of *Mesogomphus lineatus* embryo. *Proc. First Indian symp. Odonatol.*, 115-124.
- Nath, J. and L. Butler, 1971.** Acid Phosphatase during development of the black carpet battle *attagenus megatoma* (fab). *Can. J. Biochem.*, **49**: 311-315.
- Natarajan, G.M., D. Mallikaraj, P. Vijayalakshmi, R. Pushparani, N. Parthi and R. Rajalingam, 1998.** Impact of pesticides on the haematoenzymological changes in fish. *Pesticides, Man and Biosphere, 1st Edition* Chap. APH Publishing Corporation, India. **15**: 336-350.
- Nimmi, A.J., 1990.** Review of biochemical methods and other indicators to assess fish health in aquatic ecosystems containg toxic chemical. *J.Great lakes Res.*, **16**: 529-541.
- Oluah, N.S., 1999.** Plasma aspartate amino transferase activity in the cat fish, *Clarias albopunctatus* exposure to sublethal zinc and mercury. *Bulletin of Environmental Contamination Toxicology.*, **63**: 343 – 349.
- Radhakrishnan Nair, C., 2002.** Studies on pesticide- induced changes in chosen fishes. Ph.D. Thesis, Manonmaniam Sundaranar University, Tirunelveli, India.
- Ramana Rao, S.V., B. Padmavathi, K. Sathyavelu Reddy and K. Indira, 1996.** Carbon tetrachloride induces hepatotoxicity in a fresh water fish, *Saratherodon mossambicus*. *Poll. Res.*, **15**(4): 385-387.
- Sastry, K.V. and S.K. Sharma, 1979.** In vivo effect of endrin on three phosphatases in kidney and liver of the fish *Ophiocephalus punctatus*. *Bull. Environ. Contam. Toxicol.*, **21**: 185-189.
- Seth, J.S., H.B. Tewari and P.P. Snoop, 1969.** On distribution pattern of alkaline phosphatase activity and their functional significance amongst the spinal ganglion cells of squirrel. *Acta Neurol (Belga)*, **69** : 51-57.

- Sherekar P.Y., 1986.** *Some physiological aspects of the fish, Channa orientalis exposed to pollutants. Ph. D. Thesis. Amaravathi University, Amaravathi.*
- Sherekar P.S. and K.M. Kulkarni, 1987.** *Studies on acid and alkaline phosphatase activity of methyl parathion exposed fish Channa orientalis (SCH). Uttar Pradesh J. Zool., 7(2): 154-159.*
- Thomas, P.C. and T.L. Murthy, 1976.** *Acid phosphatase activity in a freshwater air breathing fish Heteropneustes fossilis and the effect of certain organic pesticides on it. Indian J. Biochem. Biophys., 13 : 347-349.*
- Venkateshwaralu, P., V.J. Shobha Rani, C. Janaiah and M.S.K. Prasad, 1990.** *Effect of endosulfan and kelthane on haematology and serum biochemical parameters of the teleost, Clarias batrachus (Linn). Indian J. Comp. Anim. Physiol., 8(1): 8-13.*
- Verity, M. and A. Reith, 1967.** *Effects of mercurial compounds on structure linked latency of lysosomal hydrolase. Biochem. J., 105: 685-690.*
- Verma, S.R., S. Rani and R.C. Dalela, 1981.** *Pesticide induced physiological alterations in certain tissues of a fish, Mystus vittatus. Toxicol Lett. (AMST), 9(4): 327- 332.*