International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 295-304
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Biochemical Changes of Fresh Water Crab, *Paratelphusa Jacquemontii* in Response to the Combination of Chlorpyrifos and Cypermethrin (Nurocombi) Insecticide

Shanmugavel.K, S.M.Fazildheen, Narayanaswamy.Y, D. Usha, Shanmuganathan. A, Ganapiriya.V, Maharajan. A*
PG & Research Department of Zoology, Khadir Mohideen College, Adirampattinam, Thanjavur Dist, Tamil Nadu

Received: 3 Oct 2018 / Accepted: 6 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: athimaha@yahoo.co.in

Abstract
Nurocombi is used as an insecticide in large-scale commercial agricultural applications as well as in consumer products for domestic purposes in India. The present research is to evaluate the effect of sub lethal concentration of nurocombi in biochemical changes of the fresh water crab, *Paratelphusa jacquemontii* after 0,7,14,21 and 28 days. The order of percentage in the concentrations of the TP, TC and TL in different tissues at the end of 28 DoE was found to be GL>TS>MU>HP>VD, GL>HP>MU>VD>TS and GL>MU>HP>TS>VD. Results of the present study revealed that sublethal doses of Nurocombi significantly alter the biochemical composition of various body tissues, particularly the TP levels in the MU tissues. The nurocombi bioaccumulation capacity of this species contributes to its suitability as a bioindicator for the presence of pollutants in aquatic systems.

Keywords
Paratelphusa jacquemontii, Nurocombi, biochemistry

*****

INTRODUCTION

The nutritional value of different species of fishes depends 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 fashioned by the toxicant (Rao, 2006). 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 possible ways to mitigate adverse effects (Matos *et al*., 2007). Biochemical constituents like glycogen, protein and lipid are considered as sensitive indicators of metabolic activities. *B.cunicularis* exposed to endosulfan (Shanmugam and Venkateshwarulu, 2000 Structurally modern pesticides belong to four different chemical groups, the organochlorines (e.g., DDT) which includes chlordane, toxaphene, hepachlor, lindane, telodrin, dieldrin and endosulfan, the organophosphates (e.g., malathion, diazinon, etc), the carbamates (e.g., Sevin or carbaryl) and the synthetic botanicals (e.g., Pyrethoids). Each group of chemicals differ significantly in its spectrum of toxicity to different insects, mode of action, persistence in the environment and toxicity to mammals and fish (Mansingh, 1987).

Crabs constitute a significant portion of the freshwater ecosystem. Very often they become the victim of pesticides used against some other activity or agricultural pest. Therefore, their population in this area was found decreasing during the last decade. The toxicity of pesticides depends on many factors such as weight, size, developmental stages, time of exposure and temperature in water content of the medium. The use of native species as sentinel organisms is proposed as a more appropriate way to obtain information about a specific site. *P. jacquemontii* is a local abundant crab that is widely distributed in Thiruvarur district, and it is territorial, easy to collect and resistant to pollutants. The nurocombi bioaccumulation capacity of this species contributes to its suitability as a bioindicator for the presence of pollutants in aquatic systems, although more studies are needed. Because the biota may accumulate persistent lipophilic organic pollutants, the transfer of these contaminants in the food web, which eventually may reach humans, must be continually observed.

MATERIALS AND METHODS

Animal collection and acclimatization

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. Fresh water crab, *P. jacquemontii* of carapace size ranging from 5.6 to 6.1 and weight 45–55 g were collected from the paddy field of Muthupettai, Thiruvarur Dist, and Tamil Nadu. They were transported and kept in 100 L tank containing well aerated filtered fresh water maintained at ambient temperature (27 ± 2°**C**) for a period of one week. Before stocking, the tank was washed with 0.1% $KMnO_4$ for disinfection.

Chemicals

For preparation of stock solution 1 ml of insecticide NUROCOMBI (Chlorpyriphos (CPF) 50% and Cypermethrin (CPM) 5% EC), Cheminova, FMC Corporation, Mumbai, diluted with 1 L of Milli-Q deionised water was purchased.

Test concentration

Crabs were exposed to 0.0187 and 0.0374 ppm sublethal concentration of combined insecticide doses at 10% and 20% respectively of the Maximum Acceptable Toxicant Concentration (MATC), which was 0.187 ppm.

Test procedure

After 2 weeks of acclimatization in a holding tank, ten healthy crabs with carapace size ranging from 5.9 to 6.2 cm and weight 50 – 60 g 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. Mortality and behavior were observed everyday in each concentration. Two crabs from each aquarium were sampled at 0, 7,14,21 and 28 days post-exposure.

Tissue samples and biochemical analysis: Sample was extracted from the tissues of muscle (MU), gills (GL), hepatopancreas (HP), testis (TS) and vas deferens (VD) 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 the method of Barnes and Blackstock (1973). 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 AND DISCUSSION

Nurocombi induced changes in proximate composition

Changes in the TP Levels:

Levels of the TP in different tissues of control and exposed *P.jacquemontii* during the exposure period are depicted in Figure 1, 2, 3,4 & 5, The TP concentrations were significantly lower in test *P.jacquemontii* than those of controls on all DoE (P<0.05). 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 GL>TS>MU>HP>VD. A progressive depletion in the TP levels of test was recorded in the tissues of GL and TS during the exposure period. Significant variation in the TP content between exposure concentrations of 0.0187ppm and 0.0374 ppm was noticed (P>0.05). The levels of hepatic protein of test *P.jacquemontii* were found to be almost similar to that of control *P.jacquemontii* 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 *P.jacquemontii*. 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 (Prasath and Arivoli, 2008). In the present study, concentrations of the total protein in the tissues of gill and muscle were found to be significantly lower than those in control crabs on all sampled days ($P<0.05$). 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, in the freshwater field crab, *P. hydrodromous* following exposure to malathion by Singaraju *et al*. (1991). A marked decrement in the concentrations of the total protein in the two freshwater field crab species, *O. senex senex* (Rajendra Prasad Naidu, 1985), *Barytelphusa guerini* (Reddy *et al*., 1991) on exposure to endosulfan have been reported.

Fig.1 Changes of total protein (mg/100 mg wet weight) in gills of *P.jacquemontii* exposed to sublethal concentrations of nurocombi

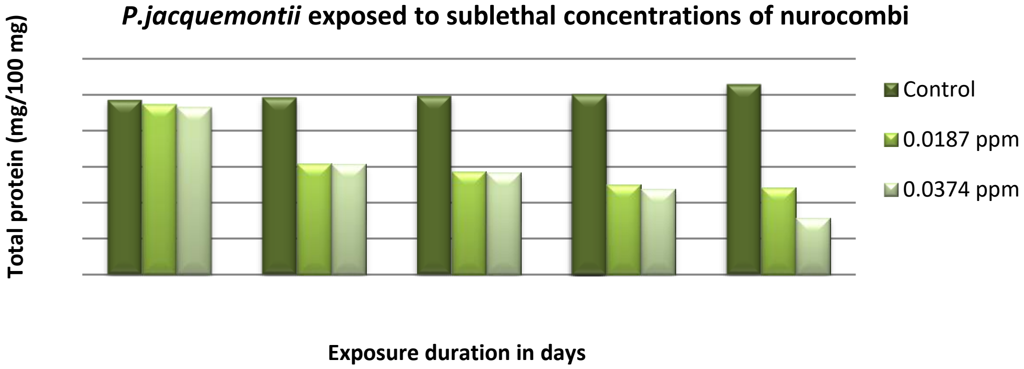


Fig. 2 Changes in total protein (mg/100mg wet weight) in hepatopancreas of P. jacquemontii exposed to sublethal concentrations of nurocombi

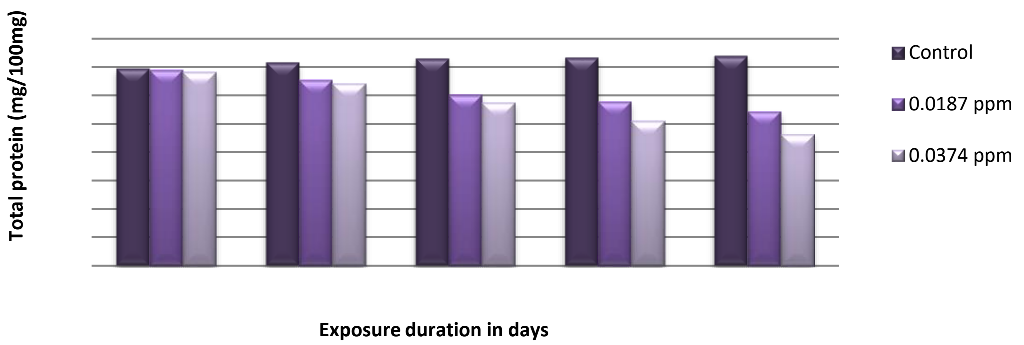


Fig:3 Changes of total protein (mg/ 100mg wet weight) in muscle of P. jacquemontii exposed to sublethal concentrations of nurocombi

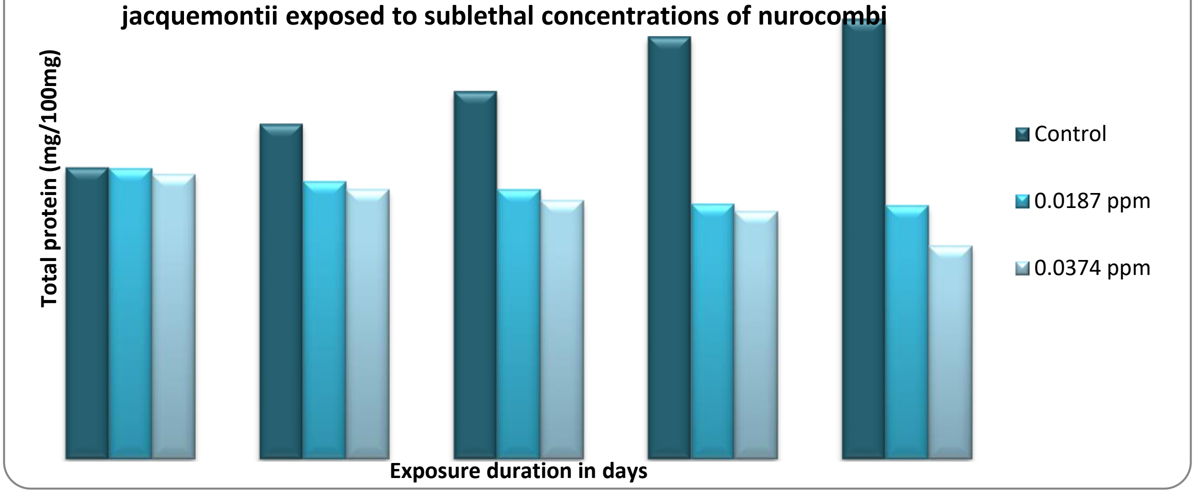


Fig.4 Changes in total protein (mg/ 100mg wet weight) in testis of *P.jacquemontii* exposed to sublethal concentrations of nurocombi

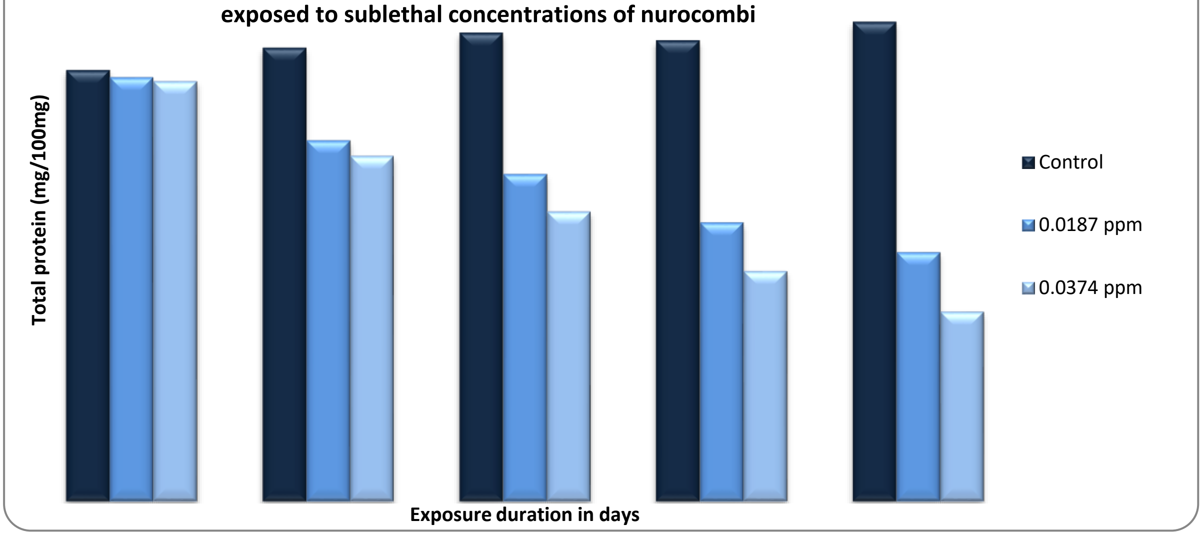
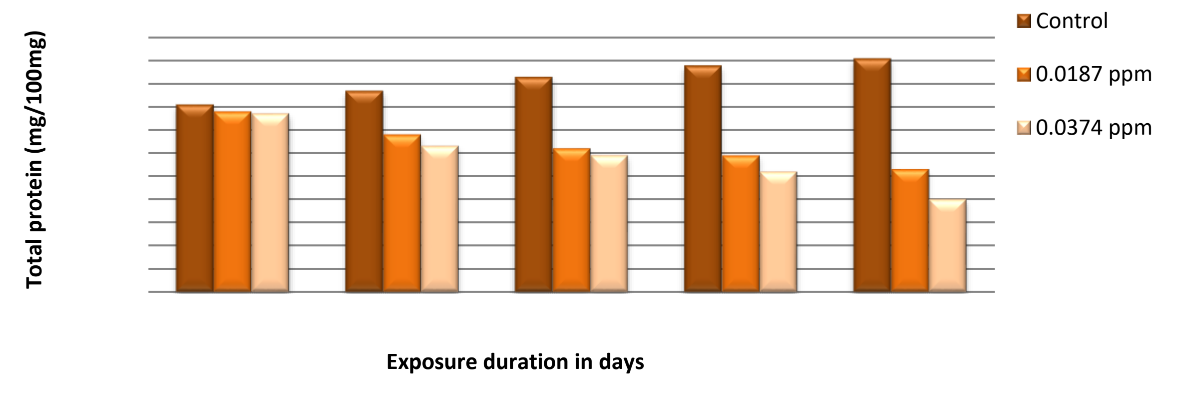


Fig. 5 Changes in total protein (mg/100mg wet weight) in vas deferens of *P.jacquemontii* exposed to sublethal concentrations of nurocombi



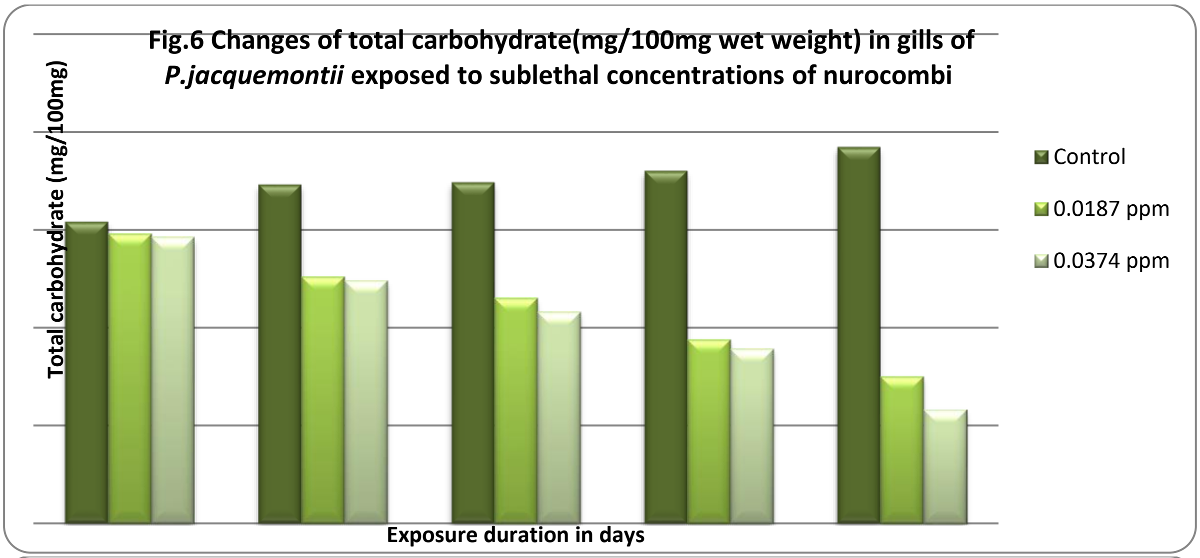


Fig.6 Changes of total carbohydrate(mg/100mg wet weight) in gills of *P.jacquemontii* exposed to sublethal concentrations of nurocombi
Total carbohydrate (mg/100mg)
Exposure duration in days
Control
0.0187 ppm
0.0374 ppm

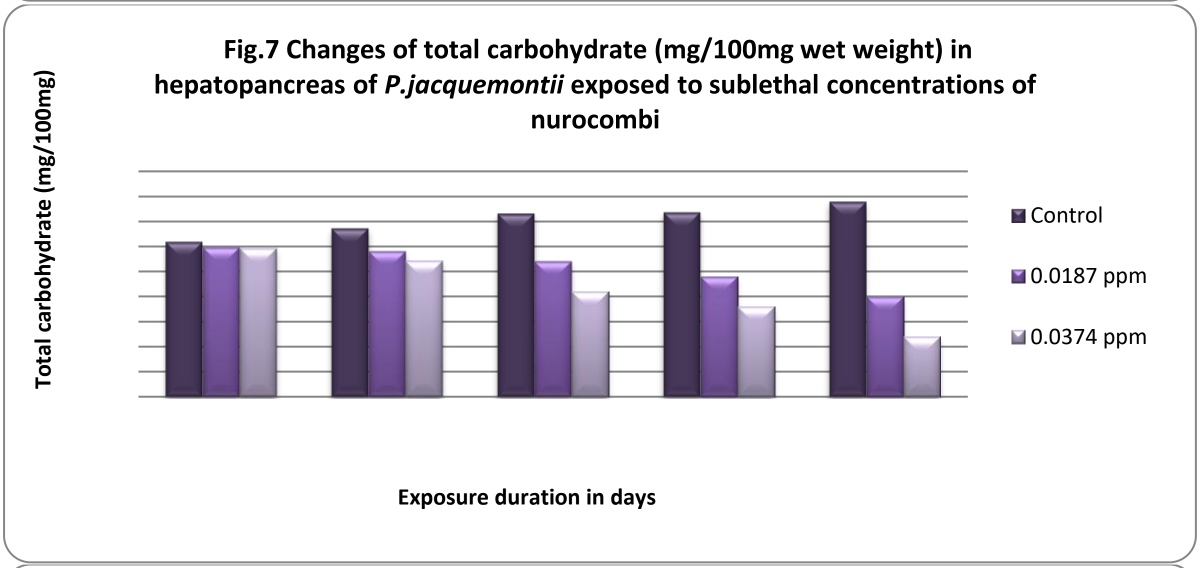


Fig.7 Changes of total carbohydrate (mg/100mg wet weight) in hepatopancreas of *P.jacquemontii* exposed to sublethal concentrations of nurocombi
Total carbohydrate (mg/100mg)
Exposure duration in days
Control
0.0187 ppm
0.0374 ppm

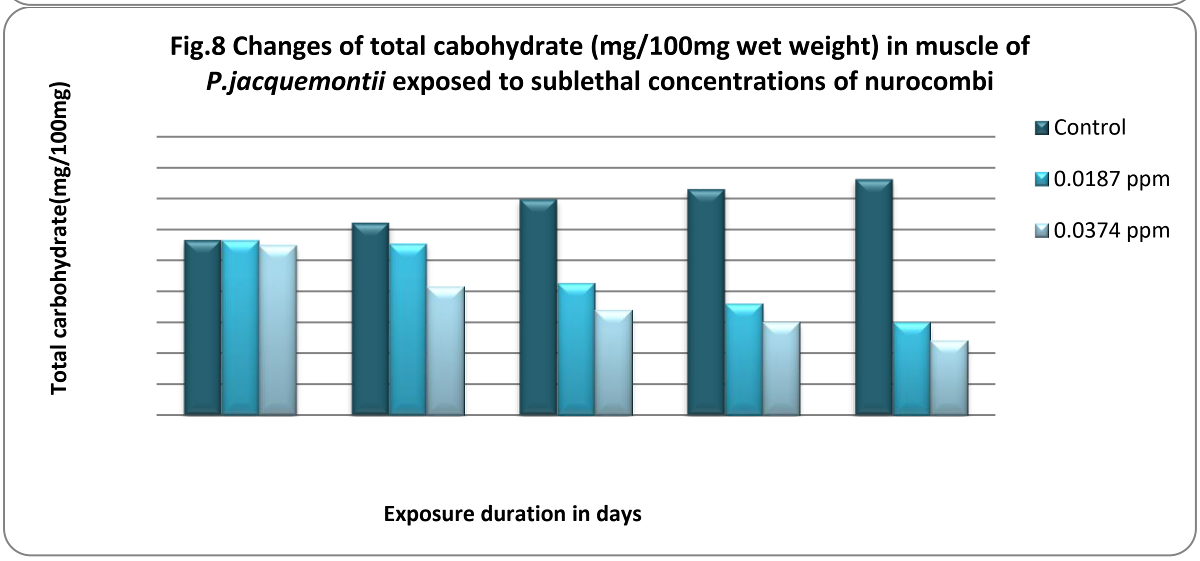


Fig.8 Changes of total cabohydrate (mg/100mg wet weight) in muscle of *P.jacquemontii* exposed to sublethal concentrations of nurocombi
Total carbohydrate(mg/100mg)
Exposure duration in days
Control
0.0187 ppm
0.0374 ppm

Fig. 9 Changes in total carbohydrate(mg/100mg wet weight) in testis of *P.jacquemontii* exposed to sublethal concentrations of nurocombi

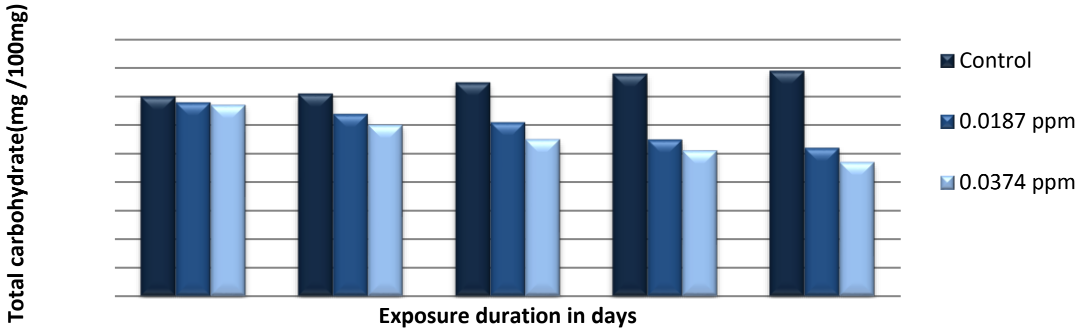


Fig.10 Changes of total carbohydrate(mg /100mg wet weight) in vas deferens of P. jacquemontii exposed tosublethal concentrations of nurocombi

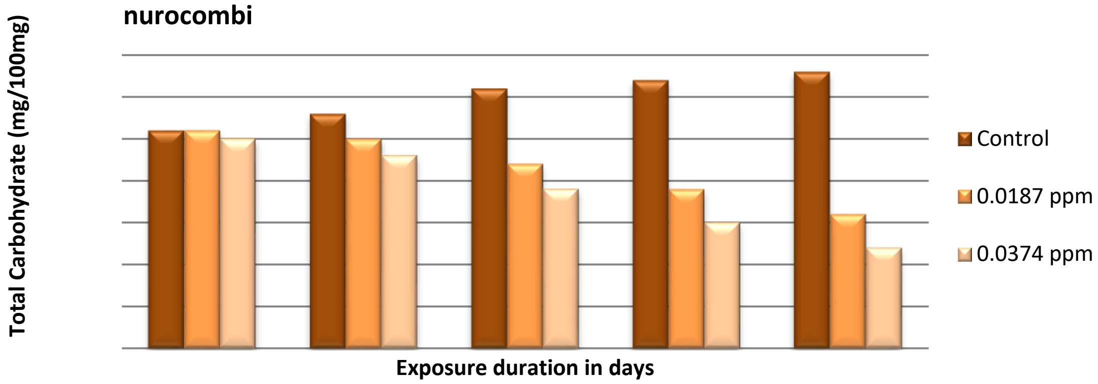
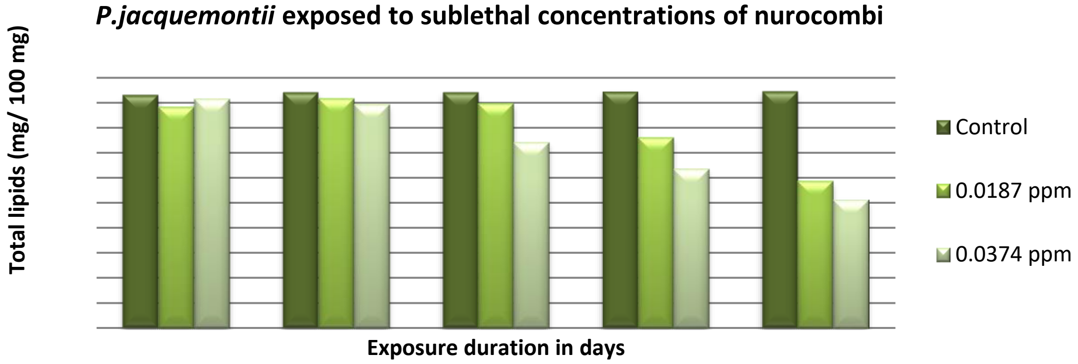


Fig.11 Changes of total lipids (mg/100mg wet weight) in gills of *P.jacquemontii* exposed to sublethal concentrations of nurocombi



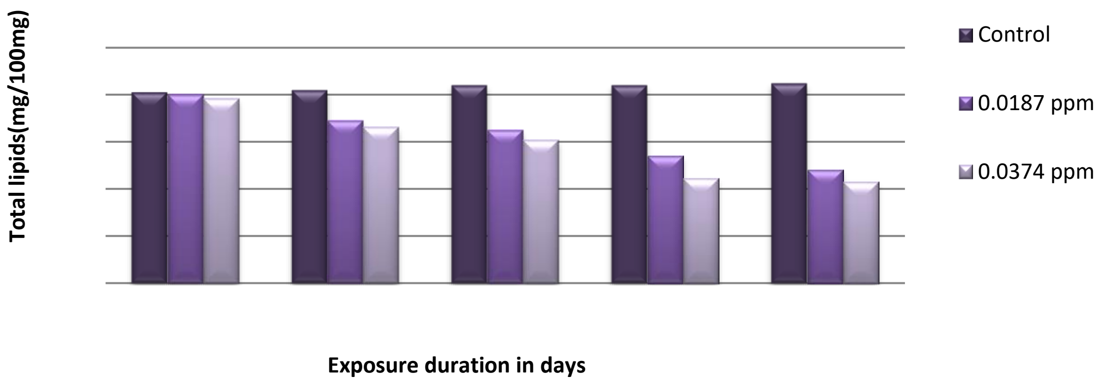


Fig.12 Changes of total lipids (mg/100mg wet weight) in hepatopancreas of *P.jacquemontii* exposed to sulethal concentrations of nurocombi

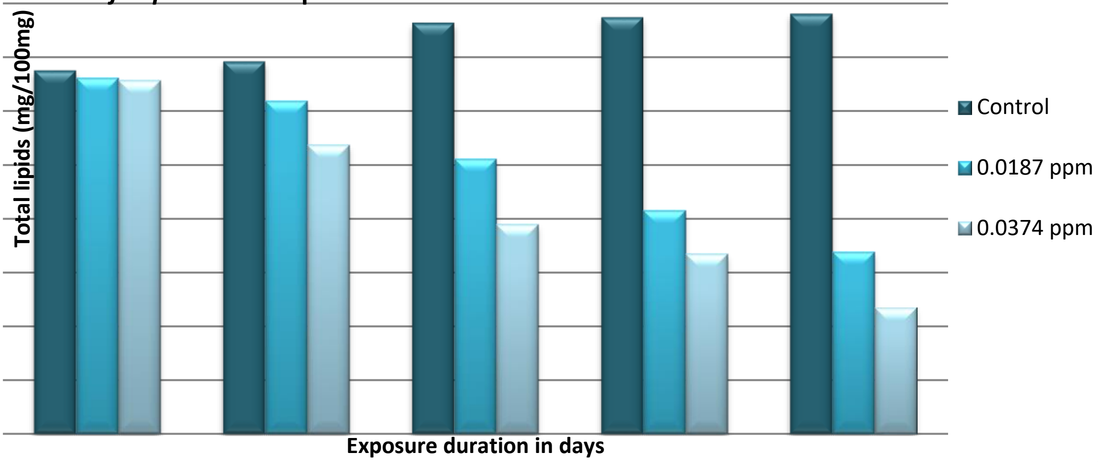


Fig.13 Changes of total lipids (mg/100mg wet weight) in muscle of *P.jacquemontii* exposed to sublethal concentrations of nurocombi

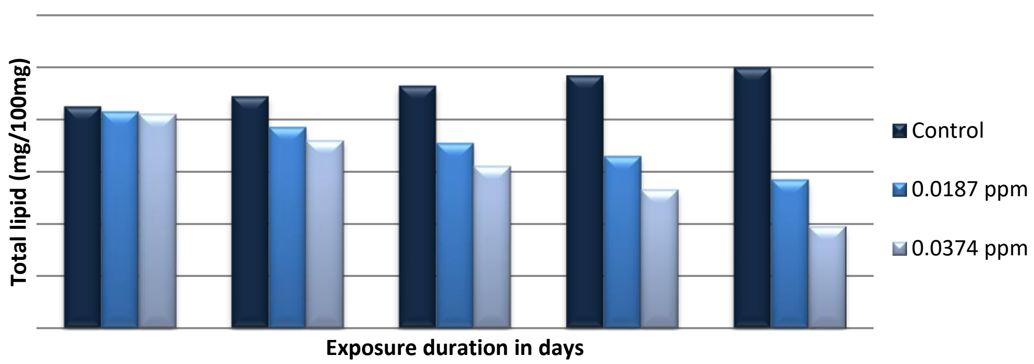
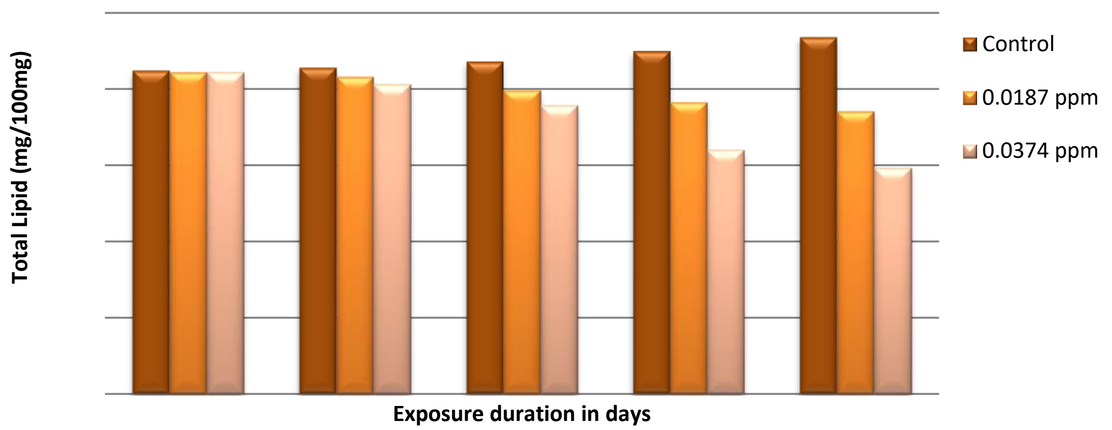


Fig. 14 Changes of total lipids (mg/100mg wet weight) in testis of *P.jacquemontii* exposed to sublethal concentrations of nurocombi

Fig. 15 Changes of total lipid (mg/ 100mg wet weight) in vas deferens of P. jacquemontii exposed to sublethal concentrations of nurocombi



Changes in the TC Levels:

Levels of the TC in different tissues of test *P.jacquemontii* and controls during the exposure period are shown in Figure 6, 7, 8, 9 & 10. The TC concentrations were significantly lower in test *P.jacquemontii* than those of controls on all DoE. The depletion in the TC levels in the MU of test *P.jacquemontii* was significant with the progress in the period of exposure. The levels of the TC in the HP of test *P.jacquemontii* 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 GL>HP>MU>VD>TS. Carbohydrate metabolism is broadly divided into the anaerobic segment or glycolysis in which the breakdown of glucose or glycogen through Embden–Meyerhaf pathway occurs and the aerobic segment that consists of oxidation of pyruvate to acetyl co-A to be utilized through citric acid cycle (Nelson and Cox, 2005). Insecticidal respiratory stress has been found to lead to a hypoxic/anoxic condition (Dezwaan and Zandee, 1972) and pesticides are also known to inhibit energy production by suppressing aerobic oxidation of carbohydrates leading to energy crisis in animals (Kohli *et al*., 1975). As a consequence of hypoxia, the metabolic pathway is shifted from aerobiosis to anaerobiosis and a strong suppression of the specific activities of enzymes involved in glycolysis and glycogen metabolism. These conditions might have depleted the total carbohydrate levels in the shrimps exposed to endosulfan in order to meet the increased energy demands as carbohydrates form the major source of energy under stressful conditions (Hochachka and Somero, 1984). Carbohydrate metabolism is not considered to be a major energy source in fish (Walton and Cowey, 1982), but its importance increases during hypoxia because of activation of anaerobic glycolysis. This may explain the observed depletion of the total carbohydrate levels in test shrimps during the later stages of exposure as a result of increased demand of these molecules to provide energy for the cellular biochemical processes under hypoxic conditions induced by endosulfan. The crustacean hepatopancreas is the vital organ involved in such diverse metabolic activities as synthesis and secretion of enzymes and it is also the major organ of detoxification (Thaker and Hariots, 1989). In *P.homarus homarus* also, a decrease in percentage of total carbohydrate in muscle and hepatopancreas has been induced by copper (Maharajan et al., 2012). Glycogen plays an important role as a readily mobilized storage form of carbohydrate in muscle (Stryer, 1988), which decreases during toxicity as evidenced also in *P. jacquemontii*.

Changes in the TL Levels:

Levels of the TL in different tissues of the test *P.jacquemontii* 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 *P.jacquemontii* exposed to sub-lethal doses of nurocombi were significantly lower than those in controls (P<0.05). The percent decrease in the hepatic lipid was higher in the LI than in the tissues of MU and GL and the order of percent decrease on

28 DoE was found to be GL>MU>HP>TS>VD. The concentrations of the total lipid decreased in all the tissues significantly with the progress of exposure period irrespective of exposure concentrations. The hepatopancreas of crustaceans is analogous to the liver of vertebrates and is the centre of lipid metabolism (Chang and O'Connor, 1983); higher levels of the lipid could be expected in the hepatopancreas compared to other tissues. The evidence of relatively higher lipid deposition in the hepatic tissues has been reported in climbing perch, *A. testudineus* exposed to pesticides (Bakthavathsalam and Reddy, 1981), in the fish, *B. conchonius* exposed to aldi carb (Pant *et al*., 1987), in the penaeid prawn, *M. monoceros* exposed to phophamidon, methylparathion and lindane (Reddy and Rao, 1989), in the freshwater prawn, *M. malcolmsonii* exposed to endosulfan (Bhavan and Geraldine, 1997). In contrast, the concentrations of the lipid decreased significantly in all the tissues of test crabs with no apparent deposition of the total lipid in the hepatopancreas in the present study. In *P. homarus homarus* the effect of copper toxicity results in the reduction of total lipids as reported in crab *Thalamita crenata* (Villalan *et al.,* 1988). Absence of such deposition of the total lipid in the hepatic tissues of *M. monoceros* exposed to endosulfan might be attributed to the differential rates of lipid metabolism in the studied tissues of shrimps (Bakthavathsalam and Reddy, 1981). A significant decrease in muscle and hepatopancreas weight may be due to its utilization for energy during detoxification mechanism.

CONCLUSIONS

These chemical stressors may cause damage at all life stages during the shrimp production. Conception of the mechanisms related to the sub-lethal effects caused by different chemicals upon crab metabolism would help to develop sensitive and precise diagnostic tools Biomarkers with a predictive capability in assessing the toxic effects, thus contributing to better pond management and sustainable aquaculture. Pesticides, as environmental stressors are known to alter serum biochemical parameters in crabs, which suggest that serum biochemical indices could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of nurocombi contamination and crab health.

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

Bakthavathsalam, R. and Reddy, Y.S. (1981). Lipid kinetics in relation to the toxicity of three pesticides in the climbing perch, *Anabas testudineus* (Bloch). *Proc. Indian. Nat. Sci. Acad*., B47: 670-676.

Barnes, H. and Blackstock, J. (1973). Estimation of lipids in marine animals and tissues. Detailed investigation of the sulphophosphovanillin method for total lipids. *J. Exp. Mar. Biol. Ecol.,* 12, 103-118.

Bhavan, P.S. and Geraldine, P. (1997). Alterations in concentrations of protein, carbohydrate, glycogen, free sugar, and lipid in the prawn, *Macrobrachium malcolmsonii* on exposure to sublethal concentrations of endosulfan. *Pestic. Biochem. Physiol.,* 58: 89–101.

Chang, E.S. and O'Connor, J.D. (1983). Metabolism and transport of carbohydrates and lipids. In: Mantel, L.H (Ed.), The Biology of Crustacea, Vol. 5. Internal anatomy and physiological regulation. Academic Press, New York, pp. 263-287.

Dezwaan, A. and Zandee, D.I. (1972). The utilization of glycogen during anaerobiosis in *Mytilus edulis* L. *Comp. Biochem. Physiol.,* 43: 47-54.

Hochachka, P.W. and Somero, G. (1984). Biochemical Adaptation. Princeton University Press, Princeton, New Jersey.

Kohli, K.K., Sharma, S.C., Bhatia, S.C. and Venkitasubramonian, T.A. (1975). Biochemical effects of chlorinate insecticides DDT and dieldrin. *J. Scient. Industr. Res.,* 34: 462-470.

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.

Maharajan, A., Rajalakshmi, S. and Vijayakumaran, M. (2012). Effect of copper in protein, carbohydrate and lipid contents of the juvenile spiny lobster, *Panulirus homarus homarus* (Linnaeus, 1758). *Sri Lanka J. Aquat. Sci*., 17, 19-34.

Mansingh, A. (1987). Pesticide residues the environment. Pesticides and Food (K Leslie ed.). Pp 18-29. Car. Food & Nutr. Instit. (WHO/PAHO) Kign.

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.

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.

Nelson, D.L. and Cox, M.M. (2005). Lehninger Principles of Biochemistry. 4th edn. W.H. Freeman and Company, New York.

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.

Rajendra Prasad Naidu, K. (1985). Impact of endosulfan on carbohydrate and protein metabolism of the freshwater field crab, *Oziotelphusa senex senex* (Fabricius), M.Phil. Dissertation. S.V. University, Tirupathi, India.

Rao, J.V. (2006). Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. *Pestic. Biochem. Physiol.*, 86: 78 - 84.

Reddy, M. S. and Rao, K.V.R. (1988). Effects of technical and commercial grade phosphamidon on the carbohydrate metabolism in selected tissues of penaeid prawn, *Metapenaeus monoceros* (Fabricius). *Bull. Environ. Contam. Toxicol.,* 40: 389-395.

Reddy, A.N., Venugopal, B.R.K. and Reddy, L.N. (1991). Effects of endosulfan 35 EC on certain aspects of protein metabolism in various tissues of a freshwater field crab, *Barytelphusa guerini*. *Pestic. Biochem. Physiol*., 39: 121-129.

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

Shanmugam, M. and Venkateshwarlu, M. (2000). Endosulfan induced changes in the tissue carbohydrate levels of fresh water crab, *Barytelphusa cunicularis*. National Symposium on Prospects of Environment in the new Millennium Mansingh A (1987) Pesticide residues the environment. Pesticides and Food (K Leslie ed.). Pp 18-29. *Car. Food & Nutr. Instit.* (WHO/PAHO) Kign.

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.

Stryer, L. (1988). Biochemistry. 3rd Edn., W.H. Freeman and Company, New York.

Thaker, A.A. and Haritos, A.A. (1989). Mercury bioaccumulation and effects on soluble peptides proteins and enzymes in the hepatopancreas of the shrimp, *Callianassa tyrrhena*. *Comp. Biochem. Physiol.,* 94: 199-205.

Villalan, P., Narayanan, K.R., Ajmal Khan, S. and Natarajan, R. (1988). Proximate composition of muscle, hepatopancreas and gill in the copper exposed estuarine crab *Thalamita crenata* (Latreille). *Pro. II Nat. Sym. Ecotoxicol.,* 55-59.

Walton, M.J. and Cowey, C.B. (1982). Aspects of intermediary metabolism in salmonid fish. *Comp. Biochem. Physiol.,* 738: 59-79.