World Journal of Environmental Pollution 6 (1): 07-14, 2016
ISSN 2222-1360

DOI: 10.5829/idosi.wjep.2016.07.14

Histological and Biochemical Changes Caused by the Pesticides Endosulfan, Chlorpyrifos and Carbaryl on the Gonads of Fiddler Crab, *Uca triangularis*

[1]*S. Sangeetha and* [2]*S. Deepa Rani*

[1]Department of Zoology, Pachaiyappas College, Chetpet, Chennai, Tamilnadu, India
[2]Department of Advanced Zoology & Biotechnology,
Sir Theagaraya College, Chennai, Tamilnadu, India

Abstract: Ecotoxicity of chemical contaminants has become one of the most critical environmental problems of this century. Pesticides used in agriculture are among the most hazardous chemicals and it reaches the lakes and rivers through agricultural runoff and affecting most of the organisms of the aquatic system. The present work is aimed to determine the toxicity of three pesticides: Endosulfan (1.3 ppm), Chlorpyrifos (1.723 ppm) and Carbaryl (0.301 ppm) on the gondal organs of the Fiddler crab, *Uca triangularis* with the help of histological and biochemical studies. Enlarged cuticular layer, degenerated oocytes, reduced epithelial layer, vacuolization and necrosis were observed in Spermatheca and Ovary. Total protein, carbohydrate and lipids were decreased significantly in pesticides treated groups at sublethal concentration and significantly decreased SDH and ACP activity and increased LDH and ALP activities were observed in Spermatheca and Ovary of *Uca triangularis* on exposure to Endosulfan and Chlorpyrifos and Carbaryl. Changes in the biochemical constituents and altered enzymatic activities indicated the metabolic stress is induced by the pesticides which had affected the reproduction ability and thus affected balance in the Ecosystem.

Key words: Endosulfan · Carbaryl · Chlorpyrifos · Toxicity · Succinate Dehydrogenase

INTRODUCTION

Physicochemical properties of the fresh water determine the diversity of organisms present in the aquatic ecosystem [1]. Polluting the aquatic ecosystem with chemical contaminants had become one of the most critical environmental problems of the century. Industrialization, production and continuous usage of chemicals become the most critical reason for the environmental degradation and thus leading to the pollution [2]. Many freshwater ecosystems facing high levels of xenobiotic chemicals as a result of the pollutants transported from individual areas into the environment [3, 4]. Pesticides used in agriculture practices are the most hazardous chemicals which may reach lakes and rivers through rains and wind, agricultural runoff and affecting most of the organisms of the aquatic ecosystem. The extensive use of pesticides to control agriculture pests poses a serious threat to many non-target organisms of the aquatic environment [5].

Crustaceans were considered as healthy food source known by their high quality protein and less contents of fat. The deleterious influence of the pesticide causes physiological, biochemical, histological and other disorders in the animal exposed. Reproduction plays a vital role in the maintenance of biodiversity and eco-balance in nature. Reproduction is a physiological process and biological need of animals for the continuity of generation and serves to replace population losses due to death and loss of biodiversity.

The present work is aimed to determine the toxic effect caused by three pesticides Endosulfan, Chlorpyrifos and Cabaryl on the gondal organs of the Fiddler crab, *Uca triangularis* with the help of

Corresponding Author: S. Deepa Rani, Research Scholar, PG & Research Department of Advanced Zoology & Biotechnology, Sir Theagaraya College, Chennai - 600021, Tamilnadu, India.
Tel: +91-7358081148.

histological and biochemical studies. In this study, the biochemical constituents such as protein, carbohydrate and lipids and the enzyme activities such as succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) activities of Ovary and Spermatheca were also studied.

MATERIALS AND METHODS

Experimental Animal: The fiddler crab, *Uca triangularis* (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, India. The crabs were collected by hand picking method and acclimatized to the laboratory. Then the crabs were immediately transferred into the experimental containers.

Selection of Pesticides: Three commonly used pesticides such as Endosulfan (organochlorine insecticide), Chlorpyrifos (crystalline organophosphate) and Carbaryl (1-naphthyl methylcarbamate) were chosen for this experimental work.

Median Lethal Concentration (LC_{50}): The acute toxicity tests were conducted in duplicates using 5L experimental containers. The duration of the test was 96h and during the study the experimental crabs were fed. A minimum of 1L water was added for 10 crabs, so that the crabs were half immersed. The water was renewed each day to avoid depletion of dissolved oxygen in the medium. Before renewal of the medium the crabs were transferred to another empty container without handling the animals by carefully slanting the container.

The mortality of *Uca triangularis* was recorded at 24, 48, 72 and 96h exposure to pesticides. The LC_{50} values were obtained by probit analysis [6]. Median lethal concentration (LC_{50}) for 24h exposure period of Endosulfan, Chlorpyrifos and Carbaryl was estimated as 1.3 ppm, 1.723 ppm and 0.301 ppm respectively (Table 1).

Experimental Design: The effects of pesticides on *Uca triangularis* was conducted by exposing the crabs (4 groups containing 10 crabs in each group) to sublethal safe concentrations (Endosulfan - 1.3 ppm, Chlorpyrifos - 1.723 ppm and Carbaryl - 0.301 ppm) for 24 hours. At the end of the treatment period the Control and treated crabs were sacrificed and Spermatotheca and Ovary were collected for the histological and biochemical analysis.

Histological Analysis: To study the histological changes in the Control and Experimental crabs (24hrs), the dissected Spermatotheca and Ovary tissues were fixed in Bouin's fluid, processed and embedded in paraffin wax. Sections were stained in hematoxylin and eosin. The slides were observed and photomicrographs were taken using a Nikon micro photographic unit [7].

Biochemical Analysis: The Protein content of the tissue extracts was estimated by Bradford [8] method using Coomassie Brilliant blue (CCB). The Carbohydrate content of the tissue extracts was estimated by the method of Roe [9] and lipid content was estimated by Folch *et al.* [10].

The effects of pesticides on mitochondrial enzymes such as LDH, SDH were analyzed by following King [11] and Nachlas *et al.* [12] methods. Acid and alkaline phosphatases (ACP and ALP) were assayed by the method of Tenniswood *et al.* [13].

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

RESULTS

Histology

Spermatheca: The Spermatheca of control crabs showed the presence of the outer cuticular, middle muscular and inner epithelial layers that stained well with haematoxylin and eosin. The outer cuticular layer was distinct and stained dark pink in colour. The muscular layer was deeply stained. The epithelial lining of the Spermatheca was clearly recognizable with columnar cells exhibiting dark pink in colour. The lumen of the Spermatheca was filled with free sperm, sperm mass and spermatophores besides the Spermathecal fluid (Figures 1a & 1b).

However the effect of Endosulfan (Figures 1c & 1d), Chlorpyrifos (Figures 1e & 1f) and Carbaryl (Figures 1g & 1h) on the Spermatheca showed differences in the constituent layers and the luminal contents on comparing with that of the control. Among the three layers, the outer cuticular layer was slightly enlarged. The luminal content consisting of granular substances, sperm mass and spermatophores exhibited much difference in shape and size.

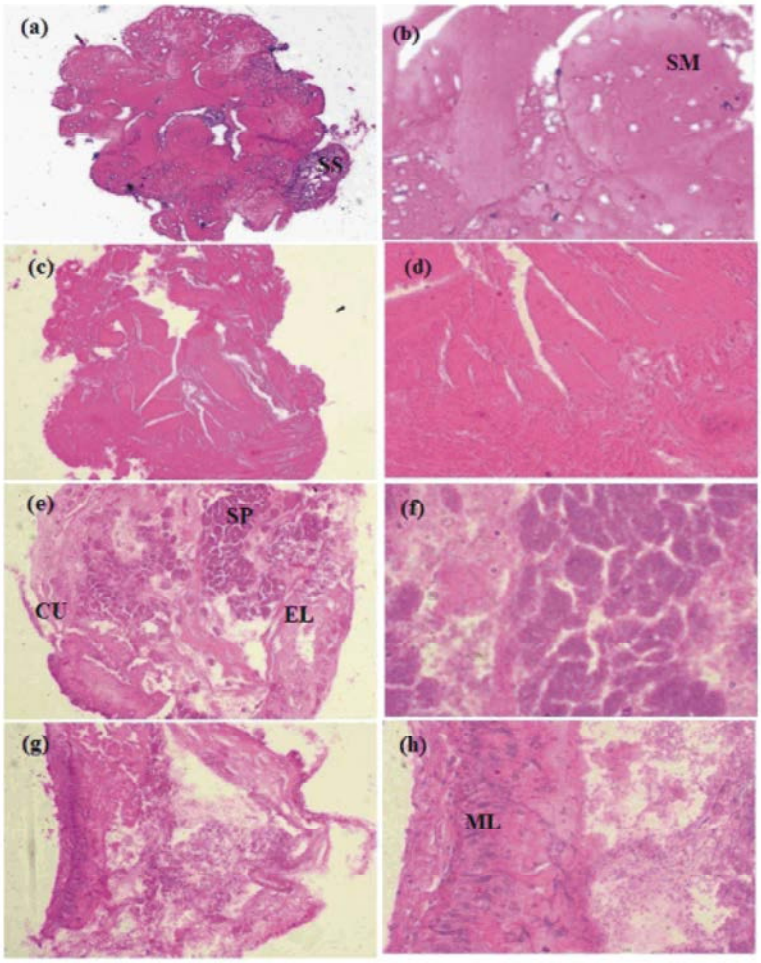

Fig. 1: Histological changes in the Spermatheca of control and Endosulfan, Chlorpyrifos and Carbaryl exposed Fiddler crab, *Uca triangularis*

Ovary: The Ovary of control crabs showed many ovarian lobes covered by a thin connective tissue. A muscular layer was present below the connective tissue and it was followed by germinal epithelial tissue. The germinal epithelium was a reticular mesh, radiates from the centre towards the periphery of ovarian lobe, finally merging with outer layers. The developing oocytes from the germinal epithelium were found to emerge from the centre of each ovarian lobe. At the advanced stage of ovarian development, the oocytes were larger in size and were almost spherical in shape. The ooplasm was heavily laden with yolk granules. The nucleus was distinct with one or two darkly staining nucleolus. The follicle cells were not found. The ooplasm was completely free from the homogenous refractile condition. The yolk globules and granules stained dark red or pink. The radiating germinal epithelial fibres were reduced at mature stage (Figures 2a & 2b).

The ovaries which were soft, shiny, lobulated and dark yellow in colour provided with dense chromatophores in the control groups showed changes by exhibiting faint colour and possession of the chromatophores, turgid condition of the Ovary and also in collapsible nature. The cross section showed moderate enlargement and distention of the oocytes. The outer connective layer was slightly reduced. Small vacuoles were observed in the ooplasm. Moderate necrosis was observed in the oocyte (Figures 2c & 2d, 2e & 2f, 2g & 2h).

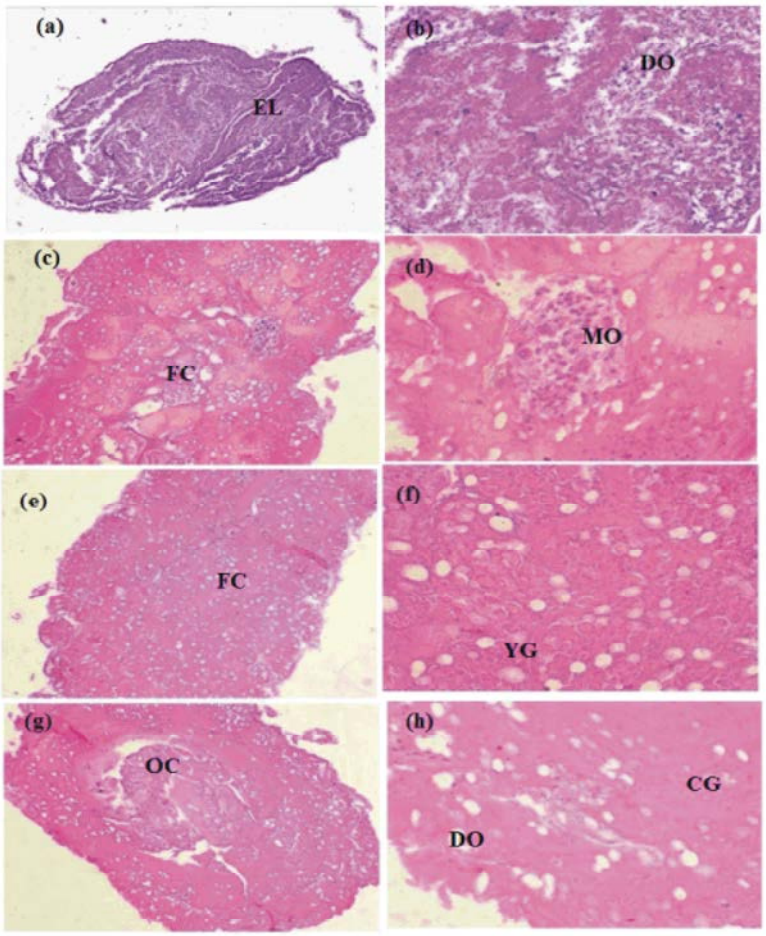

Fig. 2: Histological changes in the Ovary of control and Endosulfan, Chlorpyrifos and CArbaryl exposed Fiddler crab, *Uca triangularis*

Total Protein Content: In the Spermatheca of the control crabs, the protein content was 63.63 mg/g wet weight of tissue whereas the crabs treated with Endosulfan, Chlorpyrifos and Carbaryl the protein level was 60.65, 58.08 and 52.83 mg/g wet tissue. In 24h of exposure maximum decrease was observed in spermatheca of Carbaryl treated groups which was statistically significant (P=0.001).

The protein content of the Ovary of control crabs was 82.25 mg/g wet weight of tissue. In the experimental crabs, the protein level was reduced to 70.71, 67.83 and 55.27 mg/g wet weight of tissue in Endosulfan, Chlorpyrifos and Carbaryl groups respectively. Statistically significant (P = 0.001) decrease was observed in 24h of Carbaryl treatment (Table 2).

Total Carbohydrate Content: In the control crabs, the carbohydrate content of Spermatheca was 10.65 mg/g wet weight of tissue while in the crabs treated with Endosulfan, Chlorpyrifos the carbohydrate content was reduced to 9.22, 7.75 mg/g wet weight of Spermatheca and in Carbaryl treated groups it was further reduced to 5.87 mg/g wet weight of tissue. The significant (P=0.001) decrease in carbohydrate content was in Carbaryl for 24h of exposure period.

The carbohydrate content of the Ovary of the control crabs was 15.21 mg/g wet weight of Ovary. In the Ovary of crabs treated with Endosulfan, Chlorpyrifos and Carbaryl, the carbohydrate content was reduced to 13.74, 12.63 and 9.36 mg/g wet weight. The maximum decrease was observed in Ovary of Carbaryl treated crabs and it was statistically significant at P=0.003 (Table 2).

Table 1: The LC_{50} values (24h) and regression equations for *U. triangularis* treated with pesticides

Pesticides	LC_{50} (ppm)	Upper confidence Limits (ppm)	Lower Confidence Limits (ppm)	Regression results	Slope function	R^2
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

Table 2: Total Protein, Total Carbohydrate and Total Lipids (Mean ±S.D) in the Control and Endosulfan, Chlorpyrifos and Carbaryl treated Fiddler Crab, *Uca triangularis*

	Total Protein (mg/g)		Total Carbohydrate (mg/g)		Total Lipid (mg/g)	
Eperimental Groups	Spermatheca	Ovary	Spermatheca	Ovary	Spermatheca	Ovary
Control	63.63±0.60	82.25±0.57	10.65±0.36	15.21±0.78	25.99±1.22	70.15±1.02
Endosulfan	60.65±1.29	70.71±2.07	9.22±0.76	13.74±0.81	22.72±1.01	67.87±1.78
Chlorpyrifos	58.08±1.16	67.83±1.38	7.75±0.51	12.63±0.96	18.61±1.13	64.27±1.06
Carbaryl	52.83±1.31	55.27±1.05	5.87±0.59	9.36±0.79	16.56±1.08	62.1±1.08
F- Value	18.9	17.2	10.636	11.832	17.659	12.762
Sig.	0.001	0.001	0.001	0.003	0.001	0.002

Table 3: Levels of SDH, LDH, ACP and ALP activities (Mean ±S.D) in the Control and Endosulfan, Chlorpyrifos and Carbaryl treated Fiddler Crab, *Uca triangularis*

	SDH (MIU /min /mg protein)		LDH (µg / 100 mg)		ACP (µg PNPP to PNP / 100 mg)		ALP (µg PNPP to PNP / 100 mg)	
Eperimental Groups	Spermatheca	Ovary	Spermatheca	Ovary	Spermatheca	Ovary	Spermatheca	Ovary
Control	7.71±0.95	3.27±0.71	5.22±0.47	5.11±0.50	5.31±0.44	6.84±0.72	2.97±0.51	5.34±0.46
Endosulfan	6.04±1.07	3.15±0.32	4.37±0.58	5.30±0.46	6.59±0.53	6.67±0.76	3.15±0.32	6.07±0.71
Chlorpyrifos	4.04±0.77	2.97±0.51	4.12±1.18	6.98±0.76	6.16±0.71	5.67±0.31	3.57±0.60	6.52±0.32
Carbaryl	3.43±0.50	2.77±0.42	3.62±0.69	3.67±0.49	5.70±0.58	5.11±0.21	3.27±0.71	5.48±0.42
F- Value	16.777	12.114	15.732	15.442	9.114	10.127	15.965	8.867
Sig.	0.002	0.003	0.001	0.001	0.005	0.004	0.001	0.002

Total Lipid Content: From the biochemical results, the lipid content of Spermatheca of the control crabs was 25.99 mg/g wet weight of tissue. The lipid content was reduced in the Endosulfan, Chlorpyrifos and Carbaryl treated crabs to the level of 22.72, 18.61 and 16.56 mg/g wet weight of Spermatheca and it was statistically significant (P=0.001).

The Ovary of control crabs the lipid content was 70.15 mg/g wet weight of Ovary whereas in the crabs treated Endosulfan, Chlorpyrifos and Carbaryl, the lipid content was 67.87, 64.27 and 62.1 mg/g wet weight of Ovary respectively. Significant (P=0.002) decrease in lipid content was observed in Carbaryl treated groups (Table 2).

Succinate Dehydrogenase Activity: In the control crabs, the mean SDH activity in the Spermatheca was 7.71 MIU/min/mg protein. In the crabs exposed with Endosulfan, Chlorpyrifos the SDH activity was reduced to 6.04, 4.04 MIU/min/mg protein and further reduced to 3.43 MIU/min/mg protein when treated with Carbaryl. Statistically significant (P=0.002) decrease in the SDH activity was observed in Carbaryl treated groups.

The Ovary of *U. triangularis* treated with Endosulfan, Chlorpyrifos and Carbaryl revealed the SDH activity of 3.15, 2.97 and 2.77 MIU/min/mg protein when compare to the control crabs with 3.27 MIU/min/mg protein SDH activity. The decrease in SDH activity was statistically significant (P=0.003) in treated groups (Table 3).

Lactate Dehydrogenase Activity: Spermatheca of the control crabs had the mean LDH activity of 5.22 µg/100 mg wet tissue. In the Endosulfan, Chlorpyrifos and Carbaryl treated has LDH activity of 4.37, 4.12 and 3.62 µg/100 mg wet tissue and the LDH activity was significantly (P=0.001) decreased in treated groups.

In the Ovary of control LDH activity was 5.11 µg/100 mg wet tissue and Endosulfan, Chlorpyrifos exposed crabs, the LDH activity increased to 5.30, 6.98 µg/100 mg wet tissue and in Carbaryl exposed crabs the LDH activity decreased to 3.67 µg/100 mg wet tissue and it was statistically significant at P=0.001 (Table 3).

Acid Phosphatase Activity: The ACP activity of Spermatheca of control crabs was 5.31 µg PNPP to

PNP/100 mg wet tissue. In the crabs treated with Endosulfan, Chlorpyrifos and Carbaryl, the ACP activity was 6.59, 6.16 and 5.70 µg PNPP to PNP/100 mg wet tissue. The enzyme activity was significantly increased at P=0.005.

In the Ovary of control crabs ACP activity was 6.84 µg PNPP to PNP/100 mg wet tissue. In Endosulfan, Chlorpyrifos and Carbaryl exposed crabs, the ACP activity was 6.67, 5.67 and 5.11 µg PNPP to PNP/100 mg wet tissue. The decrease in ACP activity was statistically significant (P=0.004) (Table 3).

Alkaline Phosphatase Activity: In the Spermatheca of the control crabs, the mean ALP activity was 2.97 µg PNPP to PNP/100 mg wet tissue. In Endosulfan, Chlorpyrifos and Carbaryl treated crabs the enzyme activity was 3.15, 3.57 and 3.27 µg PNPP to PNP/100 mg wet tissue. The enzyme activity was statistically significant at P=0.001.

The ALP activity was found to be 5.34 µg PNPP to PNP/100 mg wet tissue of Ovary of control crabs. In the crabs treated with Endosulfan, Chlorpyrifos and Carbaryl the ALP activity was increased to 6.07, 6.52 and 5.48 µg PNPP to PNP/100 mg wet tissue. There was statistically significant (P=0.002) increase in enzyme activity (Table 3).

DISCUSSION

Histological changes provides an early indication of pollution hazard and also useful data on nature and degree of damage to cells and tissues [14]. Chourpagar and Kulkarni [15] observed histological changes in the tissues of freshwater female crab, *Barytelphusa cunicularis* when exposed to heavy metal pesticides.

Shinde *et al.* [16] identified the rupturing of membrane in the oocytes, Vacuolization in the peripheral oocytes and disturbances in the supporting connective tissue after acute and chronic exposure of sugar industrial effluent in crab *Barytelphusa gurini.* The results of Sakhare and Kamble, [17] showed that ovarian cells of freshwater crab, *Barytelphusa cunicularis* affected with water contaminant and had changes in reproductive capacity of organism. Water with physicochemical changes caused changes in the histopathology on developing oocytes under stressful conditions. The Ovary of the Brackish water fiddler crab *Uca annulipes* exposed to Cadmium showed degeneration of oocytes, vacuolization, damage in epithelial layers which resulted in declined reproductive activity [18]. The reports of Shaikh *et al.* [14] also supported these histological changes. In the present study luminar content consisting of granular substances, sperm mass and spermatophores exhibited much difference. In the Ovary, the outer connective layer slightly reduced and vacuolization and Moderate necrosis were observed in the oocyte indicating declined reproductive activity in *Uca triangularis* when exposed to different pesticides.

The freshwater crabs *Barytelphusa cunicularis* exposed to sublethal concentration of mercuric chloride showed decrease in protein, glycogen and lipid content in Ovary and Spermatotheca which was due to increased glycogenolysis, glycolysis, proteolysis and lipolysis under stress to meet increase energy demands for survival [19]. The results of Sekar *et al.* [20] showed that toxicity of the textile dye industry effluent (TDIE) on the crab *Spiralothelphusa hydrodroma* (Herbst) and decrease in Protein, carbohydrate and lipid content in different tissues (Ovary, Spermatheca, hepatopancreas, muscle, gills, brain, thorasic ganglia and eyestalk) studied.

In the present study the fiddler crab, *Uca triangularis* was treated with Endosulfan, Chlorpyrifos and Carbaryl and revealed a significant decrease in total protein, carbohydrate and lipid content in experimental crabs exposed to Carbaryl. This might be due to the direct effect of the pesticide on protein metabolism or due to enhanced proteolytic activity as a consequence of increased metabolic demands following exposure to the toxic stress of pesticides. The decrease in the total carbohydrate content might be due to the rapid utilization to meet the energy demands. Decreased in carbohydrate content might indicate an immediate utilization to meet the excess demand of energy under toxic stress. This condition happened by rapid glycogenolysis and inhibitions of glycogenesis through activation of glycogen phosphorylase and depletion of glycogen transferase respectively or through stress induced increase in catacholamines. The decrease in lipid content was due to the accelerated hydrolysis of lipid in order to cope with stress induced energy demand.

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. Stress exerted by exposure of freshwater crabs *paratelphusa (Barytelphusa) jacquemontii* to insecticide Endosulfan drained into water bodies had altered activities of enzyme constituents, which indicated the significant influence of toxic nature of this insecticide to crabs [21]. The results of Chourpagar and Kulkarni [22] showed elevation of LDH Activity in the Ovary, gills, hepatopancreas, muscle and Spermatheca of Freshwater Crab, *Barytelphusa cunicularis* (Westwood) treated with

copper sulphate suggesting bias towards the anaerobic glycolytic pathway. The results of Mayekar *et al.* [23] had revealed that low SDH activity and high LDH activity in the gills, hepatopancreas and muscle tissues of Female Crab *Scylla serrata* exposed to Nickel and the higher values appeared to be the probable cause of cellular damage and physiological stress. Decreased SDH activity and increased LDH activity was observed in *Uca triangularis*, treated with Endosulfan, Chlorpyrifos and Carbaryl. This might be due to the physiological disturbances caused by the pesticide which affects enzyme activity. The pesticide can bring about distortions in the cell organelles which may inhibit the activity of enzymes. The non-availability of oxygen, inhibition of SDH and simultaneous elevation of LDH may suggest a bias towards the anaerobic glycolytic pathway.

Mayekar *et al.* [23] had reported that exposure of nickel on the Ovary of Female Crab *Scylla serrata* showed increased ACP and ALP activity. The results of Narra *et al.* [24] showed increased ACP activity and decreased ALP activity in the tissue of *Barytelphusa guerini,* exposed to Chlorpyrifos. Similar reports were observed in fresh water crab, *Spiralothelphusa hydrodroma* treated with the Cypermethrin [25]. Patil *et al.* [21] also reported elevated level of ACP and ALP activity in the different tissues of freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to Malathion. Increased ALP and decreased ACP activity in Spermatheca and Ovary of *Uca triangularis* on exposure to Endosulfan and Chlorpyrifos and Carbaryl was observed. The increase in ACP activity was inferred as an altered metabolism due to stress induced by the toxic pesticides present in the aquatic eco system.

CONCLUSION

The overall results concluded that usage of pesticides affect the aquatic organisms which will lead to the loss of animal population in that aquatic environment. So the usage of pesticides in the agricultural practices should be altered and proper remedial measures taken to avoid the release of pesticides into the aquatic system.

ACKNOWLEDGMENT

The authors were thankful to Dr. P. Senthilkumaar, Associate Professor and HOD, PG & Research Dept of advanced Zoology & Biotechnology and Management of Sir Theagaraya College, Chennai – 600021 and to Dr. R. Arasu, Principal, University of Madras, Arts and Science College, Nemmeli, Chennai for his constant support throughout this work.

Conflict of Interest: There is no conflict of interest.

REFERENCES

1. Venkatesharaju, K., P. Ravikumar, R.K. Somashekhar and K.L. Prakash, 2010. Physicochemical and bacteriological investigation on the river Cauvery of Kollegal stretch in Karnataka. Kathmanadu University. Journal of Science, Engineering and Technology, 6: 10-15.
2. Jothinarendiran, N., 2012. Effect of dimethoate pesticide on oxygen consumption and gill histology of the fish, *Channa punctatus*. Current Biotica, 5: 500-507.
3. Brack, W., K. Schirmer, T. Kind, S. Schrader and S. Schuurmann, 2002. Effect-directed fractionation and identification of cytochrome P450A-inducing halogenated aromatic hydrocarbons in contaminated sediment. Environ. Toxicol. Chem., 21: 2654-2662.
4. Diez, S., M. Abalos and J.M. Bayona, 2002. Organotin contamination in sediments from the Western Mediterranean enclosures following ten years of TBT regulation. Water Res., 36: 905-918.
5. Warren, C.E., 1971. Biology and water pollution control. W.B. Saunders Co. Philadelphia.
6. APHA (American Public Health Association), 1992. Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C. 18th Ed, pp: 1200.
7. Maharajan, A., Y. Narayanasamy, V. Ganapiriya and K. Shanmugavel, 2015. Histological alterations of a combination of Chlorpyrifos and Cypermethrin (Nurocombi) insecticide in the fresh water crab, *Paratelphusa jacquemontii* (Rathbun). The J. Bas. Appl. Zoo., 72: 104-112.
8. Bradford, M.M., 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing principle of protein dye binding. Anal. Biochem., 72: 248-254.
9. Roe, J.R., 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem., 20: 335-343.
10. Folch, J., S.M. Lee and G.H. Slone-Stanley, 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-508.

11. King, J., 1965. In: Practical clinical enzymology. (Eds.), D. Van Norstand Co., London.
12. Nachlas, M.M., S.I. Margulius and A.M. Selligman, 1960. A colorimetric method for the estimation of SDH. J. Biol. Chem., 235: 499-503.
13. Tenniswood, M., C.E. Bind and A.F. Clark, 1976. Acid phosphatases androgen dependent markers of rat prostate. Can. J. Biochem., 54: 340-343.
14. Shaikh, F.I., I.R. Ustad and N.T. Ansari, 2010. Effect of heavy metal on the Ovary of freshwater crab, *Barytelphusa cunicularis* (Westwood). Bioscan., 5(2): 335-338.
15. Chourpagar, A.R. and G.K. Kulkarni, 2011. Heavy metal toxicity to fresh water crab *B. cunicularis*. Rec. Res. Sci. Tech., 3(3): 01-05.
16. Shinde, V.R., M.U. Vcersh and R.S. Kulkarni, 2002. Ovarian changes in response to heavy metals exposure to the fish *Notopterus notopterus* (Pallas). J. Environ. Biol., 23(2): 137-141.
17. Sakhare, S.S. and N.A. Kamble, 2014. Aquatic Contamination: Histopathology of Female Gametocytes in *Barytelphusa cunicularis*. Univ. J. Environ. Res. Tech., 4(2): 155-164.
18. Suresh, V., A. Palraj and M. Deecaraman, 2015. Histopathological changes in the Ovary of the brackish water fiddler crab (*Uca annulipes*) exposed to cadmium. J. Glob. Biosci., 4(8): 3037-3043.
19. Chourpagar, A.R. and G.K. Kulkarni, 2013. Effect of Mercuric Chloride on the Biochemical Constituents of Some Tissues of a Freshwater Crab, *Barytelphusa cunicularis* [Westwood, 1836]. Worl. J. Fis. Mar. Sci., 5(6): 598-602.
20. Sekar, P., S. Hari Prasad and M. Decca Raman, 2009. Effect of Textile Dye Industry Effluent on the Nutritive Value of Fresh Water Female Crab *Spiralothelphusa hydrodroma* (Herbst). J. Appl. Sci. Res., 5(11): 2041-2048.
21. Patil, V.N., G.H. Kadam and P.D. Raut, 2014. Enzymological alterations produced by chronic malathion exposure in freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii*. Int. J. Sci, Environ. Tech., 3(1): 303-313.
22. Chourpagar, A.R. and G.K. Kulkarni, 2009. Toxic Effect of Copper Sulphate on Lactate Dehydrogenase Activity in a Freshwater Crab, *Barytelphusa cunicularis* (Westwood). World Journal of Zoology, 4(3): 180-183.
23. Mayekar, V., M. Pathare, G. Afonso, V.T. Mohite, Y.K. Lahir and P.D. Raut, 2012. Effects of sub- lethal dose of nickel on the biochemical parameters in the tissues of female crab *Scylla serrata* from Mumbai coast. J. Ecophysiol. Occup. Helth, 12: 69-76.
24. Narra, M.R., R.R. Regatte and R. Kodimyala, 2012. Effects of Chlorpyrifos on enzymes as biomarkers of toxicity in Fresh water field crab *Barytelphusa guerini*. Int. J. Environ. Sci., 2(4): 2015-2023.
25. Sreenivasan, R.S., P. Krishna Moorthy and M. Deecaraman, 2011. Cypermethrin Induced Toxicity to phosphatases and dehydrogenases in gills and hemolymph of fresh water crab, *Spiralothelphusa hydrodroma* (Herbst). Int. J. Biol. Med. Res., 2(3): 784-788.