Biochemical Alterations of Mangrove Crab, *Episesarma tetragonum* Exposed to Chlorpyrifos

Shanmugavel, K.and Maharajan.A
PG & Research Department of Zoology, Khadir Mohideen College, Adirampattinam- 614701,
Thanjavur Dist, Tamil Nadu.
*Author for Correspondence
A.Maharajan,

ABSTRACT

The mangrove crab, *Episesarma tetragonum* is an important human food source in parts of south India and the crab is constantly exposed to pesticide, which are used extensively to control agricultural pests. Evaluation of the toxic effect of chlorpyrifos on the experimental crab for the LC_{50} value was carried out. Effect of chlorpyrifos on the quantitative study of the nutritive value viz.. Protein, Carbohydrate, and Lipid in muscle, gill, hepatopancreas vas deferens and hemolymph was studied. It is concluded that the accumulation of chlorpyrifos in various tissues of crab to reduce the protein, carbohydrate and lipid.

Index Items :*Episesarma tetragonum*, Biochemistry, Chlorpyrifos

INTRODUCTION

Mangrove regions are highly productive and play an important role as breeding and nursery grounds for many commercially important fin fishes and crustaceans (Kathiresan and Bingham, 2001). Dissolved organic compounds of mangrove origin are an additional source of nutrition. The predators feed on the detritus feeders, which in turn form an important foot source for both aquatic as well as terrestrial animals. Faunal assemblage of mangrove includes insects, crustaceans, mollusks, fishes, snake, crocodiles, bird and mammals. Temperature, salinity, tides rainfall, winds etc. are the major environmental factors, which influence the functions and stability of the mangrove ecosystem (Taylor and Hellberg, 2003).Indian mangrove ecosystems are known to have a total of 3,985 biological species that include 919 floral species and 3,066 faunal species. Of the biological species, the faunal species occupy about 77%, and the floral species 23%. Thus, the faunal species component is about three times greater than the floral component of the mangrove ecosystem (Kathiresan, 2000; Kathiresan and Quasim, 2005).

Crabs play vital role in the maintenance, modification and regulation of the benthic environment by influencing both the abiotic and biotic components. They are plentiful and serve both as the predator and the prey and hence are positioned at different tropic levels in the ecosystem (Siddon and Witman, 2004). Many species of crabs are burrowing in nature and with their burrowing activity they often alter the surface characteristics and drive the nutrient cycling (Pandya, 2011). Wide range of studies is available on macro invertebrates as an indicator species of aquatic habitat but amongst them specifically, brachyuran crabs are an effective indicator of different changes in both abiotic and biotic factors. In the tissue protein, carbohydrate and lipids play a major role as energy precursors for aquatic organisms exposed to stress conditions (Ramalingam, 1980).

The pesticides include insecticides, herbicides, fungicides, molluscides, nematicides and heavy metals like copper, zinc, arsenic, lead, cadmium, mercury etc (Jayakumar, 2002). These pesticides are non-biodegradable and accumulate in the food chain. Mostly they are prone to affect the nervous system causing tumors in living organisms. They are not only neurotoxic but also affect other system and have shown a high degree of impact on metabolism by altering the protein, carbohydrate, and lipid (McKee and Knowles, 1986; Nagabhushanam *et al.*, 1972).

Chlorpyrifos an organophosphate (OP), used throughout the world in controlling cutworms, corn rootworms, cockroaches, flea beetles, flies, termites, fire ants and lice. It is the second largest used organophosphate insecticide in India (Mathur and Tannan, 1999). Organophosphate pesticides inhibit acetylcholinesterae activity and affect neuronal control of heart in crab (Lundebye *et al.*, 1997; Ghedira *et al.*, 2009). Narra *et al.* (2012) reported alterations in enzyme activity (increased LDH; decreased SDH and ACP) in nervous tissues of crab.There are some reports on the toxicity of organophosphate pesticides to crabs (Senthil Kumar *et al*., 2007; Ghedira *et al.*, 2009). Of them, chlorpyrifos is more toxic to crustaceans (Bharathi and Sandeep, 2005). The present work aims to study the effect of chlorpyrifos in different tissues of *E. tetragonum.*

MATERIALS METHODS

Experimental crab

The experiments were performed in accordance with local/national guidelines for experimentation in animals and all care was taken to prevent cruelty of any kind. Mangrove crabs, *Episesarma tetragonum* of carapace size ranging from 3.5 to 4cm and weight 40 to 65gm were collected from Muthupettai mangrove, Thiruvarur Dist, Tamil Nadu. They were transported and kept in 100 L tank containing well aerated filtered estuarine water maintained at ambient temperature (27± 2 °C) for a period of one week. Before stocking, the tank was washed with 0.1% KMnO4 for disinfection.

Chemical

For preparation of stock solution 1 ml of insecticide Chlorpyrifos, Jeyban, Sabari Crob Care Sciences (P) Ltd. Chennai, diluted with 1 L of Milli-Q deionised water was purchased.

Experimental Procedures

Test Concentration:

Crabs were exposed to 0.0294 and 0.0588 ppm sublethal concentration of insecticide doses at 10% and 20% respectively of the Maximum Acceptable Toxicant Concentration (MATC), which was 0.294 ppm.

Experimental design

A recirculation closed system was set up according to Muthuwan (1998). The experiment was carried out in 100 l tank. Each aquarium was filled with 100 l of estuary water. The water was continuously aerated throughout the experiment.

Test Procedure

After 2 weeks of acclimatization in a holding tank, ten healthy crabs with carapace size ranging from 3.5 to 4cm and weight 40 to 65gm were transferred to each aquarium. Three replicates were performed for test concentration and control. Crabs were fed twice daily with commercially prepared pellet feed at 10:00 and 16:00 h. Uneaten food was quickly removed from the system. The media were renewed every alternate day. The actual concentration of chlorpyrifos was measured weekly before and after its addition to maintain chlorpyrifos concentrations at the designed level. Mortality and behavior were observed everyday in each concentration. Two crabs from each aquarium were sampled at 0, 7, 14, 21and 28 days post-exposure.

Tissue samples and biochemical analysis

Sample was extracted from the tissues of muscle (MU), gills (GL), hepatopancreas (HP), vas deferens (VD) and hemolymph (HE) at different concentration and different duration. Concentrations of biochemical constituents in different tissues were estimated by following standard procedures. The total protein (TP) and the total carbohydrate (TC) concentrations in different tissues were determined according to the methods of Lowry *et al*. (1951) and Roe (1955). The total lipid (TL) content was estimated by gravimetric methanol - chloroform extraction method suggested by Folch *et al.* (1957). Total free amino acids were estimated by Ninhydrin method. Accuracy of the analytical methods was tested against prepared standards and deviations from real standard values are expressed as coefficient of variation. Fluctuations in concentrations of biochemical components in different treatment groups and organs were assessed by analysis of variance (ANOVA).

RESULTS

Changes in the TP Levels: Levels of the TP in different tissues of control and exposed *E. tetragonum* during the exposure period are depicted in Figure 1, 2, 3, 4 & 5. The TP concentrations were significantly lower in test crab than those of controls on all DoE (P<0.01). The rate of depletion was found to be highly time and tissue dependent. The order of percent decrease of the TP concentrations in different tissues at the end of 28 DoE was observed to be HP> VD> MU>HE>GL. A progressive depletion in the TP levels of test crab was recorded in the tissues of VD and MU during the exposure period. Significant variation in the TP content between exposure concentrations of 0.0294 and 0.0588 ppm was noticed (P>0.01). The levels of hepatic protein of test crab were found to be almost similar to that of control crab on 0 and 7 DoE but depletion was more prominent on 14, 21 and 28 DoE. The magnitude of depletion in the hepatic protein was directly proportional to the concentration of chlorpyrifos. Higher percent depletion in the hepatic protein was observed in test crab exposed to 0.0294ppm compared to those exposed to 0.0588 ppm of chlorpyrifos (P<0.05).

Changes in the TC Levels: Levels of the TC in different tissues of test crab and controls during the exposure period are shown in Figure 6,7, 8, 9 & 10. The TC concentrations were significantly lower in test crab than those of controls on all DoE. The depletion in the TC levels in the HP of test crab was significant with the progress in the period of exposure. Concentrations of hepatic carbohydrate in the test crab ranged from 0.198±0.001mg/100mg (0 DoE) to 0.128±0.002mg/100mg (28 DoE) over control crab (100%). Concentrations of hepatic carbohydrate in hemolymph of test crab ranged from 20.54±0.1mg% (0 DoE) to 8.57±0.32mg% (28 DoE) The levels of the TC in the MU of test crab exhibited a biphasic pattern: higher concentrations on 0 DoE and 7 DoE and lower on 14 DoE and 21 DoE and 28 DoE. The order of percent decrease in the TC levels in the studied tissues on the last day of exposure (28 DoE) was found to be HP>MU>GL>VD>HE.

Changes in the TL Levels: Levels of the TL in different tissues of the test crab and controls during the exposure period are depicted in Figure 11, 12, 13, 14 & 15. In general, the TL concentrations in all the studied tissues of crab exposed to sub-lethal doses of chlorpyrifos were significantly lower than those in controls (P<0.05). The percent decrease in the hepatic lipid was higher in the VD than in the tissues of HP,MU, and GL and the order of percent decrease on 28 DoE was found to be VD>HP>MU>GL>HE.

DISCUSSION

The estimation of biochemical constituents helps to assess the nutritive value of an organism. It has become imperative to study how the nutritive value varies with the changes in biochemical constituents of the crustaceans, which are exposed to increased environmental pollution. The nutritional value of different species of fish and shellfish depend on their biochemical components such as protein, carbohydrate and lipids. These proximate components could serve as sensitive indicators for detecting potential adverse effects, particularly the early events of pollutant damage because their alterations appear before the clinical symptoms produced by the toxicant (Almeida *et al*., 2002). It is therefore important that potential effects of acute and chronic concentrations of pollutant on proximate composition are determined and interpreted to delineate mechanisms of pollutant action and possibly ways to mitigate adverse effects (Matos *et al*., 2007). Histopathological, biochemical, and physiological changes in different species of crustaceans after exposure to endosulfan have been widely reported (Omkar *et al*., 1984; Shukla and Omkar, 1984; Selvakumar *et al*., 1996).

Biochemical changes induced by pesticidal stress is due to disturbed metabolism manifested by inhibition of enzymes, retardation of growth and reduction in the fecundity and longevity of the organism. Most of the pesticides act as metabolic depressors and generally affect the activity of biologically active molecules such as proteins, carbohydrates and lipids (Agrahari and Gopal, 2009).Protein is one of the important biochemical components and plays an important role in metabolic pathways and biochemical reactions. Under extreme stress conditions, protein supply energy in metabolic pathways and biochemical reactions. Therefore, an assessment of the TP content in different tissues could be used as a diagnostic tool for determining the physiological status of an organism .The TP content in muscle, gills, hepatopancreas, vas deferens and hemolymph was altered; the changes in protein levels were insignificant when compared to the control crab. In the present study, the total protein in the muscle, gills, hepatopancreas, vas deferens and hemolymph of *E.tetragonum* showed decreasing trend as the duration of exposure to chlorpyrifos increased (muscle: 6.53 to 3.91 mg/100mg, gills: 2.15 to 1.63 mg/100mg, hepatopancreas: 16.65 to 4.74 mg/100mg, vas deferens: 7.4 to 3.54 mg/100mg and hemolymph: 2.84 to 1.44mg/100µl). It is likely that the observed reduction in total protein of *E. tetragonum*is due to a direct consequence of the stress imposed by chlorpyrifos. The percent depletion progressively increased with DoE irrespective of exposure concentrations. A similar depletion in the total protein content in different tissues of crustaceans on exposure to various pesticides has been documented: in the freshwater prawn, *M. kistensis* on exposure to pesticides by Nagabhushanam *et al*. (1972); in the marine edible crab, *S. serrata* on exposure to dimecron, an OC pesticide, by Rao *et al*. (1987); in the white prawn, *F. indicus* on exposure to sublethal levels of phosphamidon and methylparathion by (Reddy *et al*., 1988); in the freshwater field crab, *P. hydrodromous* following exposure to malathion by Singaraju *et al*. (1991).

Total free sugars are very important biological compounds as they are the chief source of energy and also structural constituents of protoplasm. In the present study the total free sugar in the muscle, gill, hepatopancreas, vas deferens and hemolymph of *E. tetragonum*showed decreasing trend with increasing concentration of chlorpyrifos (muscle: 0.184to 0.171mg/100mg, gills: 0.163 to 0.063mg/100mg, hepatopancreas: 0.198to 0.128mg/100mg, vas deferens: 0.155to 0.11mg/100mg and hemolymph: 20.54to 8.57 mg/100mg/%).It may be due to the breakdown of glycogen to cope with the high energy demand for the detoxification process, since carbohydrate forms major source of energy under toxicity.This is consistent with the previous observations that have been reported in themarine prawn, *M. monoceros* on exposure to methyl parathion (Reddy and Rao, 1991) and in the freshwater prawn, *M. malcolmsonii* following exposure to sublethal doses of endosulfan (Bhavan and Geraldine, 1997). The depletion of the total carbohydrate may be due to its rapid utilisation to meet the reduced energetic efficiency under the impact of chlorpyrifos.

The lipids provide energy for almost all enderogonic processes and are of utmost importance in maintaining the structural and physiological integrity of cellular and subcellular structure. Lipids are important energy resources in crustaceans and are required during reproductive cycles. The TL concentrations in different tissues chlorpyrifos treated crabs in the present study were found to be significantly lower than the concentrations in the same organs of controls

(P<0.05). The TL in the muscle, gill, hepatopancreas, vas deferens and hemolymph of *E. tetragonum* showed decreasing trend as the duration of exposure in each concentration of chlorpyrifos increased (muscle: 16.22to 15.15mg/100mg, gills: 13.46to 10.7mg/100mg, hepatopancreas: 27.73 to 17.26 mg/100mg, vas deferens: 29.44 to 9.89mg/100mg and hemolymph: 6.45to 5.86mg/100mg/µl). Similar observations have been made in the freshwater prawn, *M. kistensis* on exposure to pesticides (Nagabhushanam *et al*., 1972); in the marine prawn, *M. monoceros* exposed to phosphamidon, methylparathion and lindane (Reddy and Rao, 1988; Reddy and Rao, 1989) and in the freshwater prawn, *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 1997) and in the fishes, *Saratherodon (Oreochromis) mossambicus* and *Barbus conchonius* exposed to methylparathion and aldicarb, respectively (Rao and Rao, 1981; Pant *et al*., 1987).

Protein plays a crucial role in almost all biological processes and amino acids are the building blocks of it. A large proportion of our cells, muscles, and tissue are made up of amino acid, meaning they carry out many important bodily functions, such as giving cells and their structure. They also play a key role in the transport and the storage of nutrients. Amino acids have an influence on the function of organs, glands, tendons and arteries. Pesticides, as environmental stressors are known to alter serum biochemical parameters incrabs, which suggest that serum biochemical indices could be used as important and sensitive biomarkers

CONCLUSION

Results of this study revealed that sub-lethal doses of chlorpyrifos significantly alter the proximate composition of major tissues, particularly the total protein levels in the muscle tissues and thereby reducing the nutritive value of this economically important *E.tetragonum*.

ACKNOWLEDGEMENTS

The authors are thankful to the Secretary, Principal and HOD of Zoology, Khadir Mohideen College for the facilities provided to carry out this work and encouragements.

REFERENCES

Agrahari, S. and Gopal, K. 2009. Fluctuations of certain biochemical constituents and markers enzymes as a consequence of monocrotophos toxicity in the edible freshwater fish, *Channa punctatus*. *Pest. Biochem. Physiol.,* 94: 5-9.

Almeida, J.A., Diniz, Y.S., Marques, S.F.G., Faine, I.A., Ribas, B.O., Burneiko, R.C. and Novelli, E.I.B. 2002. The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination. *Environ. Int.* 27: 673–679.

Bharathi, Ch. and Sandeep, B.V. 2005. Toxicity of organophosphate on edible crab *Paratelphusa hydrodromus.J. Ecophysiol. Occup. Hlth., 4: 247-250.*

Bhavan, P.S.and Geraldine, P. 1997. Alteration in concentrations of protein, carbohydrate, glycogen, free sugar, and lipid in the prawn *Macrobrachium malcolmsonii* to sublethal concentrations of endosulfan. *Pest. Bioch. Physiol.,* 58: 89-101.

Folch, J., M. Lees and Sloare- Stavely, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* 226: 497-509.

Ghedira, J., Jebali, J., Bouraoui, Z., Bani, M., Chouba, L. and Boussetta, H. 2009. Acute effects of chlorpyriphos-ethyl and secondary treated effluents on acetylcholinesterase and butyryl cholinesterase activities in *Carcinus maenas*. *J.Environ. Sci.,* 21: 1467–1472.

Jayakumar, S. 2002. Effect of copper and zinc toxicity on a fresh water crab, *Spiralothelphusa hydrodroma.* Ph.D. Thesis, University of Madras, India,

Kathiresan, K. 2000. A review of studies on pichavaram mangroves, southeast India. *Hydrobiologia* 430: 185-205.

Kathiresan, K. and Bingham, B.L. 2001. Biology of mangroves and mangrove ecosystems. Adv. Marine Biol., 40, 81-251.

Kathiresan K, and Quasim, S.Z. 2005. Biodiversity of Mangrove Ecosystems New Delhi: Hindustan Publishing Corporation. p 251.

Mathur, S.C. and Tannan, S.K. 1999. Future of Indian pesticide industry in next millennium. Pesticide Information,24: 9-11.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 193: 265 - 275.

Lundebye, K. Curtis, T.M., Braven, J. and Depledge, M.H. 1997. Effects of the organophosphorous pesticide, dimethoate, on cardiac and acetylcholinesterase (AChE) activity in the shore crab *Carcinus maenas*. *Aquat. Toxicol.*, 40: 23-36.

Matos, P., Fontaınhas-Fernandes, A., Peixoto, F., Carrola, J. and Rocha, E.2007.Biochemical and histological hepatic changes in Nile tilapia, *Oreochromis niloticus* exposed to carbaryl. *Pes. Biochem. Physiol.*, 89: 73–80.

McKee, M.J. and Knowles, C.O.1986. Effects of fenvalerate on biochemical parameters, survival, and reproduction of *Daphnia magna. Ecotoxical. Environ. Saf*., 12:70-84.

Muthuwan, V., 1998. Green Water Recirculation System for Intensive Marine Shrimp Culture. Ph.D. Thesis, School of Environmental, Resource and Development, Asian Institute of Technology, pp. 91-120.

Nagabhushanam, R., Deshpande, J. and Sarojini, R. 1972. Effects of some pesticides on the biochemical constituents of the freshwater prawn, *Macrobrachium kistensis*. *Proc. Natl. Symp. Ecotoxicol.*, 73-84.

Narra, M.R., Regatte. R.R. and Odimyala, R.K. 2012. Effect of Chlorpyrifos on enzymes as biomarker of toxicity in freshwater field crab *Barytelphusa guerini.* Int. *J. Environ.Sci.,* 2(4): 2015-2023.

Omkar, V.B., Upadhyay, R. and Shukla, G.S. 1984. Endosulfan induced changes in the carbohydrate metabolism of a freshwater prawn, *Macrobrachium lamarrei*. *Curr. Sci.*, 53: 280-281.

Pant, J., Tewari, H. and Gill, T.S. 1987. Effects of aldicarb on the blood and tissues of a freshwater fish. *Bull. Environ. Contam. Toxicol.*, 38 : 36-41.

Prasath, P.M.D. and Arivoli, S. 2008. Biochemical study of freshwater fish *Catla catla* with reference to mercury chloride. *Iran J. Environ. Health. Sci. Eng.*, 3: 109-116.

Reddy, M.S. and Rao, K.V.R. 1988. Effect of lethal and sublethal concentrations of phosphamidon and methylparathion on some lipid parameters in selected tissues of penaeid prawn, *Metapenaeus monoceros* (Fabricius). *Nat. Acad. Sci. Lett. India*, 11: 193-196.

Reddy, M.S. and Rao, K.V.R. 1989. In vivo modification of lipid metabolism in response to phosphamidon, methylparathion and lindane exposure in the penaeid prawn, *Metapenaeus mononceros*. *Bull. Envt. Contam. Toxicol.*, 43: 603-610.

Reddy, M.S., Rao, K.V.R. and Murthy, B.N. 1988. Changes in nitrogen metabolism of penaeid prawn, *Penaeus indicus*, during sublethal phosphamidon and methylparathion induced stress. *Bull. Environ. Contam. Toxicol.*, 41: 344-351.

Roe, J.H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, 212 : 335 – 343.

Rao, K.S., Nagabhushanam, R. and Sarojini, R. 1987. Acute toxicity of some pesticides to the marine crab, *Scylla serrata*. *Env. Ecol.*, 5: 181-182.

Rao, J.R. and Rao, K.V.R. 1981. Lipid derivatives in the tissue of freshwater teleost, *Saratherodon mossambicus*. Effects of methylparathion. *Proc. Indian. Natn. Acad. Sci.*, 47: 53-58.

Selvakumar, S., Ajmal Khan, S. and Kumaraguru, A.K. 1996. Acute toxicity of some heavy metals, pesticides and water soluble fractions of diesel oil to the larvae of some brachyuran crabs. *J. Environ. Biol.*, 17: 221-226.

Senthil Kumar, P., Samyappan, K., Jaikumar, S. and Deecaraman, M. 2007. Effect of chloropyrifos on the nutritive value in a freshwater field crab, *Spiralothelphusa hydrodroma*. *Res.J. Agricult. Biol. Sci.*, 3(6): 760-766.

Siddon C. E. and Witman J. D. 2004.Behavioral indirect interactions: Multiple predators effects and prey switching in the rocky sub tidal. *Ecology*. 85:2938-2945.

Singaraju, R., Subramanian, M.A. and Varadaraj. 1991. Sublethal effects of malathion on the protein metabolism in the freshwater field crab *Paratelphusa hydrodromous*. *J. Ecotoxicol. Environ. Monit.*, 1: 41-44.

Shukla, G.S. and Omkar, V.B. 1984. Insecticide toxicity to *Macrobrachium lamarrei* (H. Milne Edwards) (Decapoda, Palaemonidae). *Crustaceana.* 46: 283-287.

Pandya, P. J. 2011. Benthic community structure of Mahi River estuary with special reference to animal-sediment relationship. Ph. D. Thesis, the Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India. P:23-104.

Ramalingam, K. 1980. "Studies on the effects of sublethal concentration of a few toxicants onbiochemistry, physiology and histology of *Tilapia mossambica*(Peters) Ph.D. Thesis, University of Madras, Chennai.

Reddy, M.S. and Rao, K.V.R. 1991. Methylparathion induced alteration in the tissue carbohydrate catabolism of marine prawn, *Metapenaeus monoceros*. *Bull. Environ. Contam. Toxicol.*, 47: 925-932.

Taylor, M. and Hellberg, M., 2003. Genetic evidence of local retention of pelagic larvae in carribean reef fish. Science. 299: 107-109.

Fig.1.Changes of total protein (mg/100mg wet weight) in muscle of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

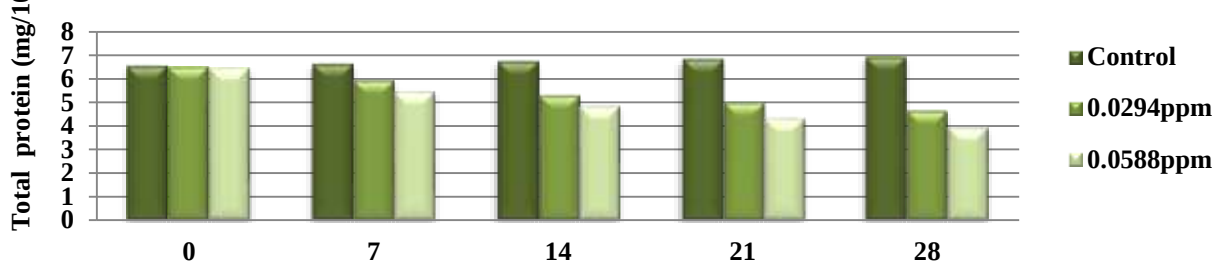

Fig.2 Changes of total protein (mg/100mg wet weight) in gills of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

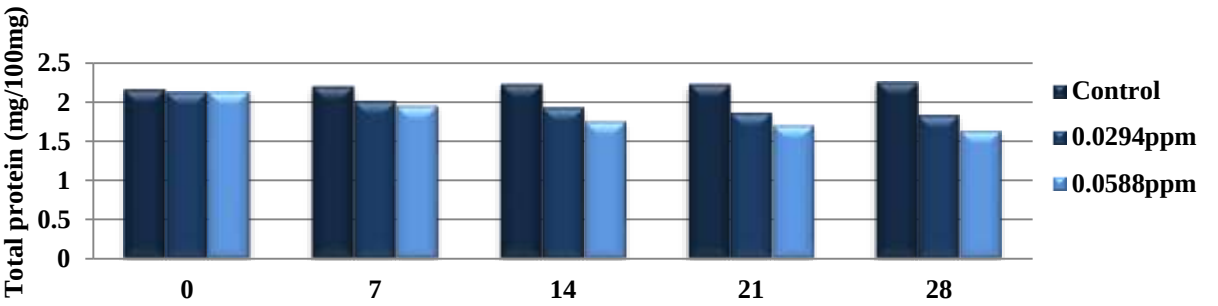

Fig.3 Changes of total protein (mg/100mg wet weight) in hepatopancreas of *E. tetragonum* exposed to sublethal concentration of chlorpyrifos

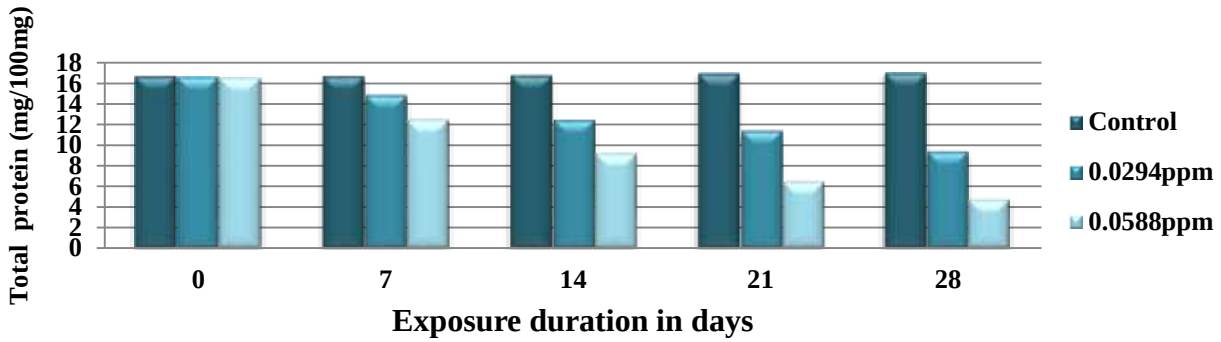

Fig.4 Changes of total protein (mg/100mg wet weight) in vas deferens of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

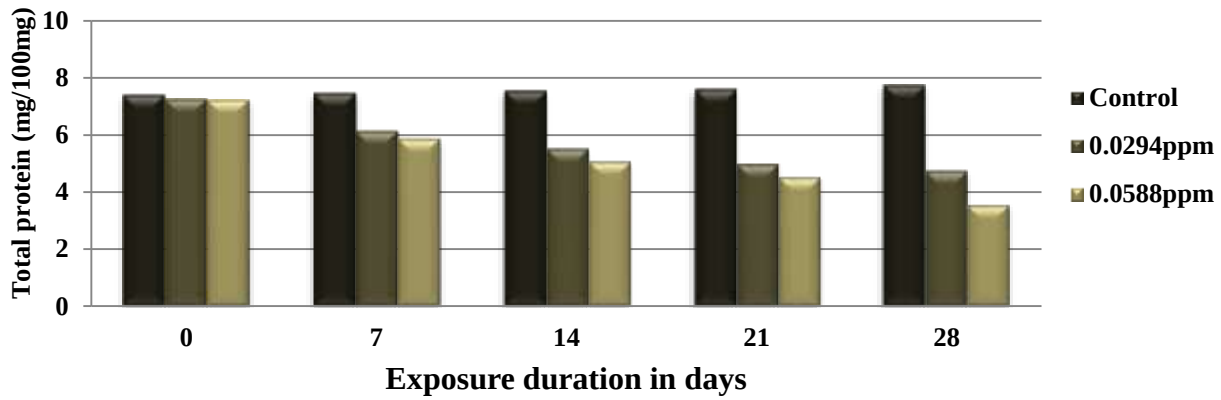

Fig.5. Changes of total protein (mg/100µl) in hemolymph of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

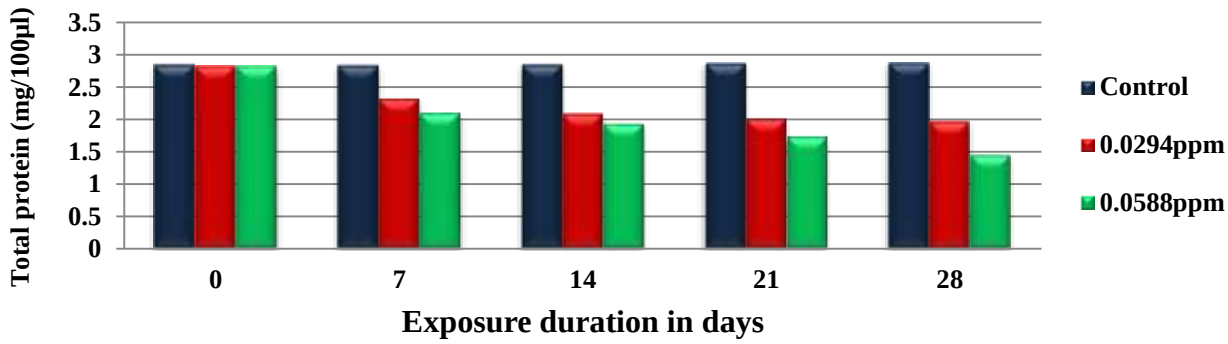

Fig.6.Changes of total carbohydrate (mg/100mg wet weight) in muscle of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

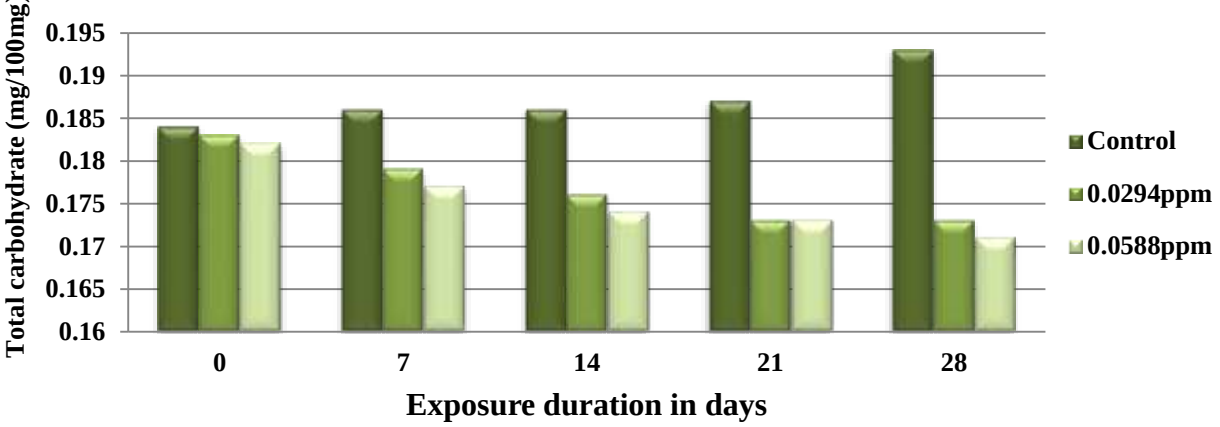

Fig.7. Changes of total carbohydrate (mg/100mg wet weight) in gills of *E.tetragonum* exposed to sulethal contration of chlorpyrifos

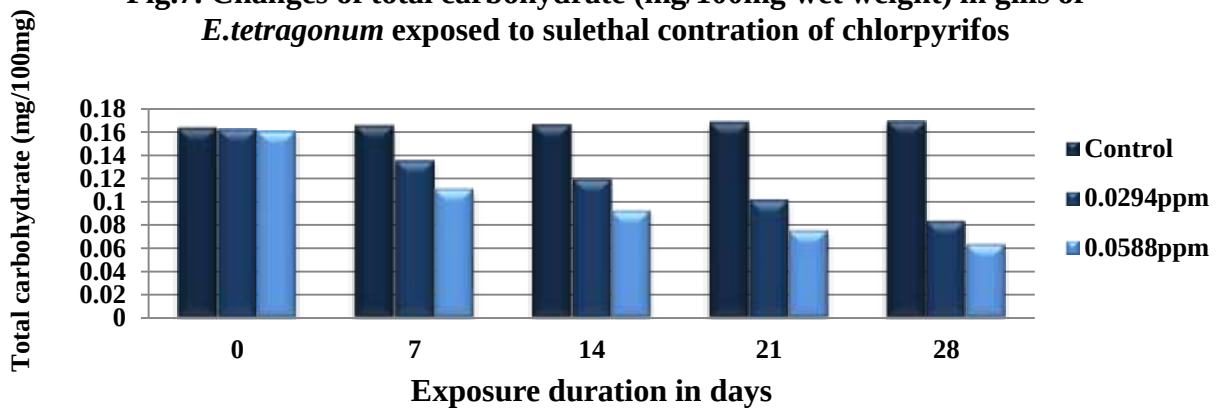

Fig.8. Changes of total carbohydrate (mg/100mg wet weight) in hepatopancreas of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

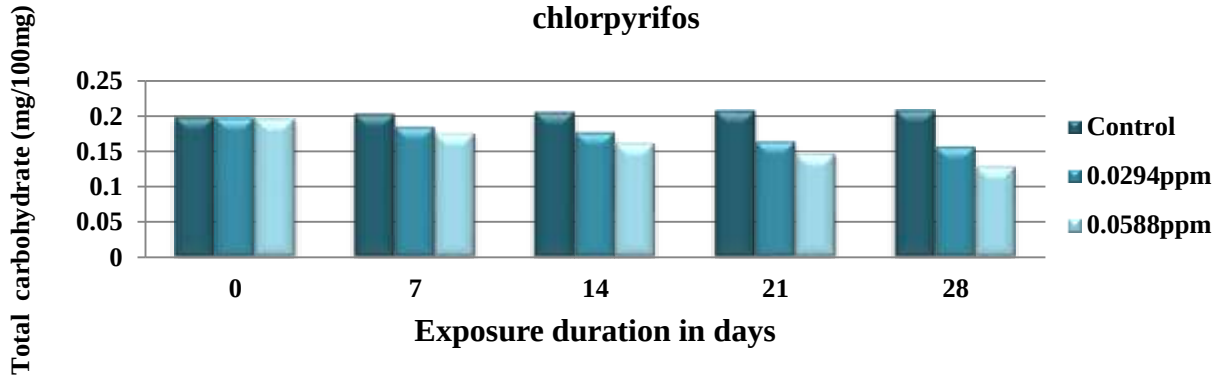

Fig.9. Changes of total carbohydrate (mg/100mg wet weight) in vas deferens of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

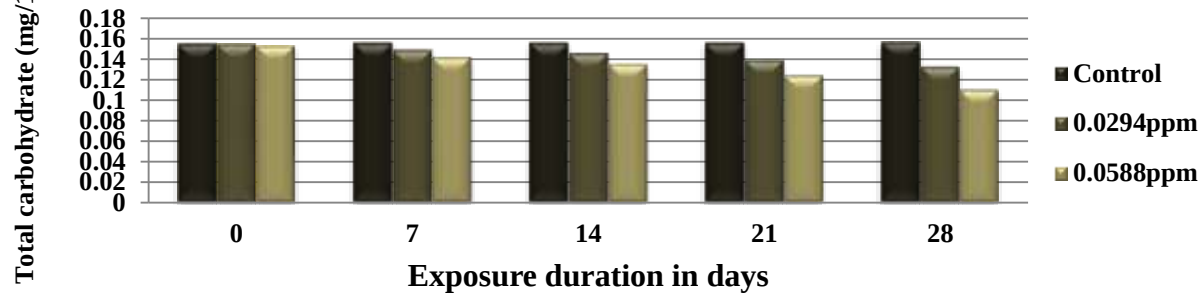

Fig.10. Changes of total carbohydrate (mg%) in hemolymph of *E.tetragonum* exposed to sulethal concentration of chlorpyrifos

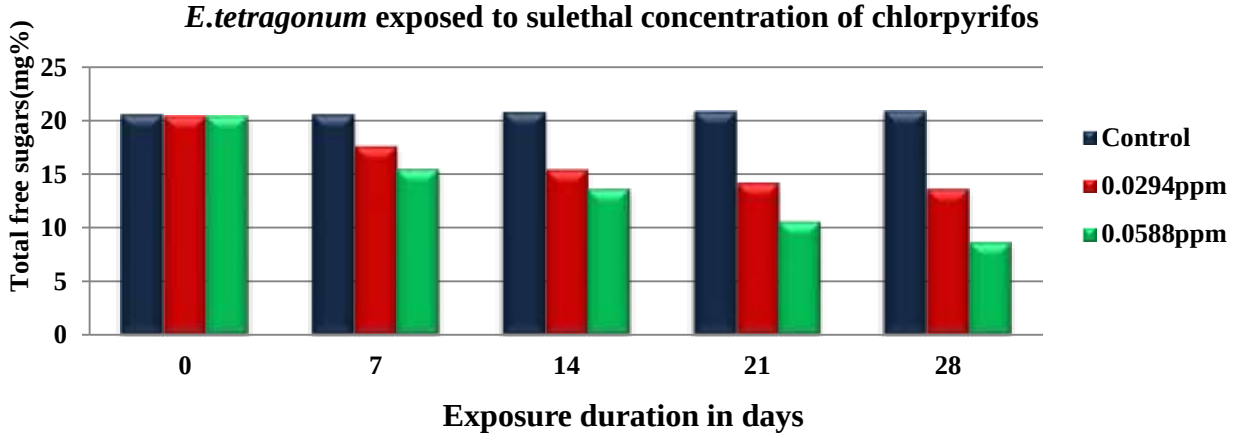

Fig. 11. Changes of total lipid (mg/100mg wet weight) in muscle of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

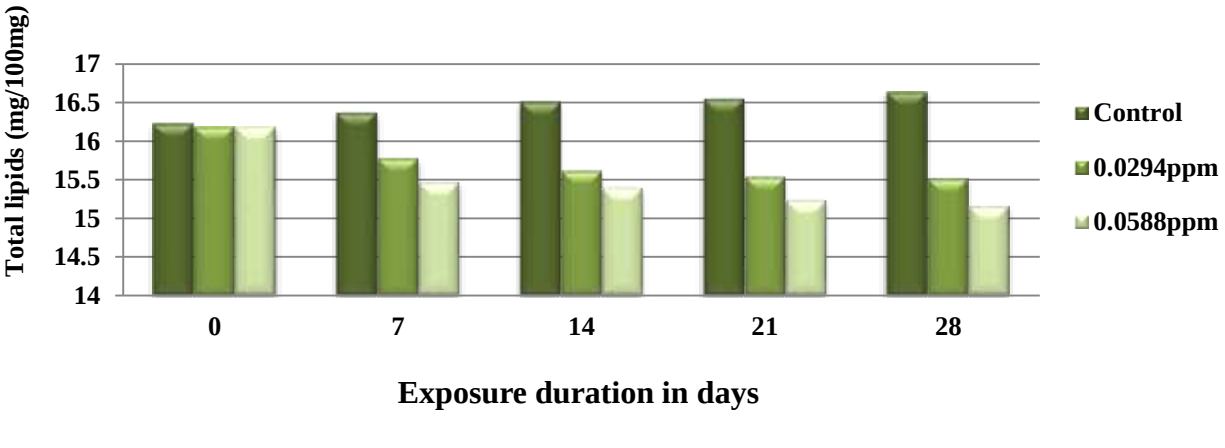

Fig.12. Changes of total lipid (mg/100mg wet weight) in gills of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

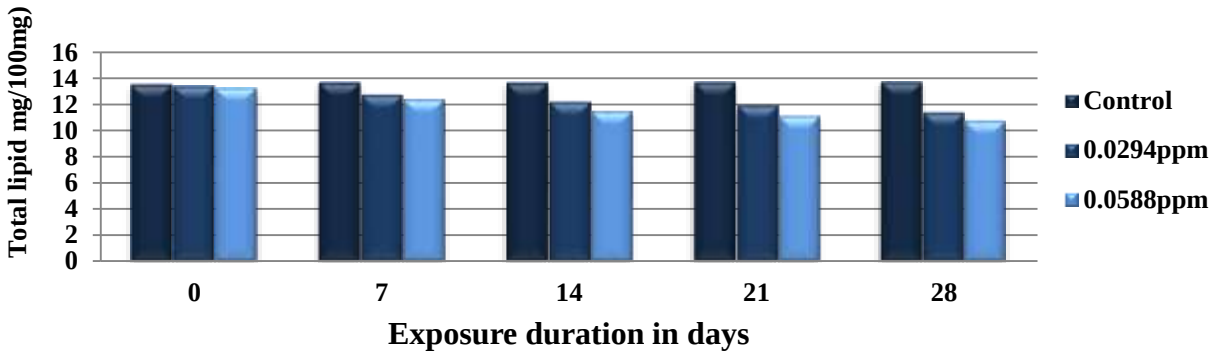

Fig.13. Changes of total lipid (mg/100mg wet weight) in hepatopancreas of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

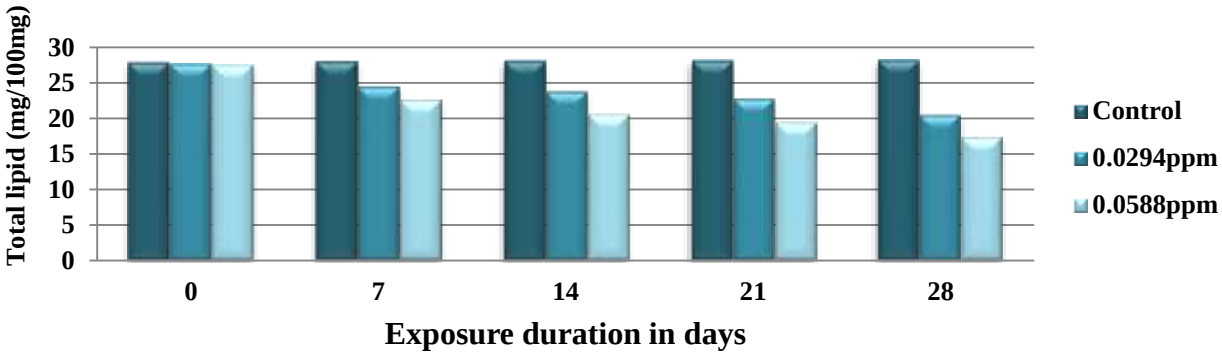

Fig.14. Changes of total lipid (mg/100mg wet weight) in vas deferens of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos

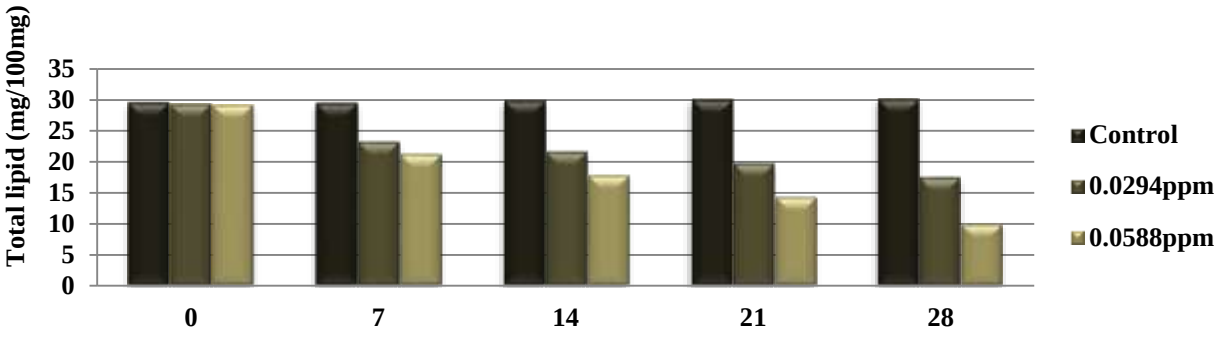

Fig.15. Changes of total lipid (mg/100µl) in hemolymph of *E.tetragonum* exposed to sublethal concentration of chlorpyrifos