Bull. Cent. Mar. Fish. Res. Inst., 1994, **46** : 51 - 56

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TOXICITY EVALUATION OF THE HOLOTHRIAN *HOLOTHURIA (MERTENSIOTHURIA) LEUCOSPILOTA* (BRANDT) AND THE EFFECT OF TOXIN ON THE PRAWN *CARIDINA RAJADHARI*

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ABSTRACT

The evaluation of lethal and sublethal concentrations is an important step for further studies on behavioural and physiological changes in the animal. In the present investigation toxicity evaluation was done using computational procedure for critical analysis of the regression line relating probits and log dose.

In the present study five size groups of the prawn *Caridina rajadhari* namely juveniles, immature females, immature males, mature females and mature males were used and the LC_{50} values were calculated upto 96 hrs. It was observed that the percent mortality of the five size groups increased progessively upto 96 hours in all concentrations of holothurian toxin and the LC_{50} values decreased with increasing exposure period. The size and sex dependent toxicity indicated that LC_{50} values followed by immature male, immature female, mature male and mature female. It was observed that males were found to be more tolerant than females in both immature aand mature stages. It was also observed that mature females were most susceptible and the juveniles are more tolerant among the test animals.

INTRODUCTION

The holothurians have certain poisonous substances in the mucous secretions of their skin, which affords excellent protection against enemies. Also, many holothurians, when attacked, can distract their attacker by ejecting their digestive tract, and in particular the Cuvierian tubules. These tubules are white, pink or red in colour. If the sea-cucumber is irritated, the Cuvierian tubules are emitted through the anus. Upon contact with the water, the tubules swell and elongate into sticky slender threads which serve to entangle any predator that attempts to annoy it. Only few of the tubules are emitted at any one time and the autotomized tubules are soon replaced by new ones. The organs of Cuvier are quite toxic, containing large concentrations of holothurin. The role of these tubules is not completely known, but they are believed to serve as a protective mechanism for sea-cucumber.

The present investigation was undertaken to study the toxicity to prawn *Caridina rajadhari* after exposing to sea-cucumber *Holothuria leucospilota* toxin.

MATERIAL AND METHODS

The freshwater prawns *Caridina rajadhri* were collected from Kham River, near Aurangabad in Maharashtra. The prawns were maintained in large glass aquaria, containing aerated tap water. The other conditions were kept constant to their minimum range (temperature 25 ± 2° C; pH 6.5 - 7.0; dissolved oxygen 5.0 ± 1.0 ml/l). They were acclimatized for seven days to laboratory conditions before subjecting them to experiments. The water in the container was changed everyday. After three days the prawns were fed with green algae. During the experimental period they were not fed.

Intermoult prawns of five different groups based on size and sex (Immature male - 18 mm, immature female - 20 mm, mature male - 23 mm, mature female - 28 mm, juvenile - 15 mm) were used measured from the tip of the rostrum upto the end of telson. Series of static bioassays were conducted under laboratory conditions as described by Finney (1971). For each experiment 20 animals approximately of similar size were exposed to 5 to 10 different concentrations of

sea-cucumber toxin. Care was taken to dechlorinate the tap water before supplying it to the experimental prawns. No artificial aeration was provided to the animals during the exposure period. Different concentrations of sea-cucumber toxin were prepared in two litres of test medium.

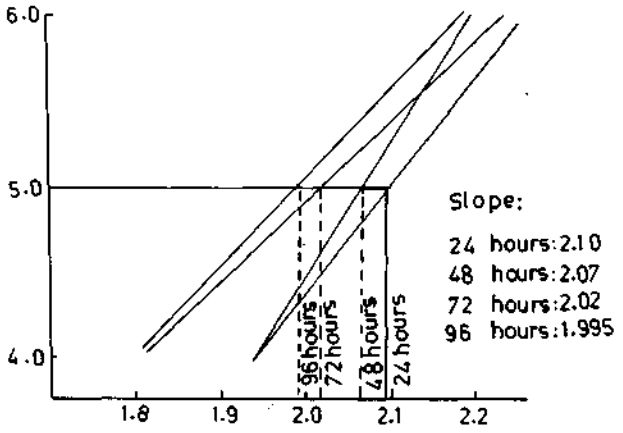

FIG 1. The effect of sea-cucumber toxin in juveniles of *Caridina rajadhari*.

The test medium was changed at every 24 hours to maintain the toxin concentration. The resulting mortality was noted to be in the range of 10 to 90% for each concentration for the durations of 24, 48, 72 and 96 hours. Individuals which were motionless and did not respond to the needle prick were regarded as dead and were removed immediately from experimental containers, because such mortality in static bioassay might deplete DO (dissolved oxygen) and affect other animals. A control set was maintained with similar number of animals and toxin free tap water. No mortality was observed in the control troughs. Each experiment was repeated thrice to obtain constant results with the toxicant.

Sea-cucumber toxin, which was used in this experiment was collected from *Holothuria leucospilota*. The specimens were collected at Ratnagiri in the west coast. The specimens were brought to the laboratory and toxin was extracted by Ferlan and Leebez (1974) method as follows.

The body wall of the sea-cucumber along with Cuverian tubules was separated from alimentary canal. The tissue was cut into small pieces and to this a small amount of water added. The suspension was kept at 4° C for 5 to 6 days. The tissue was homogenised by adding sea water and passed through a thick nylon screen. To the screened suspension distilled water was added and centrifuged. Acetone was added to the supernatant to obtain a precipitate.

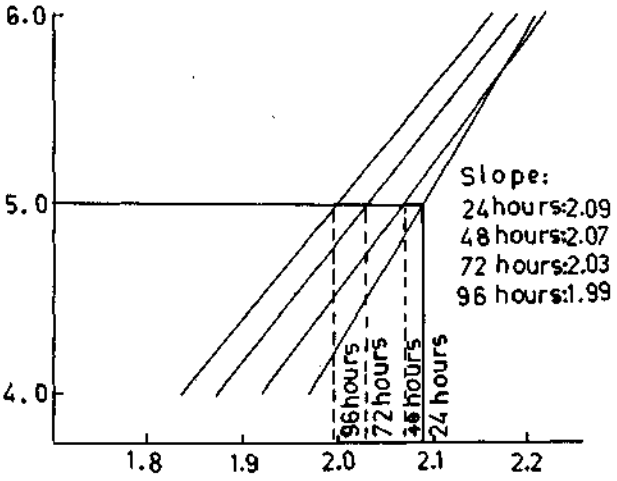

FIG. 2. The effect of Sea-cucumber toxin in immature males of *Caridina rajadhari*.

The precipitate was dissolved in distilled water, this solution is used as toxin. The data collected is elaborated statistically by means of the probits method which, by transforming the toxicity curve (% mortality/concentration) into regression lines (Mortality in probits/concentration) (Finney, 1952) which allow the "average lethal concentration" or LC_{50} to be calculated for 24, 48, 72 and 96 hours.

RESULTS

Effect of sea-cucumber toxin has been studied in five different developmental groups *viz.* juveniles, immature male, immature female, mature male and mature females to determine lethality of a prawn *Caridina rajadhari*. The LC_{50} values, along with the standard deviation, 95% fiducial limits and homogenity of all the five groups are shown in Tables 1 and 2 (Figs. 1 to 5). The safe concentrations of SCT to different stages of prawns are presented in Table 3.

Order of toxicity according to tolerance for SCT is : juveniles > immature male > immature female > mature male > mature female.

TABLE 1. *Calculation of log dose/probit regression line for some experiments in which immature male prawn* Cardina rajadhari *were exposed to different concentrations of sea-cucumber* (Holothuria leucospilota) *crude toxin in Busvine/Nash technique for a period of 48 hours*

Con. of crude toxin (ml)	No. of prawns used (n)	% dead	Corr. % kill P	log (+1) dose x	Experi-cal probit	Expected probit Y	Working probit y	Weight-ing coeffici-ent	Weight W	Wx	Wy	Wx^2	Wy^2	Wxy	y'
9.0	20	20	20	1.95	4.13	4.15	4.15	0.471	9.42	18.36	39.02	35.81	162.23	76.23	4.20
10.0	20	30	30	2.00	4.48	4.50	4.47	0.581	11.62	23.24	51.94	46.48	232.11	103.88	4.53
11.0	20	40	40	2.04	4.75	4.80	4.74	0.627	12.54	25.58	59.43	52.18	281.74	121.25	4.80
12.0	20	50	50	2.07	5.00	5.00	5.00	0.637	12.74	26.37	63.70	54.84	318.50	131.85	5.00
13.0	20	60	60	2.11	5.25	5.30	5.25	0.616	12.32	25.99	64.68	54.84	339.57	136.47	5.27
14.0	20	70	70	2.14	5.52	5.50	5.52	0.581	11.62	24.86	64.14	53.21	354.06	137.26	5.47
15.0	20	80	80	2.17	5.84	5.75	5.83	0.532	10.64	23.08	62.03	50.10	361.64	134.60	5.67
									80.90	167.48	404.94	347.20	2049.91	841.54	
									SW	SWX	SWY	Swx^2	Sw^2	Swxy	

Calculation of working pobit : Y = Yo + Kp

1. Y = 3.41 + 0.0376 x 20 = 4.16
2. Y = 3.62 + 0.0284 x 30 = 4.47
3. Y = 3.72 + 0.0256 x 40 = 4.74
4. Y = 3.75 + 0.0251 x 50 = 5.00
5. Y = 3.68 + 0.0262 x 60 = 5.25
6. Y = 3.54 + 0.0284 x 70 = 5.52
7. Y = 3.27 + 0.0320 x 80 = 5.83

Calculation of x and y

$$\bar{x} = \frac{Swx}{Sw} = 2.0702$$

$$\bar{y} = \frac{Swy}{Sw} = 5.005$$

Calculation of 'b'

$$b = \frac{Swxy - x\,Swy}{Swx^2 - x\,Swx} = \frac{3.23322}{0.483} = 6.6940$$

Calculation of regression equation

$$Y = (\bar{y} - b\bar{x}) + bx$$

$$Y = -8.852918$$

Calculation of improved expected probit Y'

$$Y' = (\bar{y} - b\bar{x}) + bx$$

Y' = -8.852918 + 6.6940 x 1.95 = 4.20
Y' = -8.852918 + 6.6940 x 2.00 = 4.53
Y' = -8.852918 + 6.6940 x 2.04 = 4.80
Y' = -8.852918 + 6.6940 x 2.07 = 5.00
Y' = -8.852918 + 6.6940 x 2.11 = 5.27
Y' = -8.852918 + 6.6940 x 2.14 = 5.47
Y' = -8.852918 + 6.6940 x 2.17 = 5.67

TABLE 2. *LC_{50} values calculated for different stages and sexes of freshwater prawn* C. rajadhari *after exposure to sea-cucumber* (Holothuria leucospilota) *toxin for periods of 24, 48, 72 and 96 hours*

Exposure period in hours	Regression equation $y = (\bar{y}-b\bar{x}) + bx$	LC_{50} values ml	Variance	Chi-square value	Fiducial limits upto 95% confidence	
					M1	M2
Juvenile						
24	-9.5838689 + 6.93355 x	12.685	0.00025644	-0.1921016	11.680831	13.786
48	-11.6840 + 8.0458 x	11.847	0.05227898	3.0303835	10.823883	12.638
72	-4.2701355 + 4.5864 x	10.500	0.0057873	3.0780055	9.0695746	11.849
96	-5.5475 + 5.3061 x	9.723	0.000443419	1.5612474	8.7378723	10.816
Immature male						
24	-12.52799 + 8.3860 x	12.306	0.000190042	1.1122421	11.554387	13.0975
48	-8.852918	11.734	0.0466457	1.5421789	10.798963	12.740892
72	-7.4966 + 6.1541 x	10.730	0.00032239	1.0655537	9.7793927	11.73894
96	-5.0146 + 5.0466 x	9.647	0.00048529	1.5455749	8.5883219	10.782834
Immature female						
24	-9.1127 + 6.8032 x	11.869185	0.000301674	-0.5895264	10.740091	12.789347
48	-7.1124 + 5.8909 x	11.379429	0.000374252	7.3302916	9.9973453	12.204756
72	-5.7404 + 5.347 x	10.201822	0.00044812	0.780883	8.9576432	11.22007
96	-8.0086 + 6.5962 x	9.3785451	0.000301021	19.188214	8.5611095	10.155246
Mature male						
24	-7.2877 + 5.9109037 x	11.99	0.000352592	4.5440398	10.959073	13.255589
48	-7.2812 + 6.1488 x	10.00	0.000321242	-18.9828	9.123652	10.880281
72	-3.1344 + 3.1285865 x	6.0	0.00123272	0.0878551	4.9641308	7.00686132
Mature female						
24	-8.30 + 6.705188 x	11.55	0.000280274	11.578083	10.771694	12.762654
48	-5.2761 + 5.1629902	9.78	0.000462467	13.583486	8.6891869	10.831154
72	-3.4202 + 4.4823 x	7.56	0.000624215	-1.35077	6.6454271	8.5244643
96	-0.5664 + 3.1721 x	5.89	0.0123777	0.2908994	4.7771907	0.7719131

From the observed data it appeared that freshwater prawn *C. rajadhari* in all the developmental stages is sensitive to SCT. From

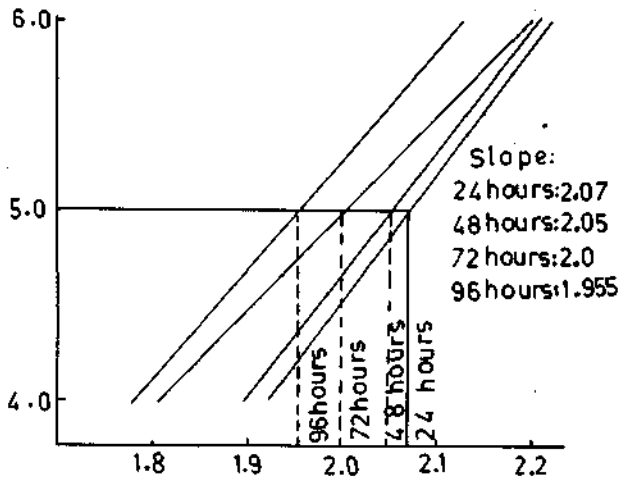

FIG. 3. The effect of sea-cucumber toxin in immature female of *Caridina rajadhari*.

the LC_{50} values observed, it was clear that 24 hours LC_{50} values in all the cases were the highest, followed by 48, 72 and lower for 96 hours among all exposure periods. The percentage mortality of all the five size groups increased progressively upto 96 hours in all concentrations of SCT. The LC_{50} values decreased with increasing exposure periods.

TABLE 3. *Safe concentration of sea-cucumber* Holothuria leucospilota *toxin calculated for freshwater prawn* Caridina rajadhari

Stage	Safe concentration
Juveniles	3.100027
Immature males	3.2005579
Immature females	3.1376557
Mature males	2.0868099
Mature females	2.1036518

From the data it seems the toxicity of SCT ranged from 5.0 ml to 14.0 ml/L for the five groups of the prawn *C. rajadhari*. The results in relation to size and sex indicated that LC_{50} values decreased as the size of animal increased. Juveniles had the highest LC_{50} values being the most tolerant stage among all the five groups of the prawn tested. They were followed by immature males, immature females, mature males and mature females. In this study, males were found to be more tolerant than females in both immature and mature stages. It was also observed that mature females were the most susceptible to SCT.

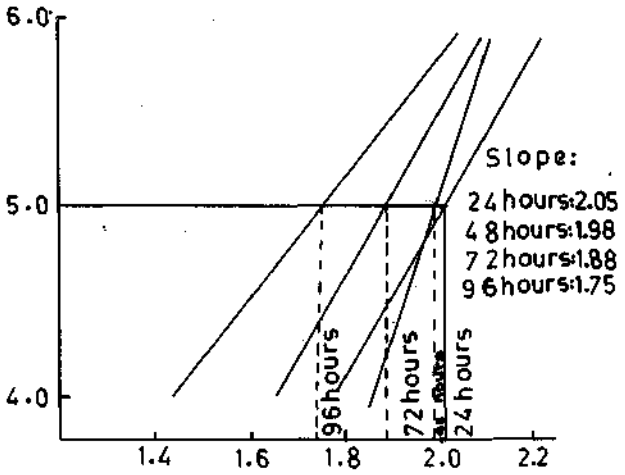

FIG. 4. The effect of sea-cucumber toxin in mature male of *Caridina rajadhari*.

Thus the above results indicate that mature prawns were the most susceptible as compared to other groups. The juveniles are more tolerant among the test animals.

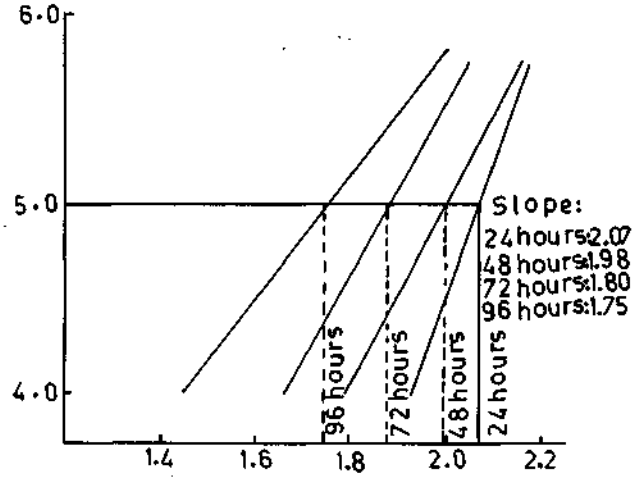

FIG. 5. The effect of sea-cucumber toxin in mature female of *Caridina rajadhari*.

DISCUSSION

Mortality of test organism is a more sensitive measure of toxicant. The physiological responses are dose dependent, the evaluation of LC_{50} concentration of sea-cucumber toxin is an

important step before carrying further studies on physiological changes in the animals. Unless and until the lethal and sublethal concentrations of SCT are known, it becomes difficult to choose the concentration, which may be effective at physiological levels and enable to study the physiological responses of the prawn to SCT. The bioassay tests in the present study have been carried out under static laboratory condition. The laboratory bioassays provides quickest and the most reliable information about the toxicity of SCT in respect of several life history stages of prawn *Caridina rajadhari*. The effect of toxin depend upon their concentration in specific target organs and tissues of the prawn. The study of the toxicity to various developmental stages exposed to SCT indicated that LC_{50} values decreased as the time of exposure increased and also the percent survival rate of the prawns decreased with the increasing concentrations. There was not much difference between 24 to 96 hours LC_{50} which indicates culmination of the acute mortality with first 96 hours. The comparative tolerance in the various developmental stages of prawn *C. rajadhari* to SCT reveals that juveniles show more tolerance among all the five groups. They were followed by juveniles, immature male, immature female, mature male and mature females. Mature females are more sensitive.

The present investigation on *C. rajadhari* indicates that juveniles are most tolerant than the adult animals. It was observed that the smaller animals show higher metabolic rate than that of larger animals. Thus due to the rapid metabolism SCT may be metabolized and excreted in larger amount and as such the SCT residues are lesser in the body of smaller prawn. In mature prawns most of the energy is utilized for reproductive purpose and comparatively less for metabolic rates (Prosser, 1973). It was also observed that like-size dependence there is a sex dependent tolerance phenomenon of SCT in *C. rajadhari*. Males are more tolerant than the females in both immature and mature groups. It may be concluded that SCT is not metabolized in mature prawns as rapidly as in the immature and juveniles and is accumulated in large amounts in the body. This may be the causative factor for the greater sensitivity of mature or larger prawns than that of immature or smaller prawns. The data in the present investigation is insufficient for a comparison, because adequate information on toxicity is not available. However the results show that the sea-cucumber toxin is poisonous to the prawn *C. rajadhari*.

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