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BIOCHEMICAL ALTERATIONS IN CERTAIN TISSUES OF FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII* (DE MAN) EXPOSED TO PERMETHRIN

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ABSTRACT: The toxicity of permethrin to juveniles of *Macrobrachium rosenbergii* was determined by 96-h bioassays. Mortality was recorded and test solutions were changed completely each day up to 96-h. The LC_{50} value was 0.02µg/L. Juveniles of *Macrobrachium rosenbergii* were exposed to three sublethal concentrations of permethrin (1.3, 2, and 4 ng/L) for 21 days. Samples were taken from the hepatopancreas, gills, and muscle of representative prawns from each test and control group on the 1, 8, and 21 days after exposure. The total protein concentration was found to be higher in the prawns hepatopancreas particularly those exposed to 1.3 ng/L of permethrin, than in the control. However, the total protein concentration in the gills and muscle was found to be lower than that in the control. The total protein content of gills and muscles was the lowest in prawns that had been exposed to 4 ng/L of permethrin. Permethrin at the tested concentration decreased total carbohydrate, glycogen and free sugar content in hepatopancreas, gills, and muscle. The total lipid was found to be higher in the hepatopancreas of the test prawns, especially those exposed to 4 ng/L of permethrin, than in the hepatopancreas of controls. These results indicate that permethrin is too toxic to *Macrobrachium rosenbergii* since it seriously impairs the metabolic functions, resulting in alterations in major biochemical consitituents, particularly in the muscle.

Key words: Biochemical, certain tissues, freshwater prawn, permethrin

INTRODUCTION

The pyrethroid insecticide permethrin is used to control pest insects in agriculture, household, forestry, and in public health programs, including head lice control. It is toxic to honey bees and other beneficial insects, fish, aquatic insects, crayfish, and shrimp. For many species, concentrations of less than one part per billion are lethal. Permethrin has been found in streams, ground water, and rivers. It washed down to streams and estuaries its residues could adversely affect populations of commercially important crustaceans (Cox, 1998). Although the toxic effects of pesticides on various biochemical constituents have been documented in aquatic nontarget organisms (Geraldine *et al.* 1999; Galindo-Reyes *et al.* 2000), there is a paucity of data relating to the effects of such pesticides on biochemical constituents in crustaceans, in general, and in *Macrobrachium* species, in particular. Therefore, the present investigation was undertaken to study the alterations in biochemical constituents of the prawn *Macrobrachium rosenbergii*, following exposure to three levels of sublethal concentrations from permethrin.

MATERIALS AND METHODS

Healthy juveniles of the freshwater prawn *Macrobrachium rosenbergii* were collected from Maryut Fish Farming Company, Alexandria, Egypt. The juveniles were acclimatized to laboratory conditions with dechlorinated tap water for 2 weeks in 25-L glass tanks at 22 ±2°C. The prawns were fed with Tetramin R Fish Flakes. The water was renewed daily . Permethrin , [3- phenoxybenzyl (1RS,3RS;1RS,3SR) – 3 - (2, 2- dichlorovinyl) -2, 2 dimethylcyclopropanecarboxylate], (99% purity) from Zeneca Agrochemicals Co. (UK), was dissolved in ethanol to prepare solutions of the required concentrations.

Acute toxicity tests were performed at 22 ±2°C in 1-L beakers, filled with 500 ml of dechlorinated tap water. The solution was daily discarded from each beaker, and replaced prompt by a freshly prepared dilution of the pesticide. Gentle aeration was provided with 1-ml glass pipettes connected to an aquarium air pump. Juveniles of *Macrobrachium rosenbergii* were tested at a density of 5 individuals (1.3g each)

per beaker. Tests were conducted at concentrations of 0.0, 0.0001, 0.001, 0.01, and 0.1μg/L of permethrin for exposure period of 96-h. For each test, three replicates were randomly distributed and then exposed to each cocentration. LC50 value and 95% confidence intervals was calculated using the Trimmed Spearman-Karber Method (Hamilton *et al.* 1977).

Based on the 96-h LC50 value, three sublethal concentrations

[1/15(1.3ng/L);1/10(2ng/L);1 /5(4ng/L)] were chosen. The prawns used in this study were divided into four groups, each comprising 25 intermolt juveniles of *Macrobrachium rosenbergii* (1.0-1.3g). One group served as control; the other three groups were exposed to the sublethal concentrations of permethrin. Each of them exposed to one of previous chosen concentrate. Each group comprised 5 beakers (1-L), each beaker housing 5 juveniles. The experiment was carried out for a period of 21 days, since the intermolt period of the prawn is 21± 1 under laboratory conditions. The entire toxic medium in each aquarium was gently changed completely daily and replaced by medium containing freshly prepared sublethal concentrations of permethrin, with minimal disturbance to the prawns. During the course of the experiment, the toxic medium was aerated and the animals were fed with Tetramin R Fish Flakes. Sampling was drawn on days 1, 8, 15, and 21. On each sampling day, prawns in each group were sacrificed. From each prawn, sample material was obtained from the hepatopancreas, gills, and muscle for biochemical analysis.

The concentrations of the soluble and insoluble fractions of proteins were estimated by the method of Lowry *et al.* (1951), and finally represented as total protein. The total carbohydrate content in the hepatopancreas, gills, and muscle were estimated by the method of Roe (1955). The concentration of glycogen was estimated by the method of Carrol *et al.* (1956). The total free sugar content in the hepatopancreas, gills, and muscle was calculated by subtracting the value for total glycogen content from the value for total carbohydrate content. Lipid was extracted essentially as described by Folch *et al.* (1957) and estimated by the method of Barnes and Blackstock (1973). The observed data were analyzed statistically ($n = 5$) by adopting the Student *t* test.

RESULTS AND DISCUSSION

The 96-h LC50 of permethrin to juveniles of *Macrobrachium rosenbergii* and their 95% confidence intervals is presented in Table 1. Juveniles were sensitive to permethrin, with 96-h LC50 of 0.02 µg/L.

In recent years, environmental pollution by pesticides has been increasingly documented. This poses a grave threat to man's resources since pesticide pollution may affect a number of physiological functions, including growth and reproductive of aquatic organisms (Lightner *et al.* 1996; Galindo-Reyes *et al.* 2000). The LC50 of permethrin to juveniles of *Macrobrachium rosenbergii* in this study is well below the corresponding values found for other insecticides with postlarvae and juveniles of crustaceans. Juarez and Sanchez 1989 reported a 96-h LC50 of 20 ng/L metamidophos to *Macrobrachium rosenbergii* postlarvae. Bhavan *et al.* 1997 reported that the 96-h value for endosulfan to juvenile of the *Macrobrachium* malcolnsonii was 160 ng/L. This show that permethrin is highly toxic to crustaceans than other insecticides belong either organophosphates or chlorinated hydrocarbons insecticides.

The total protein content of the hepatopancreas particularly those exposed to1.3 ng/L of permethrin, was found to be higher ($P<0.005$) than that in the hepatopancreas of controls on sampling days 1 and 8 (Table 2). However, after 15 and 21 days of exposure, the total protein concentration of the hepatopancreas was lower than that in the untreated of controls (Table 2). The total protein concentrations in the gills and muscle of the test prawns did not significantly differ from the value in controls on day 1 (Table 2). However, on all other sampling days, the total protein content in these tissues was found to be lower ($P<0.005$) than that in controls (Table 2).

The concentration of total proteins in the gills and muscle of test prawns were found to be lower than those in controls on all sampling days (Table 2). A Similar finding has been noted in the freshwater prawn *Macrobrachium kistensis* exposed to organochlorine insecticides (Nagabhushanam *et al.*1987). A possible explanation for these findings is that proteolytic activity was induced in these organs due to the stress factor.

The concentration of total protein in the hepatopancreas of test prawns showed a biphasic pattern, that is, it was higher than in control prawns on sampling days 1 and 8 and lower on days 15 and 21 (Table 2). The observed higher initial concentration of protein suggest that there was an initial enhanced synthesis of protein, possibly to repair damaged cell organelles, to serve as a compensatory pool to restore enzymes lost due to tissue necrosis and to meet the increased demand to detoxify the insecticide; such hypotheses have been advanced to explain the findings in fish *Puntius conchonius* exposed to phosphomidon (Gill *et al.* 1990).

The total carbohydrate content was decreased on all sampling days in the hepatopancreas, gills and muscle of prawns exposed to the three sublethal concentrations of permethrin (Table 3); this was prominent in prawns exposed to the highest sublethal concentration of 4 ng/L. Similarly, glycogen was found to be present in lower concentrations in the hepatopancreas, gills and muscle of the test prawns than in controls on all sampling days (Table 4). This effect was more prominent in prawns exposed to 4 ng permethrin/L.

In the hepatopancreas, gills and muscle of test prawns, especially those exposed to the highest sublethal concentration of permethrin, the total free sugar level was found to be lower than that in the untreated controls on all sampling days (Table 5).

The concentrations of total carbohydrate and glycogen in the hepatopancreas, gills and muscle of test prawns were found to be lower than those in the same organs of controls on all sampling days (Table 3). Carbohydrates represent the principal and immediate energy precursors for organisms exposed to stress. Insecticidal stress has been found to lead to a hypoxic/ anoxic condition; this promotes anaerobic glycolysis and a decrease in oxidation metabolism (Dezwaan and Zandee 1972), necessitating the utilization of carbohydrate to meet energy demand. This mechanism was possibly operative in *Macrobrachium rosenbergii exposed to* permethrin, in the present study, and could explain the lower concentration of total carbohydrate in the various organs of test prawns than in those of controls.Exposure of the prawn

Macrobrachium rosenbergii to permethrin was found to result in depletion of glycogen reserve, probably due to glycogenolysis (Omkar *et al.*1984). In the present study, glycogenolysis may have occurred in various organs of test prawns exposed to permethrin, thereby resulting in lower concentration of glycogen in these organs than those in the same organs of controls (Table 4). Possible explanations for this depletion of glycogen include stress-induced secretion of catecholamine and glucocorticoids leading to increase glycogenolysis, as in *Salmo gairdneri* (Grant and Mehrle 1973).

In addition to the lower concentrations of total carbohydrate and glycogen, the total free sugar levels in the hepatopancreas, gills and muscle of *Macrobrachium rosenbergii* exposed to permethrin in this study were lower than the levels in the same organs of controls (Table 5). This was probably because the free sugars generated by the breakdown of carbohydrate and glycogen were rapidly utilized to derive energy to counter the stress. A similar phenomenon has been noted in blue carb *Callinectes sapidus* exposed to pentachlorophenol (Colianese and Neff 1982).

The concentration of total lipid in the hepatopancreas of prawns exposed to the sublethal concentrations of permethrin was found to be higher ($P<0.005$) than that controls (Table 6). This was most obvious in the case of prawns that had been exposed to the highest sublethal concentration (4ng/L) of permethrin. In all test prawns, especially those exposed to 4 ng/L of permethrin, the total lipid content of gills, and muscle was lower than that in controls on all sampling days (Table 6).

Lipids are reported to serve as an alternate source of energy in crustaceans, particularly during stress conditions (Chang and O'Connor 1983). Thus, in the present study, the lower concentrations of lipid in the gills and muscle of test prawns (Table 6), in comparison to the concentrations of lipid in the same organs of controls, might reflect an accelerated hydrolysis of lipid in order to cope with the increased energy demand occurring due to permethrin toxicity. A Similar observation has been made in the fish *Sarotherodon mosambicus* exposed to methyl parathion (Rao and Rao 1981). In the present study, the lipid concentration in the hepatopancreas of test prawns was

found to exceed that in the hepatopancreas of controls (Table 6).

This was possibly due to an enhanced rate of lipogenesis or due to channelization to the hepatopancreas of lipid breakdown components from other tissues. A Similar finding has been reported in the brine shrimp *Artemia* exposed to cypermethrin (Piska *et al.* 1988).

From the above discussion, it is clear that permethrin toxicity seriously impairs various metabolic function of the prawn *Macrobrachium rosenbergii*, and this is reflected in alterations in various biochemical constituents. Thus, all possible measures should be taken to ensure that permethrin and other pesticides do not pollute fisheries, aquaculture farms, and other resources.

Table 1: 96-h LC_{50} Value and 95% Confidence Intervals for Juveniles of *Macrobrachium rosenbergii* exposed to permethrin

Insecticide	LC_{50} µg/L	95% Confidence Intervals µg/L
Permethrin	0.02	0.016-0.025

Table 2: Concentration of total protein (mg protein/g wet tissue) in various tissues of the prawn *Macrobrachium rosenbergii* exposed to sublethal concentrations of permethrin

Permethrin ng/L	Days after exposure	Total protein(mg/g) GL	MU	HP
0	1	140±7.5	215±9.8	180±8.6
	8	150±7.0	230±9.6	200±9.2
	15	165±7.8	236±9.2	200±9.6
	21	168±7.6	240±9.8	205±9.8
1.3	1	150±0.62	240±0.69	200±7.8[a]
	8	147±0.59	235±0.12[b]	220±8.2[a]
	15	140±0.10[c]	210±0.18[a]	180±6.5[a]
	21	126±0.13[a]	200±0.10[a]	180±6.4[a]
2	1	139±7.5	214±9.7	190±5.4[a]
	8	135±6.7[a]	180±9.8	210±5.8[a]
	15	132±6.5[a]	160±5.6[a]	175±6.5[a]
	21	130±6.4[a]	150±5.5[a]	165±5.8[a]
4	1	140±7.2	215±9.8	200±9.6
	8	125±5.6[a]	170±7.8	200±9.3
	15	124±5.4[a]	140±6.2[a]	150±5.5[a]
	21	120±5.2[a]	135±5.8[a]	126±5.2[a]

Mean ± SD (n = 5). A, P< 0.005; b, P < 0.01; c, P < 0.025; d, P< 0.05; other values are not statistically significant. GL, gills; MU, muscle; HP, hepatopancreas. Designations apply to all subsequent Tables.

Table 3: Concentration of total carbohydrate (mg carbohydrate/g wet tissue) in various tissues of the prawn *Macrobrachium rosenbergii* exposed to sublethal concentrations of permethrin

Permethrin	Days after	Total carbohydrate(mg/g)		
ng/L	exposure	GL	MU	HP
0	1	14.8±0.38	16.5±0.44	26.0±0.70
	8	15.0±0.42	16.2±0.40	29.0±0.68
	15	15.8±0.35	16.0±0.48	28.8±0.66
	21	15.8±0.35	17.0±0.46	29.2±0.63
1.3	1	13.9±0.69	15.0±0.62	24.0±0.69
	8	14.0±0.59	14.7±0.59	23.5±0.12[b]
	15	14.4±0.50	14.0±0.10[c]	21.0±0.18[a]
	21	13.8±0.10[c]	12.6±0.13[a]	20.0±0.10[a]
2	1	13.0±0.62	14.6±0.65	23.5±0.20[b]
	8	12.8±0.18[d]	14.2±0.15[d]	20.0±0.10[a]
	15	10.8±0.12[a]	12.0±0.10[a]	19.2±0.10[a]
	21	9.0±0.10[a]	11.5±0.12[a]	18.5±0.12[a]
4	1	12.6±0.34[d]	14.5±0.59	21.0±0.43[a]
	8	11.0±0.19[a]	12.5±0.28[a]	19.2±0.40[a]
	15	9.8±0.15[a]	9.5±0.19[a]	16.0±0.63[a]
	21	6.0±0.12[a]	7.5±0.41[a]	12.0±0.48[a]

Mean ± SD (n = 5). A, $P< 0.005$; b, $P < 0.01$; c, $P < 0.025$; d, $P< 0.05$; other values are not statistically significant. GL, gills; MU, muscle; HP, hepatopancreas. Designations apply to all subsequent Tables.

Table 4: Concentration of glycogen (mg glycogen/g wet tissue) in various tissues of the prawn *Macrobrachium rosenbergii* exposed to sublethal concentrations of permethrin

Permethrin ng/L	Days after exposure	Glycogen(mg/g) GL	MU	HP
0	1	2.9±0.12	4.8±0.18	14.0±0.60
	8	2.8±0.10	4.6±0.22	14.5±0.58
	15	2.9±0.13	5.8±0.30	13.8±0.59
	21	3.8±0.14	5.8±0.28	13.8±0.62
1.3	1	2.8±0.13	4.7±0.19	13.0±0.64
	8	1.9±0.10[a]	3.6±0.10[a]	11.6±0.24[b]
	15	1.8±0.11[a]	3.2±0.12[a]	9.6±0.12[a]
	21	2.2±0.18[a]	2.5±0.10[a]	8.8±0.10[a]
2	1	2.5±0.14[d]	4.5±0.20	11.8±0.28[d]
	8	1.2±0.10[a]	2.9±0.18[a]	10.6±0.12[a]
	15	1.3±0.10[a]	2.1±0.12[a]	8.4±0.10[a]
	21	1.8±0.12[a]	2.0±0.10[a]	7.9±0.13[a]
4	1	2.5±0.12[d]	4.2±0.22	11.6±0.12[c]
	8	1.1±0.10[a]	1.9±0.18[a]	10.4±0.10[a]
	15	1.1±0.12[a]	1.6±0.14[a]	7.2±0.12[a]
	21	1.3±0.12[a]	1.5±0.10[a]	5.8±0.10[a]

Mean ± SD (n = 5). A, $P < 0.005$; b, $P < 0.01$; c, $P < 0.025$; d, $P < 0.05$; other values are not statistically significant. GL, gills; MU, muscle; HP, hepatopancreas. Designations apply to all subsequent Tables.

Table 5:Concentration of total free sugar (mg free sugar/g wet tissue) in various tissues of the prawn *Macrobrachium rosenbergii* exposed to sublethal concentrations of permethrin

Permethrin ng/L	Days after exposure	Total free sugar(mg/g) GL	MU	HP
0	1	11.2±0.30	10.8±0.20	12.0±0.48
	8	12.4±0.25	12.0±0.19	14.5±0.59
	15	13.6±0.28	13.0±0.18	14.5±0.58
	21	13.8±0.25	13.6±0.22	16.0±0.62
1.3	1	11.2±0.28	10.7±0.18	11.8±0.35
	8	11.6±0.26	10.7±0.14	12.0±0.48
	15	11.9±0.30	9.8±0.13[b]	10.5±0.10[d]
	21	11.2±0.28	9.8±0.12[b]	12.5±0.12[b]
2	1	11.1±0.30	10.6±0.18	12.0±0.40
	8	11.2±0.25	10.4±0.19	9.5±0.12[c]
	15	9.7±0.10[c]	9.6±0.10[a]	9.0±0.14[b]
	21	6.4±0.13[b]	9.5±0.10[a]	8.4±0.10[a]
4	1	10.8±0.25	10.4±0.19	8.0±0.10[a]
	8	9.6±0.18	9.6±0.11[c]	8.0±0.11[a]
	15	9.2±0.13[b]	7.5±0.10[a]	7.8±0.10[a]
	21	5.6±0.10[a]	6.9±0.10[a]	5.5±0.12[a]

Mean ± SD (n = 5). A, $P< 0.005$; b, $P < 0.01$; c, $P \leq 0.025$; d, $P< 0.05$; other values are not statistically significant. GL, gills; MU, muscle; HP, hepatopancreas. Designations apply to all subsequent Tables.

Table 6: Concentration of total lipid (mg lipid /g wet tissue) in various tissues of the prawn *Macrobrachium rosenbergii* exposed to sublethal concentrations of permethrin

Permethrin ng/L	Days after exposure	Total lipid (mg/g) GL	MU	HP
0	1	16.5±0.63	22.0±0.48	39.0±2.5
	8	17.0±0.62	25.0±0.50	39.0±2.4
	15	17.5±0.63	26.0±0.52	40.0±2.5
	21	17.6±0.60	26.0±0.48	46.0±2.7
1.3	1	16.4±0.62	21.0±0.46	40.0±2.4
	8	16.0±0.60	19.8±0.14[b]	40.0±2.5
	15	16.0±0.63	17.2±0.12[a]	45.0±2.2[d]
	21	15.3±0.63	17.0±0.10[a]	50.0±1.6[a]
2	1	16.0±0.60	20.5±0.44	41.0±2.7
	8	15.0±0.62	17.4±0.12[a]	42.0±2.0[d]
	15	14.5±0.48[d]	15.0±0.13[a]	48.0±1.4[a]
	21	14.0±0.35[c]	12.5±0.10[a]	53.0±1.2[a]
4	1	15.0±0.62	20.0±0.40	43.0±0.22[d]
	8	14.0±0.63	18.2±0.13[a]	46.0±0.20[a]
	15	11.0±0.35[c]	12.5±0.12[a]	50.0±0.20[a]
	21	9.5±0.30[a]	11.5±0.10[a]	59.0±0.18[a]

Mean ± SD (n = 5). A, P< 0.005; b, P < 0.01; c, P < 0.025; d, P< 0.05; other values are not statistically significant. GL, gills; MU, muscle; HP, hepatopancreas. Designations apply to all subsequent Tables.

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التغيرات البيوكيميائية في بعض أنسجة جمبري المياة العذبة من جنس الماكروبراكيم المعرضة لمبيد البيرمثرين

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١- معهد الدراسات العليا والبحوث- جامعة الإسكندرية.

٢- المعمل المركزى للمبيدات – مركز البحوث الزراعية – الصبحية – الإسكندرية.

تم تقدير سمية مبيد البيرمثرين ضد الجمبري من جنس الماكروبراكيم ووجد أن قيمة التركيز الذي يسبب ٥٠% موت كانت ٠,٠٢ ميكروجرام/ لتر.

تم اختبار تأثير مبيد البيرمثرين على بعض المعايير البيوكيميائية في أنسجة الجمبري وذلك باستعمال ثلاث تركيزات تحت المميتة: ١,٣، ٢، ٤ نانوجرام / لتر لمدة ٢١ يوم وأخذت عينات من أنسجة الخياشيم، العضلات والهيباتوبنكرياس على فترات ١، ٨، ١٥، ٢١ يوم لتقدير محتواها من البروتين، الكربوهيدرات، الجليكوجين، السكر الحر و الدهون. ويمكن تلخيص النتائج على النحو الآتي:

وجد أن تركيز البروتين الكلى كان عاليا في الهيباتوبنكرياس بعد يوم و٨ أيام من المعاملة خاصة عند تركيز ٢ نانوجرام / لتر من البيرمثرين، بينما كان منخفضا في كل من الخياشيم و العضلات في كل فترات المعاملة خاصة في التركيز العالي.

انخفض محتوى الكربوهيدرات و الجليكوجين بالمقارنة بالكنترول في الثلاث أنسجة خاصة عند تركيز ٤ نانوجرام / لتر من المبيد. كانت مستويات السكر الحر منخفضة عند التركيز العالي من البيرمثرين في جميع العينات المختبرة. بينما محتوى الدهون في الهيباتوبنكرياس كان عاليا في جميع العينات بالمقارنة بالكنترول منخفضا في كل من الخياشيم و العضلات.

يتضح من هذه الدراسة أن مبيد البيرمثرين سام و إن التركيزات المختبرة لها تأثير واضح على وظائف الأيض في هذة الكائنات. لذا ينصح برصد مبيد البيرمثرين في البيئة لتجنب التأثيرات الضارة على مختلف الكائنات المائية .