ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 02, pp.26508-26514, February, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IMPACT OF ENDOSULFAN, CHLORPYRIFOS AND CARBARYL PESTICIDES ON FIDDLER CRAB, *UCA TRIANGULARIS*

[1]Sangeetha, S., [2]Senthilkumaar, P. and *,[2]Deepa Rani, S.

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

ARTICLE INFO

Article History:
Received 25th November, 2015
Received in revised form
08th December, 2015
Accepted 23rd January, 2016
Published online 27th February, 2016

Key words:

Bioaccumulation, Pesticides,
Cabaryl, Endosulfan,
Neurosecretory cells, glycogenolysis.

ABSTRACT

Anthropogenic activities were major causes of stress on natural ecosystems. Pesticides based Agricultural activities have been identified as major contributors to environmental stress, which affects all ecosystem components. The present study was conducted to estimate the toxicity of three pesticides such as Endosulfan, Chlorpyrifos and Carbaryl on the Eye stalk, Thoracic ganglion, Gills of the fiddler crab, *Uca triangularis* by histological and biochemical analysis. The LC50 (24h) value for Endosulfan, Chlorpyrifos and Carbaryl was estimated at 1.3 ppm, 1.723 ppm and 0.301 ppm respectively. Experimental group of *Uca triangularis* treated with Endosulfan (1.3ppm), Chlorpyrifos (1.723ppm) and Carbaryl (0.301ppm) showed reduced neurosecretory material and the neurosecretory cells were in distorted condition. The biochemical analysis showed that decrease in protein, carbohydrate, lipid content which was due to enhanced proteolytic activity, glycogenolysis and stress caused by toxicity of pesticides.

Copyright © 2016 Sangeetha et al. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: **Sangeetha, S., Senthilkumaar, P. and Deepa Rani, S. 2016.** "Impact of Endosulfan, Chlorpyrifos and Carbaryl Pesticides on fiddler crab, *UCA triangularis*", *International Journal of Current Research,* 8, (02), 26508-26514.

INTRODUCTION

The bioconcentration and bioaccumulation potentials of pesticides in aquatic organisms are conveniently estimated from rate of pesticide dissipation and accumulation in the aquatic environment which include the amount and type of sediment present in the water and type of diet available to water-dwelling organisms (Katagi, 2010). Extremely hydrophobic pesticides such as the organochlorines and pyrethroids are susceptible to adsorb strongly to dissolve organic matter associated with bottom sediment (Aguiar, 2002). In contrast, sediment dweller may suffer from higher levels of direct exposure to a pesticide, unless it was rapidly degraded in sediment. Hydrophobic pesticides that are expected to be highly stored in tissues would not be bioconcentrated if susceptible to biotic transformation by aquatic organisms (Katagi, 2010, Singare *et al.,* 2010). Crustaceans were consumed by human beings in different forms for their delicacy and as well as for their medicinal benefits. One feature of crabs that makes them more relevant as targets of environmental studies and of regulation was that they may not only become contaminated by pesticides or other chemicals, but they constitute an important part of the human diet (Uttah, 2013). Fiddler crabs were found along sea beaches and brackish inter-tidal mud flats, lagoons and swamps. The fiddler crab, *Uca triangularis* was selected as test animal since the population of these species were on the decreasing side due to their exposures to different routinely used pesticides; Endosulfan, Chlorpyrifos and Carbaryl during agricultural practices. The overall objectives of the present study were to study the histological and nutritional changes caused by three different pesticides; Endosulfan, Chlorpyrifos and Carbaryl on the Eye stalk, Thoracic ganglion, Gills of the fiddler crab, *Uca triangularis* to assess their ecological status on marshy area of Pulicat waters.

***Corresponding author: Deepa Rani, S.**
Department of Advanced Zoology & Biotechnology, Sir Theagaraya College, Chennai, Tamilnadu, India.

MATERIALS AND METHODS

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 fiddler

crab, *Uca triangularis* was chosen for the present study. 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.

Selection of Pesticides

Endosulfan is an 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: $C_{12}H_{11}NO_2$**.**

Acute toxicity studies

The acute toxicity tests were conducted in 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 by carefully slanting the container.

Assessment of Median Lethal Concentration (LC_{50})

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 (Abbot, 1925).

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

The LC_{50} values were obtained by probit regression line, taking test concentration and corresponding percent mortalities on log value and probit scales respectively (APHA, 1992).

Experimental Design

Sublethal studies were helpful to assess the response of the test organism to the stress caused by pesticides. Acute 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 tissues namely, Eye stalk, Thoracic ganglion, Gills were collected for histological and biochemical studies.

Histological analysis

Control and Experimental crabs (3 groups) were dissected at the end of the experimental period (24 hours) and the tissues (Eye stalk, Thoracic ganglion and Gills) 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) for the neurosecretory cells and eosin. The slides were observed under the light microscope and photomicrographs were taken using a Nikon micro photographic unit (Maharajan *et al.,* 2015).

Biochemical analysis

The protein content in the tissue extracts was estimated by Bradford (1976) method using Coomassie Brilliant blue (CCB). The carbohydrate content in the extracts was estimated as per the method of Roe (1955) and lipid content was estimated by Folch *et al*. (1957).

Statistical Analysis

The data collected 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

Median lethal concentration (LC_{50})

Median lethal concentration (LC_{50}) of Endosulfan, Chlorpyrifos and Carbaryl for *U. triangularis* was observed for 24h. The LC_{50} 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. The values of LC_{50} regression result of Endosulfan, Chlorpyrifos and Carbaryl on *U. triangularis* were given in Table 1.

Histology of different tissues in *U. triangularis*

Control

Eye stalk, Thoracic ganglia of the control crabs showed darkly stained neurosecretory cells and the cytoplasm contains more amounts of neurosecretory materials (Figures 1a & 1b; Figures 2a & 2b). Each gill plate lined with cuticle enclosing within it a single layer of cell or gill epithelium stained well. The central axis contained haemocytes and fixed nephrocytes which was highly vacuolated and contains small quantities of pale brown materials (Figures 3a & 3b).

Treated Groups

Endosulfan, Chlorpyrifos and Carbaryl treated crabs showed changes in the Eye stalk, Thoracic ganglia tissues when compared to control. The neurosecretory cells were moderately stained, distorted and neurosecretory material was reduced (Figures 1c & 1d, 1e & 1f, 1g& 1h ; Figures 2c & 2d, 2e & 2f, 2g& 2h). Gill tissues showed thickening of gill epithelium and gill lamellae were deeply stained with extensive vacuolation of the Gills. Cell distortion was observed with shrinked, granulated haemocytes and nephrocytes with reduced brown material (Figures 3c & 3d, 3e & 3f, 3g& 3h).

Total Protein Content

The total protein content in the Eye stalk of the Control crabs was 23.31 mg/g wet weight of tissue. In Endosulfan (1.3 ppm), Chlorpyrifos (1.723 ppm), Carbaryl (0.301 ppm) treated groups, the protein levels found significantly ($P<0.05$) decreased as 20.56 mg/g, 19.20 mg/g and15.25 mg/g wet weight of tissue respectively (Table 2 & Figure 4).

Table 1. The LC_{50} (24h) values 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 content (Mean±SD) in different tissues of *U. triangularis* (mg / g wet weight of tissue)

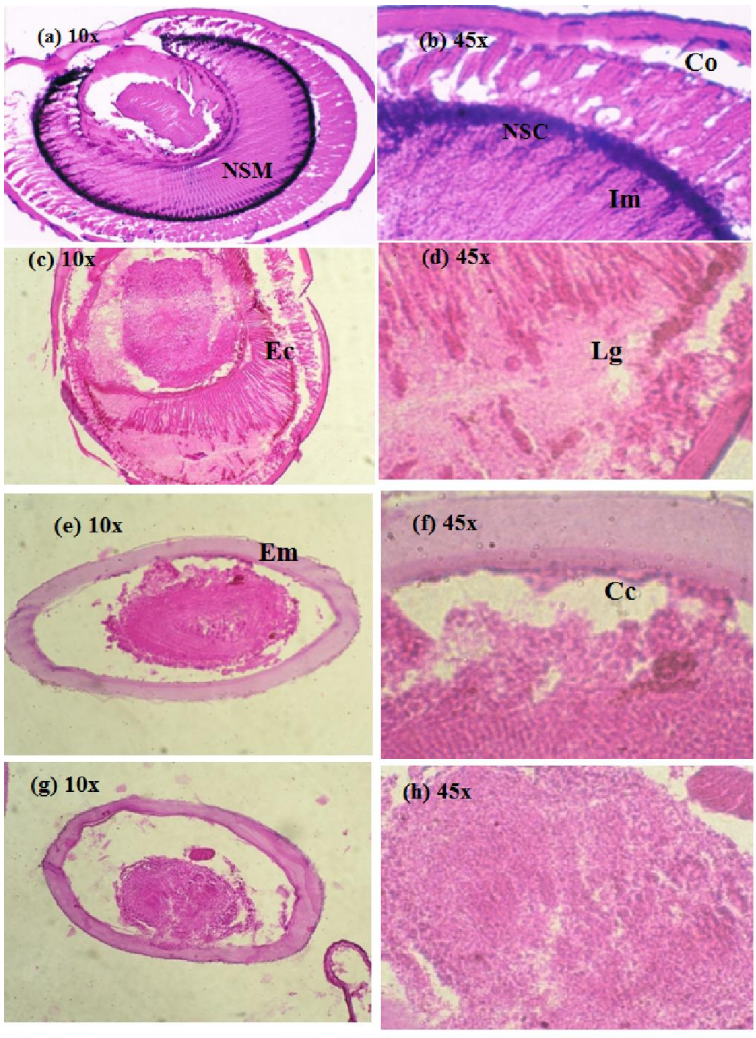
Tissues	Control	Endosulfan	Chlorpyrifos	Carbaryl	F-value	P-value
Eye stalk	23.31±0.80	20.56±0.71	19.20±0.98	15.25±0.60	13.4	0.003
Thoracic ganglia	60.15±0.91	57.36±1.08	53.83±0.79	48.55±0.87	12.5	0.003
Gills	58.12±0.90	55.76±1.05	52.87±1.32	49.55±0.93	16.2	0.001

Table 3. Total Carbohydrate content (Mean±SD) in different tissues of *U. triangularis* (mg / g wet weight of tissue)

Tissues	Control	Endosulfan	Chlorpyrifos	Carbaryl	F-value	P-value
Eye stalk	4.04±0.77	3.43±0.50	2.75±0.44	2.56±0.64	16.776	0.001
Thoracic ganglia	12.31±0.80	11.63±0.98	10.36±1.07	9.03±1.11	15.858	0.001
Gills	8.71±0.57	7.75±0.81	6.77±0.53	5.98±0.79	18.6	0.001

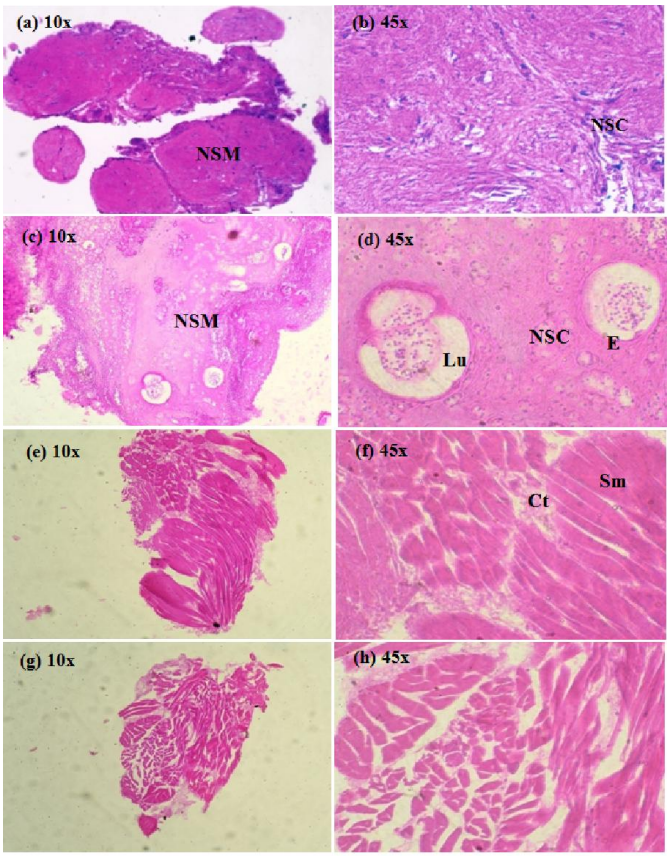
Table 4. Total lipid content (Mean±SD) in different tissues of *U. triangularis* (mg / g wet weight of tissue)

Tissues	Control	Endosulfan	Chlorpyrifos	Carbaryl	F-value	P-value
Eye stalk	5.89±0.32	5.38±0.71	4.94±0.43	3.68±0.91	17.266	0.001
Thoracic ganglia	27.92±1.27	24.83±0.96	23.16±1.07	20.22±1.28	13.711	0.002
Gills	7.75±0.72	6.64±0.37	5.34±0.46	4.47±0.51	16.815	0.001



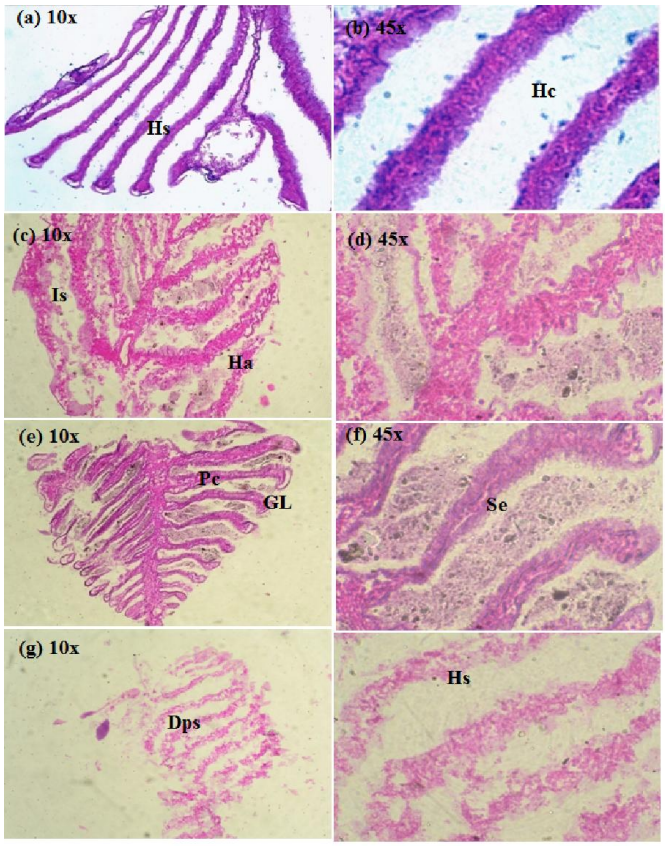
NSM - Neuro Secretory material; NSC- Neuro Secretory cells, Co-Corneal cuticle ; Im - Internal medulla; Ec - External Chaisma; Lg - Lamina ganglionaris; Em - External medulla; Cc- Crystaline cones;

Figure 1. Histological changes observed in Eye Stalk of Control, Endosulfan, Chlorpyrifos and Carbaryl Pesticides on Fiddler Crab, *Uca triangularis*



NSM - Neuro Secretory Materials; NSC - Neuro Secretory cells; Lu- Lumen; E - Epithelium; Ct - Connective Tissue ; Sm- Striated muscles

Figure 2. Histological changes observed in Thoracic ganglia of Control, Endosulfan, Chlorpyrifos and Carbaryl Pesticides on Fiddler Crab, *Uca triangularis*



Hs - Haemal space; Hc- Haemal canal; Is - Intra lamellar space; Ha- Haematocytes; Pc - Pillar cells; GL - Gill Lamellae; Se - Squamous Epithelia; Dps - Degenerated pillar cells

Figure 3. Histological changes observed in Gills of Control, Endosulfan, Chlorpyrifos and Carbaryl Pesticides on Fiddler Crab, *Uca triangularis*

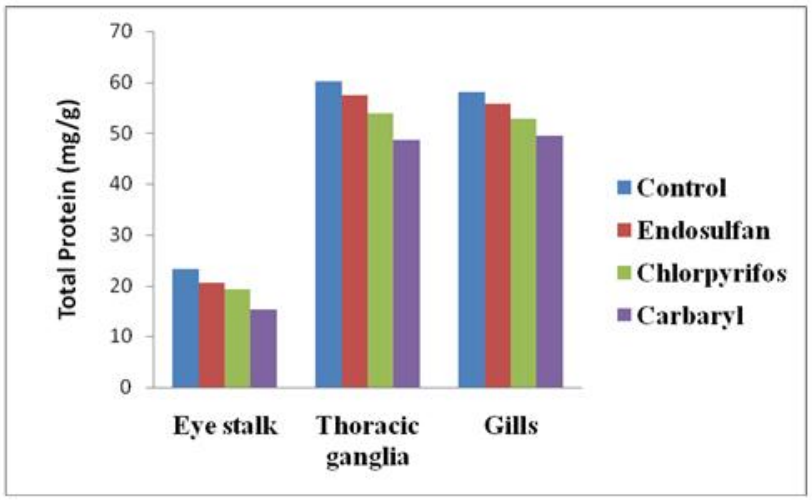


Figure 4. Total Protein content in different tissues of Control, Endosulfan, Chlorpyrifos and Carbaryl treated Fiddler Crab, *Uca triangularis*

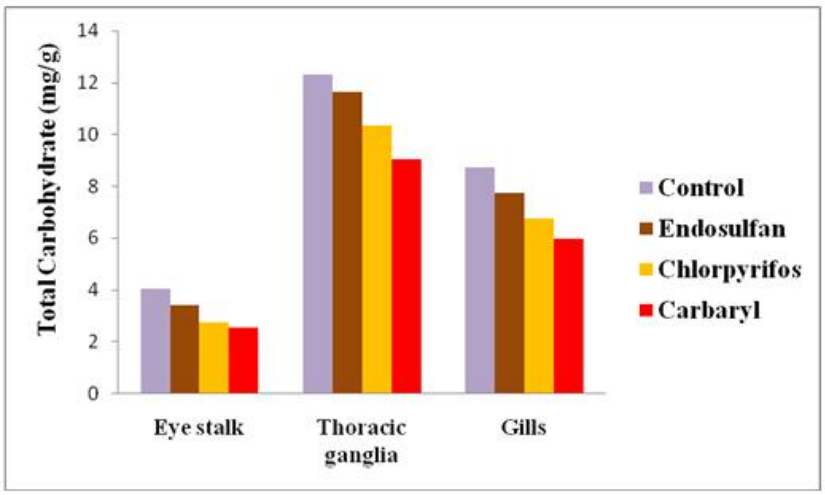


Figure 5. Total Carbohydrate content in different tissues of Control, Endosulfan, Chlorpyrifos and Carbaryl treated Fiddler Crab, *Uca triangularis*

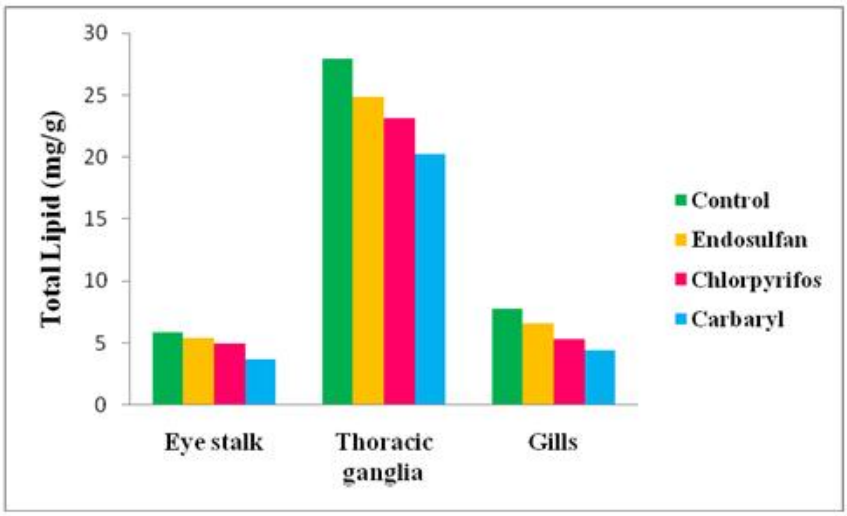


Figure 6. Total Lipid content in different tissues of Control, Endosulfan, Chlorpyrifos and Carbaryl treated Fiddler Crab, *Uca triangularis*

In Control Thoracic ganglia tissues, 60.15 mg/g wet weight of tissue was observed. In Endosulfan, Chlorpyrifos and Carbaryl treated tissues, the protein content was 57.36 mg/g, 53.83 mg/g and 48.55 mg/g wet weight respectively. The protein level was significantly (P<0.05) reduced in Carbaryl treated crabs (Table 2 & Figure 4). In the Gills of control crabs, the protein content was 58.12 mg/g wet weight of tissue. When treated with Endosulfan, Chlorpyrifos and Carbaryl, the protein content was reduced to 55.76 mg/g, 52.87 mg/g and 49.55 mg/g respectively. The significant (P<0.05) decrease in protein content was observed in Carbaryl treated crabs (Table 2 & Figure 4).

Total Carbohydrate Content

The carbohydrate content of Eye stalk of the control, Endosulfan, Chlorpyrifos and Carbaryl treated crabs was 4.04 mg/g 3.43 mg/g, 2.75 mg/g and 2.56 mg/g wet weight of Eye stalk. In 24h of treatment, significant (P<0.05) decrease was observed in Carbaryl treated crabs (Table 3 & Figure 5). In Endosulfan, Chlorpyrifos and Carbaryl treated crabs, the carbohydrate content of Thoracic ganglia was reduced to 11.63 mg/g, 10.36 mg/g and 9.03 mg/g wet weight of tissue whereas in Control crabs, the carbohydrate content was 12.31 mg/g wet weight of tissue. Statistically significant (P<0.05) decrease observed in 24h of Carbaryl treated crabs (Table 3 & Figure 5). In Gills of the control crabs, the carbohydrate content was 8.71 mg/g wet weight of tissue whereas crabs treated with Endosulfan, Chlorpyrifos and Carbaryl, the carbohydrate content was 7.75 mg/g, 6.77 mg/g and 5.98 mg/g wet weight of Gills respectively. The Carbaryl treated crabs showed significant (P<0.05) decrease in 24h (Table 3 & Figure 5).

Total Lipid Content

The lipid content of Eye stalk of the control crabs was 5.89 mg/g wet weight of tissue. When the crabs were treated with Endosulfan, Chlorpyrifos and Carbaryl, the lipid content was reduced to 5.38 mg/g, 4.94 mg/g was and 3.68 mg/g wet weight of tissue respectively. Statistically significant (P<0.05) decrease of lipid content was observed in Carbaryl exposed crabs (Table 4 & Figure 6). The lipid content of Thoracic ganglia of the Control crabs was 27.92 mg/g wet weight of tissue. While the crabs treated with Endosulfan, Chlorpyrifos and Carbaryl the lipid content was 24.83 mg/g, 23.16 mg/g and 20.22 mg/g wet weight of Thoracic ganglia. The decrease was statistically significant (P<0.05) (Table 4 & Figure 6). In the Gills of crabs treated with Endosulfan, Chlorpyrifos and Carbaryl, the lipid content reduced to 6.64 mg/g, 5.34 mg/g and 4.47 mg/g wet weight of tissue respectively when compared with Control lipid content which was 7.75 mg/g wet weight of Gills. Carbaryl treated crabs showed statistically significant (P<0.05) decrease in lipid content (Table 4 & Figure 6).

DISCUSSION

Pesticides with Chlorpyrifos and Endosulfan were used for pest control on agricultural and creates high-risk inputs in aquatic system. The effect of Carbaryl on the bioenergitics parameters on prawn, *Macrobrachium malcolmsonii* was severe in the highest sub lethal concentration, less in the intermediate concentration and least in the lowest sub lethal concentration (Saravana Bhavan *et al*., 2011). Kumar *et al*. (2010) reported the toxicity of six pesticides (Carbaryl, Chlorpyrifos, cypermethrine, dimethoate, diuron and fenarimal) in the fresh water shrimp, *Paratya australiensis* after 96h exposures. Of the six pesticides tested, cypermethrin was more toxic than Chlorpyrifos > Carbaryl > dimethoate > fenarimol > diuron. The present acute toxicity study showed that among the three pesticides tested, the toxic effect was high with Carbaryl > Endosulfan > Chlorpyrifos based on the sublethal concentration findings. The neurosecretory cells observed in the cross section of Eye stalk, Thoracic ganglia and Gills of the control crab showed darkly stained neurosecretory materials in the cytoplasm than in the experimental groups of *Spiralothelphusa hydrodroma* (Sreenivasan, 2005). Suganthi *et al.* (2015) reported that the gills showed fused lamellae, lifted lamellar epithelium, Necrotic Lamellae, increased mucus cells and Proliferated chloride cells in 120 and 150 ppm of Cobalt chloride exposed groups. In the present study, in the experimental group of *Uca triangularis* when treated with Endosulfan, Chlorpyrifos and Carbaryl, the neurosecretory cells were moderately stained, the neurosecretory cells were in distorted condition and the neurosecretory material was reduced.

The results of Patil *et al.* (2008) revealed decrease in protein, glycogen and lipid content content in the organopesticide exposed fresh water crab, *Barytelphusa guerini*. The freshwater female crab, *Spiralothelphusa hydrodroma* when exposed to textile dye industry effluent (TDIE) had reduced protein, carbohydrate and lipid levels in various tissues studied (Sekar *et al.,* 2009). Sreenivasan *et al.* (2011) reported that toxicity of Cypermethrin caused decrease in protein, csarbohydrate and lipid content in Fresh Water Field Crab, *Spiralothelphusa hydrodroma* (Herbst). The results of Chourpagar and Kulkarni (2013) showed significant decrease in protein, carbohydrate and lipid content when the Freshwater Crab, *Barytelphusa cunicularis* was treated with Merculric chloride. In the present experiment the fiddler crab, *Uca triangularis* was treated with Endosulfan, Chlorpyrifos and Carbaryl and the tissues revealed significant decrease in total protein, carbohydrate and lipid content and it was maximum reduced in Carbaryl treated experimental crabs. This reduction in biochemical components was due to the toxic 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 in the tissues of *Uca triangularis* treated with pesticides was due to the rapid glycogenolysis and utilization of carbohydrate in hypoxia to meet the energy demands. The decrease in the lipid content was due to the stress imposed by toxicity of pesticides.

Conclusion

The present research on the effect of pesticides on the fiddler crab, *Uca triangularis* clearly indicates that the test animal was sensitive to pesticide presence. Hence, fiddler crabs can be used as a bio-indicator of the toxicology of the pesticide to assess the environmental damage. They can have toxic effects

in the short term in directly exposed organisms, or long-term effects by causing changes in habitat and the food chain. Thus the development of possible remedial measure to prevent the contamination of pesticides exceeding permissible limit in the aquatic environment is needed.

Acknowledgments

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

REFERENCES

Abbot, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomo.,* 18, 265-267.

Aguiar, L.H. 2002. Effects of the organophosphate insecticide methyl parathion (Folidol 600) on the teleost freshwater, matrinxa *Brycon cephalus* (Gunther, 1869) aspects of intermediary metabolism. Ph.D thesis, Federal Univ. of Sao Carlos.

APHA, 1992. Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C. 18th Ed.

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.

Chourpagar, A. R. and Kulkarni, G.K., 2013. Effect of Mercuric Chloride on the Biochemical Constituents of Some Tissues of a Freshwater Crab, Barytelphusa cunicularis [Westwood, 1836]. *W. J. Fish and Mar. Sci*. 5 (6), 598-602.

Folch, J., Lee, S.M. and Slone-Stanley, G.H., 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497-508.

Katagi, T., 2010. Bioconcentration, bioaccumulation, and metabolism of pesticides in aquatic organisms. *Rev. Env. Contam. Toxico.,* 204, 1-132.

Kumar, A., Doan, H., Barnes, M., Chapman, J.C. and Kookana, R.S. 2010. Responses and recovery of acetylcholinesterase activity in freshwater shrimp, *Paratya australiensis* (Decapoda: Atyidae) exposed to selected anti-cholinesterase. Ecotoxicol. Environ. Safety, 73(7), 1503-1510.

Maharajan, A., Narayanasamy, Y., Ganapiriya, V. and Shanmugavel, K., 2015. Histological alterations of a combination of Chlorpyrifos Chlorpyrifos and Cypermethrin (Nurocombi) insecticide in the fresh water crab,*Paratelphusa jacquemontii* (Rathbun). *The J. Bas. Appl. Zoo.,* 72, 104–112.

Patil, C., Paul, R. and Malkanna., 2008. Neuroendocrine regulation and pesticidal impact on freshwater crab, *Barytelphusa guerini* (H. Milne Edwards). *J. Environ. Biol.,* 29(6), 887-892.

Roe, J.R. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol.Chem.* 20, 335-343.

Saravana Bhavan, P., Geraldine, P. and Sowdeswari, R. 2011. Sub-lethal impact of carbarylCarbaryl on food utilization in the fresh water prawn, *Macrobrachium malcolmsonii*. *J. Environ. Biol.,* 32, 369-373.

Sekar, P., Hari Prasad, S. and Decca Raman, M. 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.

Singare, P.U., Lokhande, R.S. and Jagtap, A.G. 2010. Study of physico-chemical quality of the industrial waste water effluent from Gove industrial area of Bhiwandi City of Maharashtra, India. *Interdi. Environ. Rev.* 11(4), 263.

Sreenivasan, R.S. 2005. A study on the effects of cypermethrin, a pyrethroid compound in *Spiralothelphusa hydrodroma*. Ph.D. Thesis. University of Madras.

Sreenivasan, R.S., Krishna Moorthy, P. and Deecaraman, M., 2011. Bioaccumulation Study on the Cypermethrin Toxicity in Fresh Water Field Crab, *Spiralothelphusa hydrodroma*(Herbst). *J. Chem. Pharm. Res.* 3(3), 397-402.

Suganthi, P., Soundarya, N., Stalin, A. and Nedunchezhiyan, S., 2015. Toxicological effect of cobalt chloride on freshwater fish *Oreochromis mossambicus. Inter. Jour. Appl, Res.* 1(3), 331-340.

Uttah, E. C. 2013. Prevalence of human edible crabs infected with paragonimus uterobilateralis metacercariae in southeastern nigeria. *Pacific. J. Med. Sci.* 11(1), 11-20.

****