

## Histological and biochemical changes in hepatopancreas of fiddler crab, *Uca triangularis* exposed to endosulfan, chlorpyrifos and carbaryl pesticides

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### Abstract

The extensive use of pesticides to control agricultural pests poses a serious threat to organisms of the aquatic environment. Chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels. In the present study the impact of Endosulfan, Chlorpyrifos and Carbaryl on hepatopancreas of Fiddler crab, *Uca triangularis* was studied. Histological alterations and biochemical changes such as acid phosphatase (ACP), alkaline phosphatase (ALP), succinate dehydrogenase (SDH), lactate dehydrogenase (LDH) activities in hepatopancreas had been carried out. The deceased hepatopancreas revealed infiltration, formation of large lumen and disappearance of haemocytes. The LDH and ACP activity was increased in Hepatopancreas while SDH and ALP activity was decreased in treated groups. Overall work concluded that histological biomarkers provide reliable data to discriminate the usage of pesticides which had direct influence on loss of aquatic animals.

**Keywords:** Endosulfan, Hepatopancreas, Alkaline phosphatase, Hematocytes, Infiltration

### 1. Introduction

The extensive use of pesticides to control agricultural pests poses a serious threat to many non-target organisms of the aquatic environment. Pesticides are commonly found in aquatic habitats, including streams, rivers, and ponds, at varying concentrations because of direct overspray, drift, atmospheric transport, agricultural and residential runoff, individual misuse, and improper disposal (Gilliom and Hamilton, 2006) [6]. The deleterious influence of the pesticide causes physiological, biochemical, histological and such other disorders in the animal exposed (Patil *et al.*, 2008) [19].

Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. Biochemical changes induced by heavy metal stress is due to disturbed metabolism manifested by inhibition of enzymes, retardation of growth and reduction in the fecundity and longevity of the organism. Animal exposed to stress conditions alter their physiological status with the help of enzymes. Toxicants like pesticides are known to secrete hyper or hypo level of enzymes (Mortimer, 2000) [15]. Enzymes catalyse physiological reactions by lowering the activation energy level that the reactants (substrates) must reach for the reaction to occur. The effect of toxicants on enzymatic activity is one of the most important biochemical parameters which is affected under stress. When an organ is diseased due to the effect of a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted (Valarmathi and Azariah, 2003) [24].

The crustacean hepatopancreas or digestive gland involves in food absorption, synthesis and secretion of digestive enzymes,

storage of lipids, glycogen and minerals. It is the main organ of reserve and detoxification of xenobiotics and is highly sensitive to physiological and environmental changes (Johnson *et al.*, 1998) [8]. Histopathological examination has been increasingly recognized as a valuable tool for the assessment of the impact of environmental pollutants on aquatic animals (Manosathiyadevan *et al.*, 2012; Chourpagar and Kulkarani, 2014; Paruruckumani *et al.*, 2015) [4, 13, 18]. The present work is undertaken to find out the Histological and Biochemical changes in Hepatopancreas of Fiddler crab, *Uca triangularis* Exposed to Endosulfan, Chlorpyrifos and Carbaryl pesticides.

### 2. Materials and Methods

#### 2.1 Experimental animal

The fiddler crab, *Uca triangularis* (Female & Male) with the carapace length ranging from 2.25 to 2.60 cm and breadth ranging from 3.0 to 3.5 cm was collected from the Pulicat marshy area, Thiruvallur District, Tamil Nadu. The crabs were collected in early morning hours or late evening hours by hand picking and stored in plastic containers and brought alive to the laboratory. The crabs were immediately transferred into experimental containers.

#### 2.2 Selection of Pesticides

Endosulfan is organochlorine insecticide and its Molecular formula is  $C_9H_6Cl_6O_3S$ . Chlorpyrifos is a crystalline organophosphate insecticide. Molecular formula is  $C_9H_{11}Cl_3NO_3PS$ . Carbaryl (1-naphthyl methylcarbamate) is a chemical in the carbamate family used chiefly as an insecticide. Molecular formula:  $C_{12}H_{11}NO_2$ .

### 2.3 Assessment of Median Lethal Concentration (LC<sub>50</sub>)

The mortality was recorded for *Uca triangularis* at 24, 48 72 and 96h exposure to pesticides were corrected for natural response by Abbot's formula (Abbott, 1925) <sup>[11]</sup>.

$$\text{Corrected mortality \%} = \frac{\text{Percentage living in Control} - \text{Percentage living in treatment}}{\text{Percentage living in Control}} \times 100$$

**Table 1:** The LC<sub>50</sub> (24h) values and regression equations for *U. triangularis* treated with pesticides

Pesticides	LC <sub>50</sub> (ppm)	Upper confidence Limits (ppm)	Lower Confidence Limits (ppm)	Regression results	Slope function	R <sup>2</sup>
Endosulfan	1.3	3.14	0.454	Y=1.82x+2.826	0.529	0.9637
Chlorpyrifos	1.723	5.958	0.868	Y=2.03x+3.7943	0.466	0.9524
Carbaryl	0.301	6.517	0.985	Y=3.78x+3.9931	0.259	0.9833

### 2.4 Experimental Design

Sublethal studies are helpful to assess the response of the test organism to stress caused by pesticides. Chronic time course study on the effects of pesticides on *Uca triangularis* were conducted by exposing to sublethal safe concentrations for 24 hours. At the end of the treatment period the Control and treated crabs were dissected and Hepatopancreas were collected for histological and biochemical studies.

### 2.5 Histological analysis

Control and Experimental crabs (3 groups) were dissected at the end of the experimental period (24 hours) and the Hepatopancreatic tissues were fixed in Bouin's fluid, processed and embedded in paraffin wax. Section of 4-6 μm thickness were made and stained in chrome-alum-hematoxylin phloxine (CHP) and eosin. The slides were observed under the light microscope and photomicrographs were taken using a Nikon micro photographic unit (Maharajan *et al.*, 2015) <sup>[18]</sup>.

### 2.7. Biochemical analysis

The effect of pesticides on mitochondrial enzymes such as LDH, SDH were analyzed by following King (1965) and Nachlas *et al.* (1960) Protocols. Acid and alkaline phosphatases were assayed following the procedure adopted by Tenniswood *et al.* (1976) <sup>[10, 16, 23]</sup>.

### 2.8. Statistical Analysis

The data collected was statistical analyzed using SPSS software (Version 15.0). Regression and Analysis of variance (ANOVA) were used to determine the significance of difference among the pesticides.

## 3. Results and Discussion

### 3.1 Histology of Control groups

The histological studies of Hepatopancreas revealed the presence of large number of elongated tubules united together by a connective tissue with outer thin cuticle and inner epithelial lining enclosing a lumen of varying shape and size. The cuticle was deeply stained than the epithelial cells. R-cells with variable numbers of cytoplasmic vacuoles, F-cells with

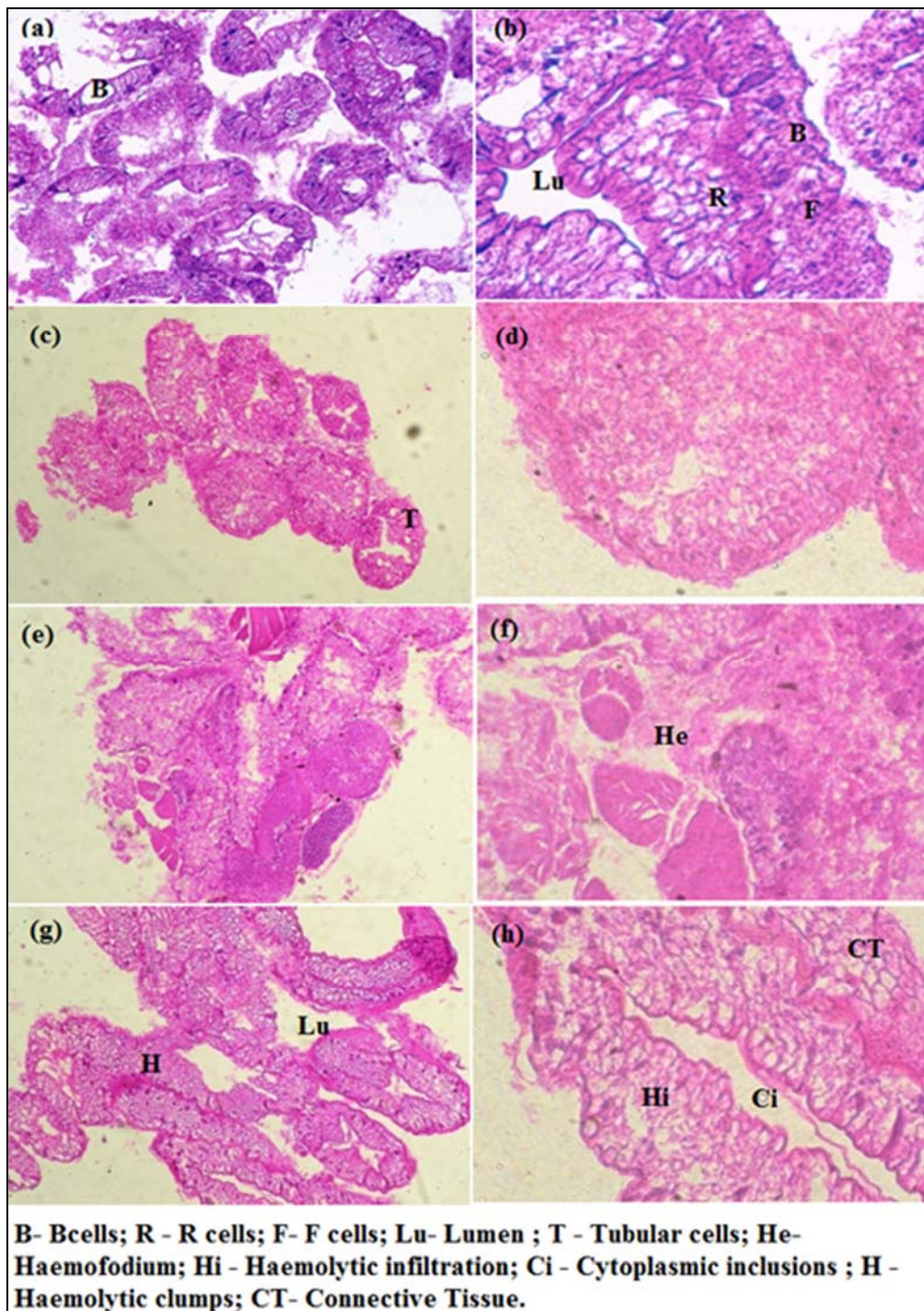
The LC<sub>50</sub> values were obtained by probit regression line, taking test concentration and corresponding percent mortalities on log value and probit scales respectively. (APHA, 1998). Median lethal concentration (LC<sub>50</sub>) of Endosulfan, Chlorpyrifos and Carbaryl for *U. triangularis* was observed for 24h. The LC<sub>50</sub> value for 24h of exposure period of Endosulfan, Chlorpyrifos and Carbaryl was estimated as 1.3 ppm, 1.723 ppm and 0.301 ppm respectively (Table 1).

deeply stained cytoplasm and Mature B-cells bordered the lumen with a vacuolar apical complex and had no apparent connection to the basal lamina. The nucleus was distinct and stained dark. Central vacuole was observed that contained brown material. Each tubule of the hepatopancreas was surrounded by haemal spaces that contained haemolymph and haemocytes. The secretory epithelial cell that lines the lumen secretes the digestive enzymes (Figures 1a & 1b).

### 3.2 Histology of Treated Groups

Kavitha *et al.* (2013) <sup>[9]</sup> reported that Field Crab, *Spiralothelphusa hydrodroma* (Herbst) exposed to Cadmium Nano Particle had abnormal structural changes in the hepatopancreas such as extensive vacuolation, necrotic lamellar lesion formation and haemocytic infiltration. Similarly, hemocytic infiltration in the interstitial sinuses, an increased number of hemocytes, thickening and ruptures of the basal laminae, and necrosis of the tubules were observed in the hepatopancreas of Giant Freshwater Prawn *Macrobrachium rosenbergii* (Li *et al.*, 2007) <sup>[11]</sup>. The results of Maharajan *et al.* (2015) <sup>[18]</sup> showed histological alterations in the hepatopancreas of fresh water crab, *Paratelphusa jacquemontii* (Rathbun) exposed to combination of Chlorpyrifos and Cypermethrin (Nurocombi) insecticide. The deceased hepatopancreas revealed infiltration, formation of large lumen and disappearance of haemocytes.

In the present study the Hepatopancreas of the crabs treated with Endosulfan, Chlorpyrifos and Carbaryl showed prominent changes (Figures 1c, 1d, 1e, 1f, 1g, & 1h). The tubules were not elongated and also not closely united by the connective tissue as found in control. The cells of the tubular epithelium were slightly reduced in length. The lumen size increased and vacuolation was more. Lighter degree of atrophy was observed. The secretory cells were reduced in size and the connective layer was slightly distorted. These histological changes in the hepatopancreas were due to accumulation of the pesticide since this organ is the center of storage, metabolism and detoxification. The rupture of basal laminae observed in the hepatopancreatic tubules suggest that tissue integrity was affected in crab.



**Fig 1:** Hepatopancreas of Fiddler crab, *Uca triangularis* exposed to Endosulfan, Chlorpyrifos and Carbaryl Pesticides

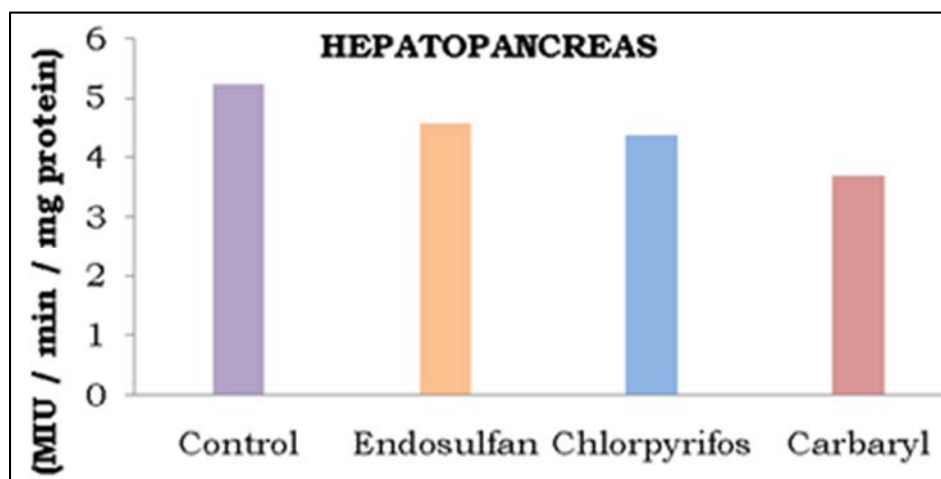
### 3.3 Succinate Dehydrogenase activity

Succinate dehydrogenase enzyme plays an important role in regulating osmoregulation and any change in its activity would disrupt the osmoregulatory mechanism. Sreenivasan *et al.* (2011) [22] reported that the Hepatopancreas and Abdominal Muscle of the Fresh Water Female Field Crab, *Spiralothelphusa hydrodroma* (Herbst) exposed to Cypermethrin had increased LDH activity and reduced SDH activity in sublethal concentration. Narra *et al.* (2012) [17] reported alterations in enzyme activity (increased LDH; decreased SDH and ACP) in nervous tissues of crab.

In the present investigation, in Hepatopancreas of the crabs treated with Endosulfan and Chlorpyrifos, the SDH activity was reduced to 4.58 MIU/min/mg protein, 4.36 MIU/min/mg protein and Carbaryl treated it was further reduced to 3.68 MIU/min/mg protein when compared to the Control group which showed the SDH activity of 5.23 MIU/min/mg protein. Significant ( $P < 0.05$ ) decrease in enzyme activity was in Carbaryl treated groups (Table 2 & Figure 2). The pesticide can bring about distortions in the cell organelles which may inhibit the activity of enzymes.

**Table 2:** Mean and SD of enzyme activities of SDH (MIU /min/mg protein), LDH ( $\mu\text{g}/100\text{ mg}$  wet weight of tissue), ACP and ALP ( $\mu\text{g}$  PNPP to PNP/ $100\text{ mg}$  wet tissue) in the Hepatopancreas of Fiddler crab, *Uca triangularis* exposed to Endosulfan, Chlorpyrifos and Carbaryl pesticides

Experimental Groups	SDH (MIU /min /mg protein)	LDH ( $\mu\text{g} / 100\text{ mg}$ wet weight of tissue)	ACP ( $\mu\text{g}$ PNPP to PNP / 100 mg wet tissue)	ALP ( $\mu\text{g}$ PNPP to PNP / 100 mg wet tissue)
Control	5.23 $\pm$ 0.51	5.56 $\pm$ 0.64	5.51 $\pm$ 0.49	7.16 $\pm$ 0.67
Endosulfan	4.58 $\pm$ 0.48	6.22 $\pm$ 0.47	5.58 $\pm$ 0.49	6.02 $\pm$ 0.54
Chlorpyrifos	4.36 $\pm$ 0.74	6.08 $\pm$ 0.53	6.64 $\pm$ 0.37	5.51 $\pm$ 0.46
Carbaryl	3.68 $\pm$ 0.56	4.99 $\pm$ 0.74	5.88 $\pm$ 0.43	5.38 $\pm$ 0.71
F-Value	14.884	10.389	9.43	15.35
P-Value	0.002	0.005	0.005	0.001



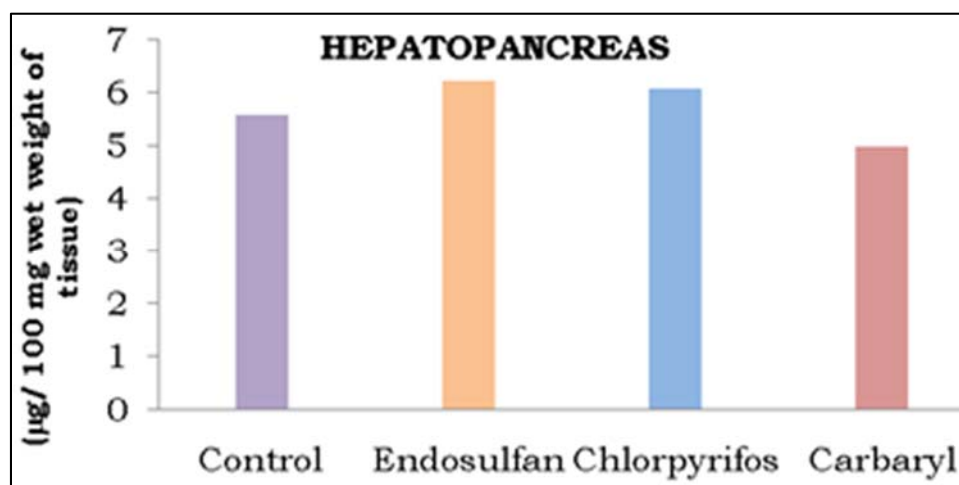
**Fig 2:** Succinate Dehydrogenase (SDH) (MIU/min /mg protein) activity in Hepatopancreas of Fiddler crab, *Uca triangularis* exposed to Endosulfan, Chlorpyrifos and Carbaryl Pesticides

### 3.4 Lactate Dehydrogenase activity

Ramanibai (1986) <sup>[20]</sup> exposed *Scylla serrata*, the estuarine crab, to copper and found that in the muscle, gills and hepatopancreas the activity of succinate SDH was decreased, whereas LDH activity showed an increase. The work of Valarmathi and Azariah (2002) <sup>[25]</sup> indicated that LDH levels were significantly elevated in the muscle, gill and hepatopancreas tissues of the crab *S. quadratum* when exposed to two sublethal concentrations of chlorine.

It was similarly observed in the Hepatopancreas of the experimental crabs, exposed to Endosulfan and Chlorpyrifos

the LDH activity was increased to 6.22  $\mu\text{g}/100\text{ mg}$  wet tissue, 6.08  $\mu\text{g}/100\text{ mg}$  wet tissue respectively while in Carbaryl treated it was reduced to 4.99  $\mu\text{g}/100\text{ mg}$  wet tissue when compared to 5.56  $\mu\text{g}/100\text{ mg}$  wet tissue in the Control crabs. The enzyme activity was statistically significant at  $P < 0.05$  (Table 2 & Figure 3). The increase in LDH activity reflected an increased dependence on anaerobic carbohydrate metabolism by hepatopancreas of the crab, *Uca triangularis* that was exposed to three different pesticides.



**Fig 3:** Lactate Dehydrogenase (LDH) ( $\mu\text{g}/100\text{ mg}$  wet weight of tissue) activity in Hepatopancreas of Fiddler crab, *Uca triangularis* exposed to Endosulfan, Chlorpyrifos and Carbaryl Pesticides

### 3.5 Acid Phosphatase activity

Ahmed *et al.* (1997)<sup>[2]</sup> pointed that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphatase. Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphate to release energy in view of impaired ATPase system during metal stress (Reddy and Bhagyalakshmi, 1994)<sup>[21]</sup>. The results of Kavithal *et al.* (2013)<sup>[9]</sup> reported that ACP and ALP activities was reduced in Cypermethrin, Treated Fresh Water Female Field Crab, *Spiralothelphusa hydrodroma* (Herbst).

In comparison with that, the Hepatopancreas of experimental crabs exposed to Endosulfan, Chlorpyrifos and Carbaryl showed ACP activity of 5.58 µg PNPP to PNP/100 mg wet tissue, 6.64 µg PNPP to PNP/100 mg wet tissue and 5.88 µg PNPP to PNP/100 mg wet tissue when compared with the Control crabs which showed ACP activity of 5.51 µg PNPP to PNP/100 mg wet tissue. The ACP activity was significantly ( $P < 0.05$ ) increased in Chlorpyrifos treated groups. (Table 2 & Figure 4) and reduced in other groups. Increase or decrease in the ACP enzyme activity represents the stress in any organism that results in metabolic burden (Hansen *et al.*, 1992)<sup>[7]</sup>.

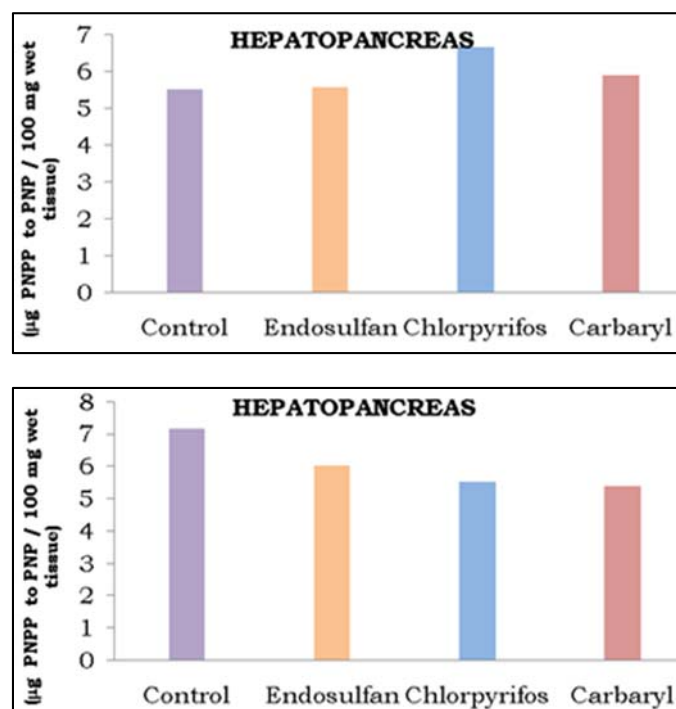


Fig 4: ACP and ALP (µg PNPP to PNP / 100 mg wet tissue) activity in the Hepatopancreas of Fiddler crab, *Uca triangularise* exposed to Endosulfan, Chlorpyrifos and Carbaryl pesticides

### 3.6 Alkaline Phosphatase (ALP) activity

The reports of Mayekar *et al.* (2012)<sup>[14]</sup> showed that Exposure of Sub-Lethal Dose of Nickel produced increased Acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in gills, hepatopancreas and muscle and relatively low SDH activity and high LDH activity were found in the tissues studied. Ahmed *et al.* (1997)<sup>[2]</sup> studied the effect of copper on oxygen consumption and phosphatase in *Scylla serrata* and concluded that there was decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph. Similar

observations were noted in the same crab in response to naphthalene (Elumalai *et al.*, 1998)<sup>[5]</sup>.

In the current study the Hepatopancreas of crabs exposed to Endosulfan, Chlorpyrifos and Carbaryl treatment had ALP activity of 6.02 µg PNPP to PNP/100 mg wet tissue, 5.51 µg PNPP to PNP/100 mg wet tissue and 5.38 µg PNPP to PNP/100 mg wet tissue respectively. However, in Control crabs ALP activity was 7.16 µg PNPP to PNP/100 mg wet tissue. There was significant ( $P < 0.05$ ) decrease in enzyme activity in Carbaryl treated groups. (Table 2 & Figure 4).

### 4. Conclusion

The results of sub lethal exposure of Fiddler crab, *Uca triangularis* to Endosulfan, Chlorpyrifos and Carbaryl pesticides causes histological and biochemical changes. From the results it was concluded that the usage of pesticides in the agricultural practices not only affect the soil but also affect the aquatic ecosystem drastically. Thus the ecological contamination of such toxic chemicals should be prevented to conserve the biodiversity of aquatic organism.

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### 6. References

1. Abbott WS. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*. 1925; 18:265-267.
2. Ahmed MR, Elumalai M, Balasubramanian SE, Balasubramanian MP. Individual and combined effect of copper and chromium on oxygen consumption and phosphatases of a marine edible crab, *Scylla serrata*. *Biochemical Letter* 1997; 55:147-152.
3. APHA. Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C. 17<sup>th</sup> Ed, 1998.
4. Chourpagar AR, Kulkarani GK. Effect of mercuric chloride on gill structure of a freshwater female crab, *Barytelphusa cunicularis* (Westwood). *Journal of Global Biosciences*. 2014; 3(2):423-427.
5. Elumalai M, Balasubramanian SE, Balasubramanian MP. Influence of Naphthalene on protein, carbohydrate and phosphatases system during the vitellogenesis in marine edible crab, *Scylla serrata*. *Bulletin of Environmental Contamination and Toxicology* 1998; 60:25-29.
6. Gilliom RJ, Hamilton PA. Pesticides in the nation's streams and ground water, 1992-2001, A summary fact sheet. Reston: US Geological Survey, 2006.
7. Hansen JI, Mustafa T, Depledge M. Mechanism of copper toxicity in the shore crab, *Carcinus maenas*: Effects Na, K-ATPase activity, haemolymph electrolyte concentrations and tissue water contents. *Marine. Biology* 1992; 114(2):253-257.

8. Johnson DJ, Alexander CG, Yellowlees D. Epithelial cytology and function in the digestive gland of *Thenus orientalis* (Decapoda, Scyllaridae). *Journal of Crustacean Biology*. 1998; 18(12):271-278.
9. Kavitha R, Deepa Rani S, Sivagnanam S, Padmaja M. Cadmium Nanoparticle Induced Histological and Biochemical changes in Hepatopancreas of Mud Crab *Scylla olivacea* (Herbst, 1796). *Journal of Academia and Industrial Research (JAIR)*. 2013; 2(3):205-209.
10. King J. In: Practical clinical enzymology. (Eds.), D. Van Norstand Co London, 1965.
11. Li N, Zhao Y, Yang J. Impact of Waterborne Copper on the Structure of Gills and Hepatopancreas and Its Impact on the Content of Metallothionein in Juvenile Giant Freshwater Prawn *Macrobrachium rosenbergii* (Crustacea: Decapoda). *Archives of Environmental Contamination and Toxicology* 2007; 52(1):73-79.
12. Maharajan A, Narayanasamy Y, Ganapiriya V, Shanmugavel K. Histological alterations of a combination of Chlorpyrifos Chlorpyrifos and Cypermethrin (Nurocombi) insecticide in the fresh water crab, *Paratelpusa jacquemontii* (Rathbun). *The Journal of Basic & Applied Zoology*. 2015; 72:104-112.
13. Manosathiyadevan M, Selvisabhanayakam V. Divya Morphological alterations and biochemical contents of the testis of adult male freshwater prawn *Macrobrachium malcolmsonii*. *Indian Journal of Fundamental and Applied Life Sciences*. 2012; 2(4):104-113.
14. Mayekar V, Pathare M, Afonso G, Mohite VT, Lahir YK, Raut PD. Effects of sub-lethal dose of nickel on the biochemical parameters in the tissues of female crab *Scylla serrata* from Mumbai coast. *Journal of Ecophysiology and Occupational Health*. 2012; 12:69-76.
15. Mortimer MR. Pesticide and trace metal concentrations in Queensland Estuarine crabs. *Marine Pollution Bulletin* 2000; 41(7-12):359-366.
16. Nachlas MM, Margulius SI, Selligman AM. A colorimetric method for the estimation of SDH. *The Journal of Biological Chemistry*. 1960; 235:499-503.
17. Narra MR, Regatte RR, Kodimiyala R. Effects of chlorpyrifos on enzymes as biomarkers of toxicity in Fresh water field crab *Barytelphusa guerini*. *International Journal of environmental sciences*. 2012; 2(4):2015-2023.
18. Paruruckumani PS, Maharajan A, Ganapiriya V, Narayanaswamy Y, Raja Jeyasekar R. Surface ultrastructural changes in the gill and liver tissue of asian sea bass *Lates calcarifer* (Bloch) exposed to copper. *Biological Trace Element Research* 2015; 168:2.
19. Patil C, Paul R, Malkanna. Neuroendocrine regulation and pesticidal impact on freshwater crab, *Barytelphusa guerini*. *Journal of Environmental Biology*. 2008; 29:887-892.
20. Ramanibai PS. Ecotoxicological studies on the coastal ecosystem of Madras, Ph.D. thesis, University of Madras, Tamil Nadu, India, 1986.
21. Reddy PS, Bhagyalakshmi A. Changes in oxidative metabolism in selected tissues of the crab, *Scylla serrata* in response to cadmium toxicity. *Ecotoxicology and Environmental Safety* 1994; 29(3):255-264.
22. Sreenivasan RS, Krishna Moorthy P, Deecaraman M. Cypermethrin Induced Toxicity to phosphatases and dehydrogenases in gills and hemolymph of fresh water crab, *Spiralothelphusa hydrodroma* (Herbst). *International Journal of Biology & Medical Research*. 2011; 2(3):784-788.
23. Tenniswood M, Bind CE, Clark AF. Acid phosphatases androgen dependent markers of rat prostate. *Canadian Journal of Biochemistry*. 1976; 54:340-343.
24. Valarmathi S, Azariah J. Effect of Copper Chloride on the Enzyme Activities of the Crab *Sesarma quadratum* (Fabricius) *Turkish Journal of Zoology*. 2003; 27:253-256.
25. Valarmathi S, Azariah J. Impact of Two Sublethal Concentrations of Copper Chloride and Chlorine on the Excretory Products of Crab *Sesarma quadratum* (Fabricius). *Turkish Journal of Zoology*. 2002; 26:357-361.