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Toxicity of Kelthane to Eyestalk Ablated and Eyestalk Extract Injected Penaeid Prawn, *Metapenaeus monoceros* (Fabricius)

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The neuroendocrine system present in the eyestalks of crustacean is involved in molting, growth, metabolism, development and physiological processes (Scharrer and Scharrer 1954; Highnam and Hill 1979). The organochlorine (OCl) insecticides are still in use because of their high initial kill, broad spectrum of action and long lasting efficacy (PAI 1987). Because of their persistent nature and longer half-life, the OCl insecticides are more likely to enter into all environments including the aquatic systems. Reports on the effects of crustacean neuroendocrine substances are mostly confined to physiology, metabolism and reproduction, but its influence on insecticide toxicity, particularly of OCl compounds received less attention. Therefore in the present investigation, the influence of the neuroendocrine system on the toxicity of kelthane to the penaeid prawn, Metapenaeus monoceros was tested.

MATERIALS AND METHODS

Healthy, intermolt prawns, Metapenaeus monoceros of 75 ± 5 mm long and weighing 2.5 ± 0.5 g were collected from Buckingham canal adjoining Kavali sea coast (lat. 14°55'N and long.80°3'E), India. They were acclimatized in the laboratory for 1 wk in seawater of 15 ± 1 ppt salinity, 27 ± 1°C temperature and pH 7.2 ± 0.2. The prawns were fed with a mixture of rice bran, powdered oil cake and minced crab muscle (1:1:1 mixture). The media in which they were placed was changed daily. Continuous aeration was provided. These prawns form the stock which were maintained continuously and used for experimentation. The experimental design consists of controls, eyestalk ablated (ESA) and eyestalk extract injected (ESEI) prawns. Unilateral eyestalk ablation was performed on control prawns by extirpating the left eyestalk at the base without prior ligation as described by Tullis and Kamemoto (1973). They form the ESA prawns. ESEI prawns were maintained by injecting eyestalk (ES) extract to 1 d

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old ESA prawn. 20 µL of ES extract was injected intramuscularly into the lateral abdominal muscle in between the pleural membrane of the second and third segments using a microsyringe. The ES extract was prepared by the method of Silverthorn (1975) using ethanol:acetone (2:1) mixture as the extraction medium (Ramanaiah 1991). Sham controls for ESA prawns were maintained by slightly injuring the eyestalks without removal. Injection of 20 µL of ethanol-acetone mixture to the ES injured prawns serve as sham controls for ESEI prawns. A small quantity of streptopencillin was added in the media containing control and experimental prawns to prevent microbial infection. The controls and experimental prawns (ESA, ESEI and sham controls) were used for toxicity evaluation 1 d after treatment to recover from shock effects.

The technical grade kelthane (1,1-bischlorophenyl,2,2,2-trichloro ethanol) of 85% purity obtained from Indofil Chemicals Ltd, Bombay (India) was used as the test chemical. A stock solution of 1000 mg/L and appropriate working concentrations were prepared by dilutions with 15 ppt seawater. The toxicity evaluation was conducted following the static bioassay procedure of Doudoroff et al (1951). The control and sham control prawns were exposed to 7 concentrations of kelthane from 0.08 mg/L to 0.20 mg/L; the ESA and ESEI prawns were exposed to 7 concentrations of kelthane each from 0.04 mg/L to 0.16 mg/L and from 0.06 mg/L to 0.18 mg/L respectively (with a difference of 0.02 mg/L). For each set and at each concentration 12 prawns of almost equal size and weight were taken and the experiment was repeated six times. The mortality rate in the kelthane exposed sham controls were similar to the mortality rates of kelthane exposed control prawns. The 96-hr LC50 values were computed following the probit arithmatical, probit graphical, cumulative mortality method, Behrens method and Karbers method as per Finney (1964). The mean LC50, standard error, slope and 95% fiducial limits were also calculated (Finney 1964).

RESULTS AND DISCUSSION

The control prawns recorded no mortality at 0.08 mg/L but 100% mortality at 0.2 mg/L concentration of kelthane. The ESA prawns recorded nil and 100% mortality at 0.04 mg/L and 0.16 mg/L concentrations of kelthane respectively, while the ESEI prawns recorded no mortality at 0.06 mg/L and 100% mortality at 0.18 mg/L kelthane (Fig 1). From the 96-hr LC50 values obtained through various methods, mean LC50 value, standard error, slope and fiducial limits for the control and experimental prawns were calculated (Table 1). These data show agreement in LC50 values obtained through

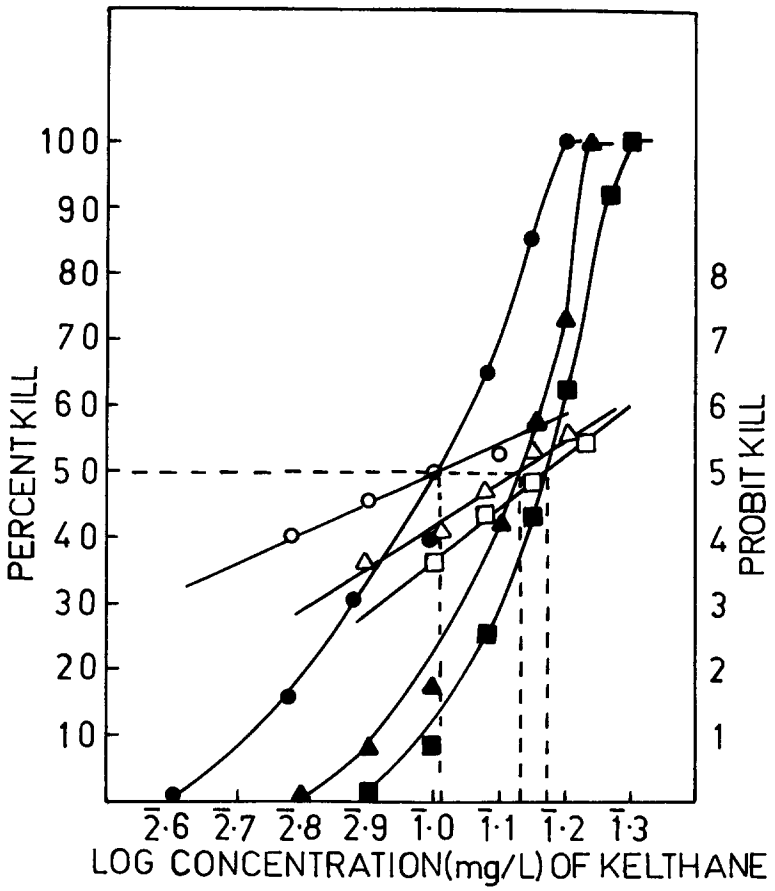

Figure 1. LC_{50} of kelthane to M.monoceros

O—O, ●—●=Eyestalk ablated prawns exposed to kelthane, △—△, ▲—▲=Eyestalk extract injected prawns exposed to kelthane and □—□, ■—■ =control prawns exposed to kelthane.

probit with the other methods confirming the probit method is still the most reliable and valid method in toxicity evaluation. The mean 96-hr LC50 value to control prawns exposed to kelthane is 0.142 mg/L, for ESA prawn it is 0.100 mg/L and for ESEI prawn it is 0.132 mg/L (Table 1). These trends demonstrate that kelthane is highly toxic to ESA prawns compared to ESEI and control prawns. The low LC50 value for ESA prawns demonstrate that the ESA prawns are more sensitive to kelthane than ESEI and control prawns. The LC50 values for ESA and ESEI prawns were highly significant (different) but the per cent change in LC50 values of ESEI prawns was low compared to ESA prawns. The high slope

Table 1. 96-hr LC50 values (mg/L) of kelthane (calculated by different methods) along with other statistical details to control, eyestalk ablated and eyestalk extract injected penaeid prawn, M. monoceros.

Condition	LC50 values								Fiducial limits	
	Probit Arithmatic method	Probit graphic method	Cumulative mortality method	Karbers method	Behrens method	Mean LC50	Standard error	Slope	Lower	Upper
Control	0.138 ±0.002	0.148 ±0.002	0.145 ±0.004	0.140 ±0.006	0.140 ±0.008	0.142 ±0.004	0.009	7.883	0.120	0.156
Eyestalk ablated	0.098 ±0.006 (-29)	0.102 ±0.006 (-31)	0.103 ±0.008 (-29)	0.095 ±0.004 (-32)	0.100 ±0.008 (-29)	0.100 ±0.006 (-30)	0.005	9.987	0.088	0.108
Es extract injected	0.128 ±0.004 (-7)	0.135 ±0.004 (-9)	0.140[a] ±0.006 (-4)	0.125 ±0.002 (-11)	0.130[b] ±0.006 (-7)	0.132 ±0.004 (-7)	0.007	8.322	0.114	0.142

Each value is the mean ± SD of 6 individual observations. Values in parentheses indicate per cent change over control. All values are significant at $P<0.001$ except a=$P<0.1$ and b=$P<0.02$. Standard error, slope and Fiducial limits were calculated on probit (Arithmatic) values.

Value for RSA prawns also confirms that kelthane is more toxic to ESA prawns compared to ESEI and control prawns.

The high toxicity of kelthane to ESA prawns should be the consequence of loss of a single dose of ES hormone. Since insecticides cause toxic stress (Matsumura 1985) in the absence of a single dose of ES hormone a cumulative effect, must be operating on ESA and kelthane exposed prawns resulting in ESA prawns becoming highly sensitive to kelthane toxicity. In ESEI prawns the toxicity of kelthane is much reduced than the ESA prawn indicating the influence of the neuroendocrine extract. In general kelthane is toxic to M. monoceros irrespective of the condition. In ESA prawns, kelthane toxicity is enhanced at low concentrations but in ESEI prawns the trend is reversed, almost reaching the control LC50 value. This indicates the ES hormones have an influence on toxicity. The fiducial (upper and lower) limits of ESA prawns exposed to kelthane showed a narrow range compared to the ESEI and control prawns exposed to kelthane (Table 1). This indicates that ESA prawns as such are highly sensitive to kelthane because of insufficient availability of RS hormones while the controls and ESEI prawns are able to withstand kelthane toxicity because sufficient amount of ES hormones are available and that they are also interacting with the kelthane molecules.

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