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CARBOSULFAN IMPACT ON ACETYCHOLINOESTRASE ACTIVITY AND BIOCHEMICAL COMPOSITION OF FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

Exposure of the freshwater prawn *Macrobrachium rosenbergii* to a lethal concentration of carbosulfan (5.0 and 15.0 µg/L) for 24 hr lead to inhibition in AChE activities from 34% to 46%. While, at concentration 25.0 µg/L the moribund animals showed more inhibition, reaching to 52% and more highly significantly reduction in the AChE activity. Recovery of AChE activity to normal levels from a 24hr-exposure to 5 µg/L carbosulfan required approximately 12 day. Sublethal chronic exposure (21d) at a concentration of 1.66 µg/L caused significantly reduction in muscle AChE activity of 16% and highly significant reduction in hepatopancreas AChE activity of 35%. Also, exposure of *M. rosenbergii* to sublethal concentration of carbosulfan (1.66 µg/L) for 96 hr severely affected various physiological mechanisms which was reflected in alterations in concentrations of various biochemical constituents such as protein, carbohydrate and lipids. The total protein concentration was more significantly increased in gills; however, the total protein concentration in the hepatopancreas and muscle of test prawns was lower on all sampling days, than that in same tissues of controls. Carbohydrate of test prawn was lower in gills, hepatopancreas and muscles when compared to control. Total lipid in hepatopancreas was significantly increased as consequence of 96hr of 1.66 µg/L carbosulfan. On the contrary, total lipid in muscles was significantly decreased than control. These data strongly suggested that carbosulfan is toxic to *M. rosenbergii* since it seriously impairs the metabolic functions, resulting in alterations in major biochemical constituents, particularly in the muscle. Since the nutritive value of this prawn was greatly affected by exposure to carbosulfan.

1. INTRODUCTION

Organophosphorous (OP) and carbamate (CB) pesticides are commonly used in agriculture practices as insecticides because they are, in general, more biodegradable and have less persistence in the environment than organochlorine pesticides. However, they are toxic to non target species at low concentrations. The toxicity of these pesticides is mainly due to the inhibition of AcetylCholinEsterase (AChE) activity, which is responsible for terminating the transmission of nerve impulses. The inhibition of AChE provokes an accumulation of acetylcholine at the nerve synapses and disruption of the nerve function (Peakall, 1992; Sturm & Hansen, 1999 and Varó, *et al*., 2002).

The recent development of biomarkers based on the study of biological responses of organisms to pollutants has provided the biochemical tools essential to the implementation of programs for monitoring

of contaminants effects. Cholinoesterase inhibition has been used for years as a marker of exposure to insecticides, not only in humans but also in wild life in order to monitor the effects of contaminants on living organisms (de la Torre *et al.*, 2002; Forget *et al.,* 2003; Barata *et al.*, 2004; Bonacci *et al.,* 2004; Ashauer *et al.,* 2006; Frasco *et al.,* 2006; Fernandez, 2007 and Uereb *et al.,* 2007).

Degradation of the insecticides in the aquatic environment results in reduced exposure and allows organisms to recover from the poisoning. Recovery of AChE activity following exposure to insecticides has been found to be a process that takes time, depending on factors such as type of insecticide, test species, and the extent of depression of AChE activity (Abdullah *et al*., 1994).

There are several studies which described the effects of sublethal exposure to contaminants on the biochemical composition of freshwater and marine crustaceans (Dickson *et al.,* 1982; Radhakrishnaiah & Busappa, 1986; Wany & Stikle, 1988; Surendranath *et al*., 1991; Torrebalance *et al.,* 1991 & 1992; Bhavan & Geraldin, 1997 & 2002; Sharshar 2000). Biochemical responses in prawns to various contaminants were reviewed by Bainy (2000) and Lingot *et al*. (2000).

Although the toxic effect of pesticides on various biochemical constituent have been documented in aquatic non target organisms *(*Rafi *et al.,* 1991; Reddy *et al.,* 1991 a & b). There is paucity of data relating to the effects of such pesticides on biochemical aspects in crustacean in general and in *Macrobrachium* species in particular.

Since, they seriously impair the metabolic functions, resulting in alterations in major biochemical constituents such as carbohydrate levels which were studied in hepatopancreas and muscles. The most important pollutants uptake from water is route, of gills which is the primary target organ and may be one of the first organs to exhibit symptoms of sublethal toxicity. Furthermore, there are biochemical components other than carbohydrates such as protein and lipids that also act as energy sources.

In recent year, the monoculture of prawn *M. rosenbergii* in ponds and its polyculture with fishes have been found profitable as a protein rich source of food by population (Kanaujia & Mohandy, 1996). The prawns were then used to monitor the aquaculture pond with pesticides.

The present work aims to study the change in AcetylCholinoEstrase (AChE) activity of freshwater prawn *Macrobrachium rosenbergii* after acute lethal exposure (24 hr exposure) to different concentrations of carbosulfan followed by 21 day recovery period for understanding nature of intoxication. Also, assess the effect on AChE activity of *M. rosenbergi*i after exposed to sub lethal chronic concentration of carbosulfan (1.66 µg/L) for 21 day .

Moreover, alteration of the biochemical constituents of some organs of *M. rosenbergii* exposed to sublethal concentration of carbosulfan (1.66 µg/L) for 96 hr was investigated.

2. MATERIALS AND METHODS

2.1. Animals

Healthy juveniles of the freshwater prawn *Macrobrachium rosenbergii* were obtained from the hatchery of Saft-Khaled Fish Farming at Behera Governorate, Egypt. They were transferred from the farm in large plastic bag filled to its third volume with water and the other 2/3 part was left free to keep the prawn alive until reaching to the laboratory. The specimens were put in glass aquarium of 70 x 35 x 35 cm. It was filled with dechlorinated tap water and was supplied with air pump for continuous aeration and heater to maintain constant temperature of 28°C which was suitable to this type of prawn. It was covered to prevent the escape of specimens. Suitable amount of

dried prawn powder minced fresh fish were used daily as food while the uneaten food and excrements were removed by siphoning. Approximately while half of water volume was replaced with fresh dechlorinated tap water each day.

2.2. Determination of AChE activity in whole tissue of prawn

2.2.1. Acute experiment

In this experiment, about 80 samples were used. They were divided into 10 groups, one group served as control and the other groups were exposed to nominal concentrations of 5.0, 15.0, 25.0 µg/L of carbosulfan. The exposed prawns were observed for mortality for up to 5 hr., after 24 hr. dead animals were removed and while the moribund and survivor individuals were frozen at –20°C for AChE analysis. Also, control prawn were removed after 24 hr. and frozen.

2.2.2. Recovery experiment

Prawns were exposed to 5.0 µg/L of carbosulfan for 24 hr. and thereafter transferred into clean water and maintained for a period of 12 day. During this period water was changed every day and a sample of prawns were taken every 4 days and frozen until analysis.

2.2.3. Chronic experiment

50 samples were used and divided into two groups; one group served as control (free from insecticide) and the other group was treated with sublethal concentration of carbosulfan (1.66 µg/L) for a period of 21 days. This experiment was carried out under semi static conditions, whereby the solution was renewed every 24 hr. After this period, the prawns from control and treated groups were dissected and the muscles and hepatopancreas were frozen until analysis.

2.2.4. Assay of AChE activity

Juvenile prawns were pooled (2-4 individuals) in triplicate and homogenized in cold 0.1 M phosphate buffer pH 8. AChE analysis was conducted using the calorimetric method of Ellman *et al*. (1961) using acetylthiocholine as substrate, in a 5 ml test tube, 20 µl of the prepared tissue homogenate was added to 2.9 ml of 0.1 M phosphate buffer pH 8 and 0.1 ml mM DTNB reagent solution (39.5 mg of 5.5 dithiobis –2-nitrobenzoic acid). To the above mixture, 0.02 ml of the substrate (0.075 M) acetylthiocholine iodide was added. The optical density of the developed yellow color was recorded after 10 min against blank, which contained the entire reagent except the enzyme at 412 nm, using a spectronic 20.

While the Lowery *et al.* method (1951) was used for quantitative determination of protein, using bovine serum albumin as the standard. All spectrophotometric measurements were triplicated and the average change in absorbance per minute was determined. AChE activities were expressed as microunit of substrate per gram protein (µU/g protein). A unit is defined as the conversion of 1 mol of substrate to products in 1 min.

Analysis of variance (ANOVA) was used to determine if significant group differences ($p \leq 0.05$).

2.2.5. Effect of carbosulfan on biochemical constituents of prawn

About 80 specimens were divided into two equal groups, one group served as control and the second group was exposed to sublethal concentration of carbosulfan 1.66 µg/L (1/10 of 96 hr.LC_{50}) for 12, 24, 48 and 96 hr. On each time interval, gills, hepatopancreas and muscles were removed by dissection from 10 prawns and kept frozen until analyzed. During the test the mean water temperature was 28 ±2 °C.

Protein was determined by Bradford method (1976), 1 g of the sample was

extracted with Tris Hcl (0.2 M, pH 7.9). The sample was kept at 4°C over night, filtered by cheese cloth and centrifuged at 10000 rpm for 25 min, 100 µl of extract was added to 1 ml Bradford reagent and measured at 595 nm (Beckman DU 7400 spectrophotometer)

Carbohydrate was determined by adding nearest 1 g of sample to known volume of hydrochloric acid (1 N) and sealed tightly and kept in the oven at 90 °C over night, and filtered .For the precipitation of protein, a saturated solution of lead acetate di basic was dropped one by one wisely till turbidity, and was filtered. Then 1 ml extraction plus 1 ml phenol (5%) and 5 ml Conc. H_2SO_4 respectively were left for 10 min then measured at 490 nm (Beckman DU 7400 spectrophotometer).

Lipid was determined by using Sokselet method (AOAC, 1984).

3. RESULTS

3.1. Effect of acute lethal concentrations of carbosulfan on the activity of AChE

The effects of exposure to different lethal concentrations of carbosulfan on the activity of acetylcholinesterase (AChE) in *M. rosenbergii* were studied as shown in table (1). At 5.0 and 15.0 µg/L of carbosulfan concentrations, AChE activity of moribund investigated prawns was inhibited from 34% to 46% and the activity of AChE was found to be highly significant. While, at concentration 25.0 µg/L the moribund animals showed more inhibition that reached to 52% and was more highly significantly reduced in the AChE activity (Table 1,& Fig.1). On the other hand, the present study revealed that at higher concentrations of carbosulfan (5.0, 15.0 & 25.0 µg/L), death of the exposed prawns was accompanied by large depression in AChE activity.

Recovery is defined as a significant increase in AChE activity as a function of time that occurs following cessation of exposure to anticholinesterase agent (pesticides). At the end of 24 hr exposure period, prawn subjected to 5.0 µg/L carbosulfan exhibited significantly depressed AChE activity (34% inhibition). AChE activity remained further depressed (38 % inhibition) 4 days after the transferred into uncontaminated water (Fig. 2). Measured AChE activities on and subsequent to 12 day were not considered significantly different from those in control animals.

The present results indicate that complete recovery was attained approximately 12 day following 24 h exposure to 5.0 µg/L carbosulfan (Table 2).

Table (1): Mean AChE activity of *Macrobrachium rosenbergii* after exposure to various concentrations of carbosulfan (24 hr.).

Insecticides	Concentrations	AChE activity [a] (µU/g protein)	% inhibition [b]
Carbosulfan	Control	60.76 ± 3.81	-
	5.0 µg/L	40.03 ± 1.22***	34
	15.0 µg/L	32.68 ± 1.48***	46
	25.0 µg/L	29.02 ± 1.23****	52

[a] Mean value of three replicates± S.D
[b] Compared with control
*** Highly Significant P < 0.001
**** More highly Significant P < 0.0001

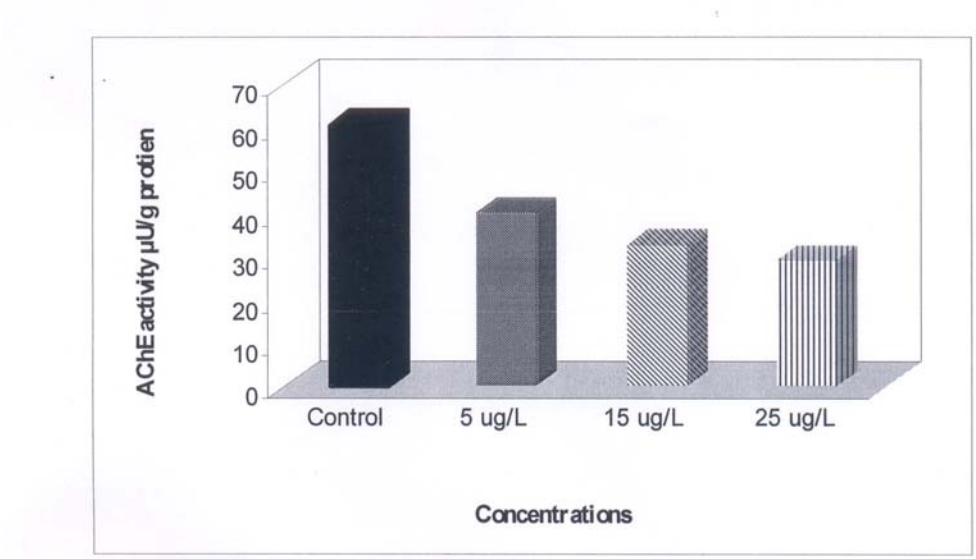

Fig. (1): AChE activity of *Macrobrachium rosenbergii* after exposure to various concentrations of carbosulfan (24 hr).

Table (2): Recovery of AChE activity in *Macrobrachium rosenbergii* subsequent to 24-hr exposure to 5.0 µg/L carbosulfan.

Insecticides	Time interval (Day)	AChE activity [a] (µU/g protein)		% Inhibition [b]
		Control	Experimental	
Carbosulfan	4	64.47 ± 2.56	37.61 ± 2.67***	42
	8	59.85± 2.06	44.4 ± 1.18***	25
	12	60.96 ± 1.96	54.41 ± 3.74 [n.s]	10

[a] Mean value of three replicates± S.D
[b] Compared with control
[n.s] Non significant P > 0.05
***Highly significant P < 0.001

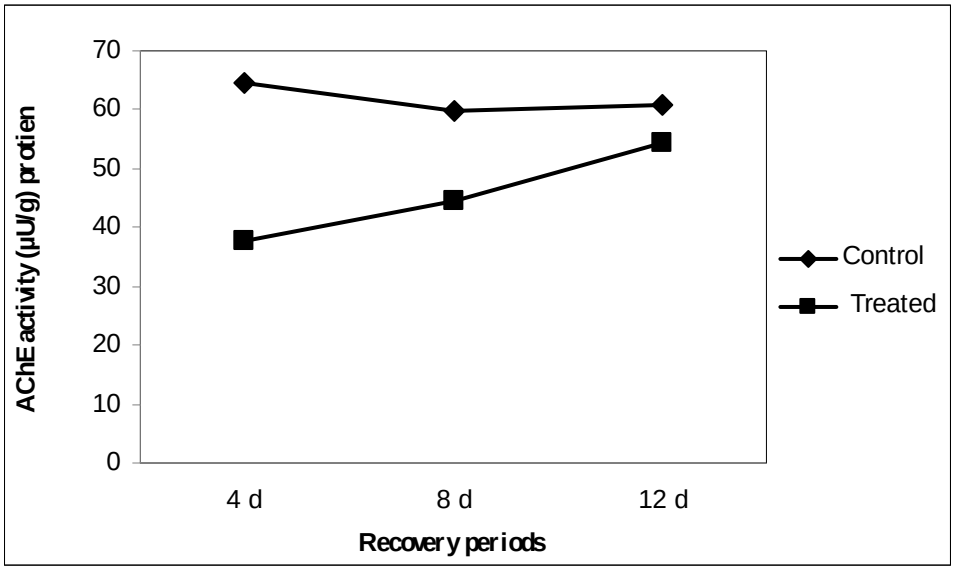

Fig. (2): Recovery of AChE activity in *Macrobrachium rosenbergii* subsequent to 24-hr exposure to 5.0 µg/L carbosulfan

3.2. Effect of chronic sublethal concentration of carbosulfan on the activity of AChE in muscle and hepatopancreas

The amount of AChE activity was determined in muscles and hepatopancreas of freshwater prawn in control and after exposure to 1.66 µg/L of carbosulfan for 21 day (Table 3). The AChE from muscles showed the highest activity compared to hepatopancreas. After exposure to 1.66 µg/L carbosulfan the muscle AChE activity was significantly reduced (16% inhibition) while, the reduction in hepatopancreas AChE activity was 35% significant compared to the control at 21 day (Table 3 and Fig. 3). The mortality rate was determined as 40%.

3.3. Effect of chronic sublethal concentration of carbosulfan on biochemical constituents

As it is clear from the results in table (3) and figure (3), the most remarkable effect of carbosulfan on gills tissue was statistically more significant increased in protein concentration ($P < 0.01$). However, Carbohydrate concentration was highly significant decreased ($P < 0.001$) in carbosulfan-exposed group than in control after 12 hr., but more significant decrease ($P < 0.01$) after 24, 48 and 96 hr. After 96, hr carbosulfan treatment showed a slight decrease in lipids concentration was observed, but there was no significant decrease after 48 hr. with values corresponding to control prawns.

After 96 hr. of sublethal concentration of carbosulfan exposure, protein and carbohydrate concentration in hepatopancreas was more significantly lower when compared to control prawn (Table 5). On the contrary, total lipids in the hepatopancreas was significantly increased as a consequence of 96 hr. of carbosulfan exposure.

The protein level was 23.53 g/100g in the prawn muscles of the control specimens. Meanwhile, it was significantly decreased to 19.58 g/100g after treatment with 1/10 LC_{50} carbosulfan for 96 hr (Table 6 and Fig.6). Also, carbohydrate percentage in muscle of

treated prawns with carbosulfan showed significant decrease from that of control after 24, 48 and 96 hr. The lipids content in muscles of the control prawn was 14.34 g/100g and significantly decreased to 12.11 and 11.27 g/100g after treatment with sublethal concentration of carbosulfan for 48 and 96 hr.

Table (3): Mean AChE activity in muscles and hepatopancreas of *Macrobrachium rosenbergii* after exposure to 1.66 µg/L of carbosulfan for 21 days.

The tissue	AChE activity [a] (µU/g protein)		% Inhibition [b]
	Control	Treated	
Muscles	77.04 ±1.0	64.61 ± 4.08*	16
Hepatopancreas	3.69 ± 0.52	2.38 ± 0.57*	35

[a] Mean value of three replicates± S.D
[b] Compared with control
* Significant $P < 0.05$

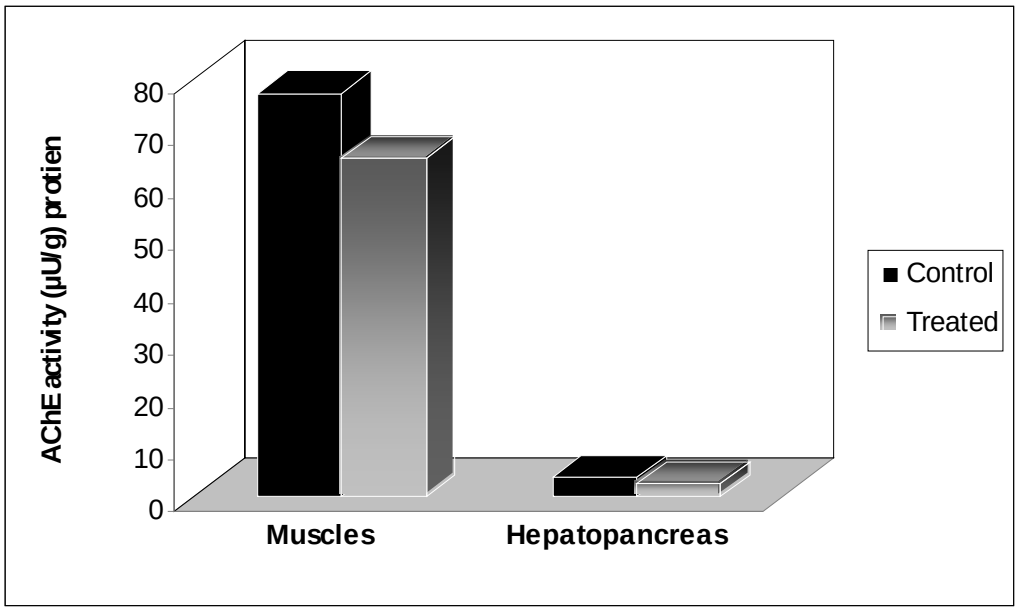

Fig. (3): Mean AChE activity in muscles and hepatopancreas of *Macrobrachium rosenbergii* after exposure to 1.66 µg/L of carbosulfan for 21 day.

Table (4): Effect of sublethal concentration of carbosulfan on biochemical constituent of gills of *Macrobrachium rosenbergii.*

Biochemical Constituents	Control	Time exposure			
		12 hr	24hr	48 hr	96 hr
Protein	14.63 ± 0.59	15.81 ± 0.28 *	16.42 ± 0.52 *	16.69 ± 0.74 *	19.90 ± 1.60 **
Carbohydrate	36.19 ± 1.04	26.67 ± 1.17***	24.55 ± 0.67**	30.27 ± 1.20**	31.34 ± 1.11**
Lipids	18.38 ± 0.74	17.76 ± 0.8 n.s	17.22 ± 1.15 n.s	17.09 ± 1.32 n.s	16.45 ± 0.8 *

Each value of three replicates ± SD
n.s Non significant P > 0.05
*** Significant P < 0.05**
More significant P < 0.01**
***** Highly significant P < 0.001**

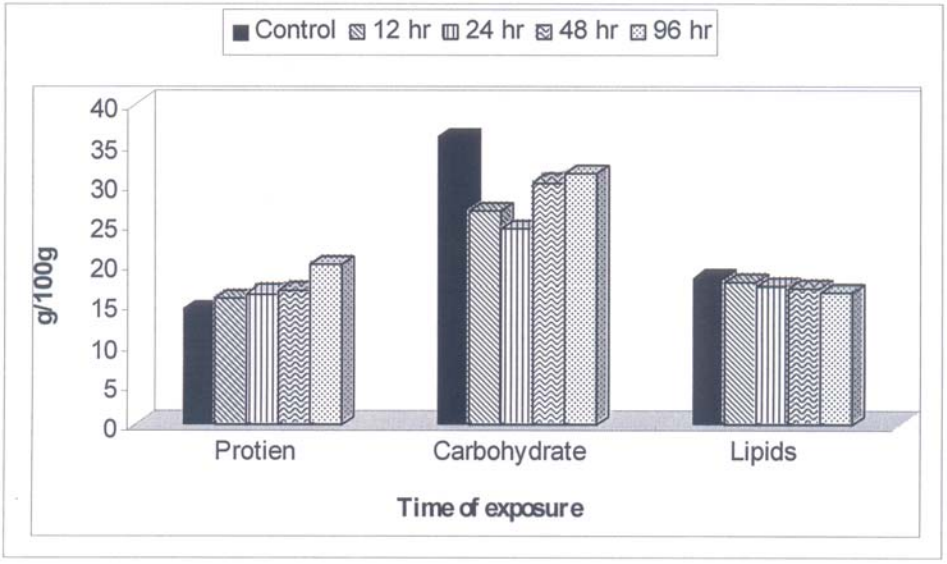

Fig. (4): Biohemical constituent of gills of prawn *Macrobrachium rosenbergii* exposed to 1/10 LC_{50} of carbosulfan.

Table (5): Effect of sublethal concentration of carbosulfan on biochemical constituent of hepatopancreas of *Macrobrachium rosenbergii*.

Biochemical Constituents	Control	Time exposure			
		12 hr	24hr	48 hr	96 hr
Protein	19.48 ± 0.71	14.52 ± 0.48 **	12.35 ± 1.01***	15.57 ± 1.24**	15.45 ± 1.12**
Carbohydrate	28.28 ± 0.14	27.25 ± 0.36***	27.28 ± 1.03***	26.72 ± 1.01**	25.83 ± 1.28*
Lipids	19.38 ± 0.6	20.43 ± 1.08 n.s	20.92 ± 1.06 n.s	21.40 ± 1.15 n.s	22.16 ± 0.6 *

Each value of three replicates ± SD
n.s Non significant P > 0.05
* Significant P < 0.05
More significant P < 0.01**
*** Highly significant P < 0.001

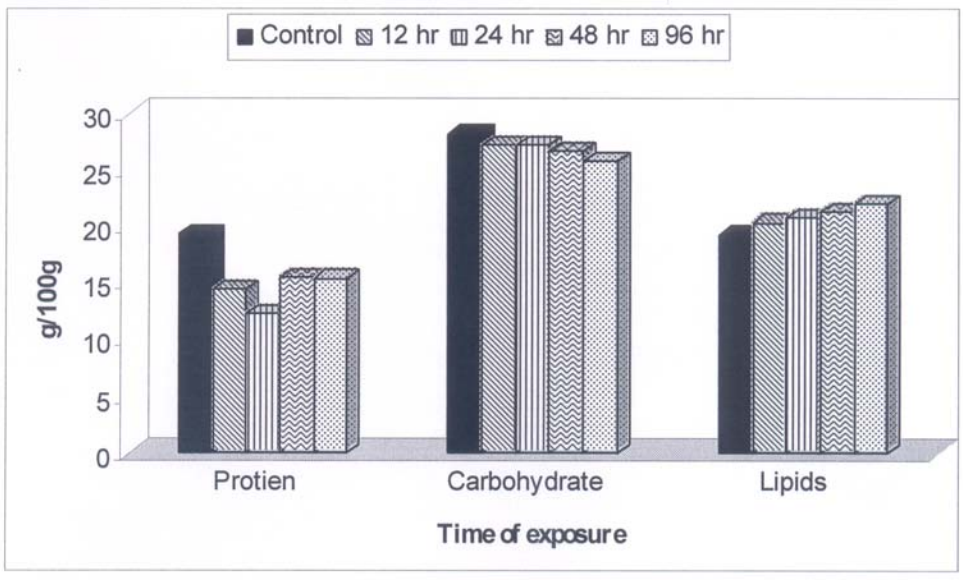

Fig. (5): Biochemical constituent of hepatopancreas of prawn *Macrobrachium rosenbergii* exposed to 1/10 LC_{50} of carbosulfan.

Table (6): Effect of sublethal concentration of carbosulfan on biochemical constituent of muscles of *Macrobrachium rosenbergii*.

Biochemical Constituents	Control	Time exposure			
		12hr	24 hr	48 hr	96 hr
Protein	23.53 ± 1.39	22.14 ± 0.6 $^{n.s}$	21.42 ± 0.96 $^{n.s}$	20.91 ± 0.56 $^{n.s}$	19.58 ± 0.68 *
Carbohydrate	23.72 ± 1.11	22.21 ± 0.83 ^{n}	21.27 ± 1.05 *	20.42 ± 0.95 *	20.15 ± 1.08 *
Lipids	14.34 ± 1.01	13.85 ± 1.08 $^{n.s}$	13.08 ± 1.66 $^{n.s}$	12.11 ± 1.00 *	11.27 ± 0.58 *

Each value of three replicates ± SD
$^{n.s}$ Non significant P > 0.05
* Significant P < 0.05

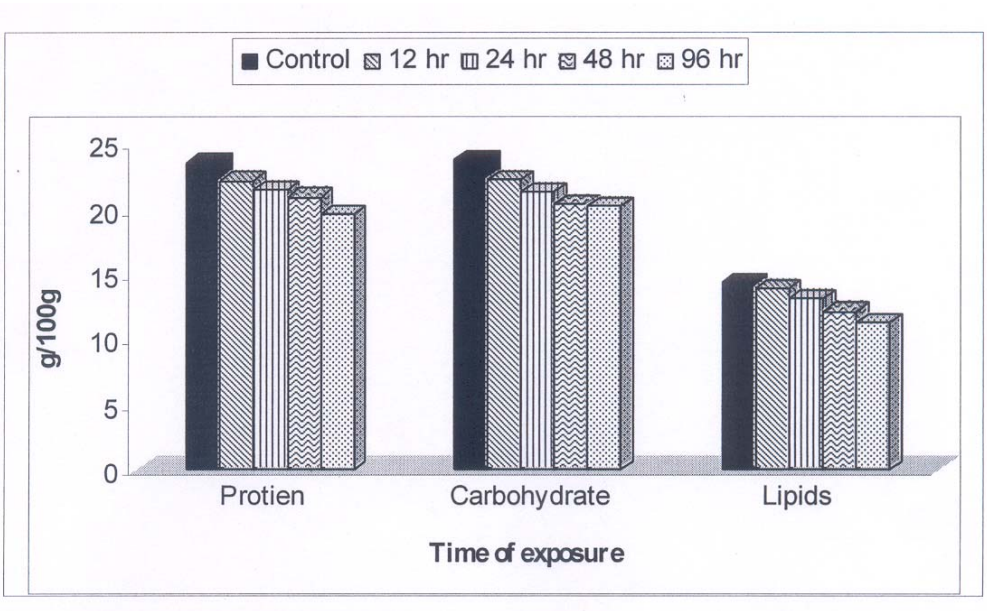

Fig. (6): Biochemical composition of muscles of prawn *Macrobrachium rosenbergii* exposed to 1/10 LC_{50} of carbosulfan.

4. DISCUSTION

The present study showed a clear variability in AChE activity patterns of affected prawn exposed to different insecticides under various concentrations. Significant depression in AChE activity was observed not only at acute lethal concentrations, but also at sublethal concentrations.

Organophsphorous (OP) and carbamate (CB) compounds produce specific inhibition of AChE, which in some cases is accompanied by the inhibition of neuro-target esterase (NTE). The modification of NTE activity (esterase activity at pH 8) is responsible for the appearance of the syndrome of delayed neurotoxicity induced by some OP compounds (Repetto *et al.,* 1988).

The inhibition of AChE activity and accumulation at the synaptic junctions caused by carbosulfan exposure may lead to the observed behavior as hyperexcitability, restriction and lost coordination and equilibrium and ultimated of the organism (Ecobichon, 1996 and Fulton & Key, 2001)

In this study, the activity of AChE in *M. rosenbergii* was significantly inhibited after 24 hr of exposure to all concentrations of carbosulfan. It was found that deaths of exposed prawns were accompanied by depression in AChE of 52% for carbosulfan. There is some controversy in the literature as to the extent of AChE depression required to cause death in aquatic organisms. Some animals are able to survive with more than 50% AChE inhibition but this is an indication of life-threating situation (Ludke *et al*., 1975). In crustaceans, lethal effects have been described at a AChE inhibition of 40% or below (Bocquene *et al*., 1991 and Escartin & Porte, 1996), whereas other studies have reported that a 30% reduction in AChE activity is not lethal (Lunebye *et al*., 1997). Studies with *Penaeus duoraum* showed a mean inhibition of AChE activity of 75% in moribund shrimp following 48 hr exposure to 1000 µg/L malathion (Coppag & Matthews, 1974). Reddy and Rao, (1988) noted inhibition in AChE activity of 64 % and 54 % in the nervous tissue of the prawn *Metapenaus monceros* following 48 hr exposure to lethal concentrations of methyl parathion (120 µg/L) and phosphamidon (1200 µg/L) respectively. Abdullah *et al*. (1994), showed that the organophosphorous insecticide profenofos induced inhibitory effect on the AChE activity of the freshwater shrimp (*Paratya australiensis*) ranging from > 40% inhibition at sublethal concentration (0.1 to 1.0 µg/L) to > 80% at lethal concentration (10 µg/L.). Varó *et al*. (2002) showed that AChE activity of both *Artemia salina* and *Artemia parthenognetica* was significantly inhibited after 24 hr of exposure to all concentrations of dichlorvos and chlorpyrifos by more than 80 % inhibition at the lowest concentration.

In the present study, the AChE activities of hepatopancreas and muscles of prawns exposed to sublethal concentration of carbosulfan have significant decrease compared to those prawns in control solution. This finding agrees with Chin *et al.* (2006) who found, that the OP insecticide trichlorofon induce a significant inhibition in haemolymph, muscles and hepatopancreatic AChE activities of *M. rosenbergii* following 24 hr to more than 0.2 and 0.4 mg/L trichlorfon respectively. These facts suggest that the insecticide might have been acting to reduce ventilation of the gills of *M. rosenbergii* by causing an accumulation of AChE at neuromuscular junctions.

In the present study, recovery of AChE activity to normal levels following 24 hr exposure to 5.0 µg/L of carbosulfan appeared to take 12 day for carbosulfan. It has been found that, AChE activity remained further depressed after being transferred into uncontaminated water, presumably due to the presence of the activated form of the insecticide within the shrimp following in

vivo activation of insecticide to its Oxon form (Murty, 1986). Abdullah *et al*. (1994) found that recovery of AChE activity to normal level from 24 hr exposure to 0.1 µg/L profenofos required less than 9 days. Also, Reddy and Rao (1988) observed that the penaeid prawn, *Metapenaeus monoceros* required 7 days to recover their AChE activity to normal levels, subsequent to 48 hr exposure to sublethal concentrations of phosphamidon and methyl parathion.

In this study, the carbamate insecticide carbosulfan appeared to alter the concentrations of major biochemical constituent in different tissues of *M. rosenbergii*. The concentration of total protein of muscles and hepatopancreas in test prawns was found to be lower than those in control on all exposure times; a possible explanation for this finding is that proteolytic activity was induced in these organs due to the stress.

Bhavan and Geraldine (1997) stated that, the concentration of total protein in muscles and gills of *Macrobrachium malcolmsonii* exposed to sublethal concentration of endosulfan was decreased than that in control. Similar findings have been noted in the freshwater prawn *Macrobrachium kistensis* on exposure to naphthalene and pesticides (Nagabhushanam *et al.,* 1987 and Jaiswal *et al.,* 1991), in *Macrobrachium malcolmsonii* exposed to carbaryl (Bhavan & Geraldine, 2002). Reddy *et al.* (1991) stated that the reduced protein level infreshwater field crab *Barytelphusa guerini* exposed to endosulfan because of physiological compensatory mechanisms was activated to compensate for osmoregulatory problems (arising out of leakage of ions and other essential molecules) by enhancing the free amino acid level in blood.

In this study, the concentration of total protein in gills of test prawns exposed to carbosulfan was found to be higher than in control in all exposure times, this may be due to initial enhanced synthesis of protein possibly to repair damaged cell organeles to serve as compensatory tool to restore enzyme loss due to tissue necrosis and to meet the increased demand to detoxify the insecticide.

Because of carbohydrate represents the principal and immediate energy precursor for organisms exposed to stress (Umminger, 1970) and insecticidal stress has been found to lead to an hypoxic / anoxic condition (Dezwaan and Zandee, 1972). This promotes anaerobic glycolysis and decrease in oxidative metabolism (Reddy, 1986) which necessitate the utilization of carbohydrate to meet energy demand. Also,carbohydrate may also be mobilized into the haemolymph as free sugar to meet and increase energy demand, as observed in *Channa punctatus* exposed to endosulfan (Murthy & Davi, 1982). Thus ,the concentration of total carbohydrate in the hepatopancreas, gills and muscles of test prawns was found to be lower than those in the same organs of control on all times of exposure. This result agrees with the previous findings of Surendranath *et al.* (1991) and Bhavan & Geraldine (1997 & 2002).

Also, the concentration of lipid in muscles and gills of test prawns was lower than in the same organs of control. Similar observation has been found in *Macrobrachium malcolmsonii* exposed to endosulfan and carbaryl respectively (Bhavan & Geraldine, 1997 & 2002). Lipids are reported to serve as alternate source of energy in crustaceans, particularly during stress condition (Chang & O'Connor, 1983), which might reflect an accelerated hydrolysis of lipid in order to cope with the increased energy demand occurring due to carbosulfan toxicity. In contrast to the present results, Surendranath *et al*. (1991) reported that the total lipids in muscles rose when the penaeid prawn *Metapenaeus monoceros* was exposed to kelthane.

But, the lipid concentration in the hepatopancreas of test prawns was found to be higher than that of hepatopancreas of control; similar findings have been made in the prawn *Macrobrachium malcolmsonii* exposed to endosulfan (Bhavan & Geraldine, 1997), brine shrimp *Artemia* exposed to

cypermethrin (Piska *et al*., 1988), in the freshwater murrel and in climbing perch *A. testudinus* exposed to pesticides (Bakthavathalam & Reddy, 1981). This was possibly due to canalization to the hepatopancreas of lipid breakdown component from other tissues, the subsequent derangement in lipid due to the rapid penetration of carbosulfan through the biological membrane and its specific affinity toward lipid. Bhavan *et al*. (1997) have been found that endosulfan accumulates to a greater extent in the hepatopancreas than in the gills and muscle of *Macrobrachium malcolmsonii* .

The concentration of major biochemical constituents especially total protein content in the muscle was lower than in the control, since this reflects a loss of nutritive value of the prawn *Macrobrachium rosenbergii* and poses a serious threat to its potential use as a food stuff. Thus, all possible measures should be taken to ensure that carbosulfan and other pesticides do not pollute fisheries, aquaculture farms and other resources.

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