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Effects of Phosphamidon and Lindane on the Limb Regeneration of Penaeid Prawn, *Penaeus monodon*

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Hazards of environmental contamination through indiscriminate use of a variety of pesticides have attracted global attention. Determination of acute toxicity levels has little relevance in the estimation of ecologic consequences. Crustacean limb bud regeneration is considered to be a sensitive parameter for assessing the water quality of the aquatic environment (Fingerman & Fingerman 1978). Hence in the present investigation an attempt was made to understand the toxic impact of sublethal concentrations of phosphamidon, an organophosphorous insecticide, and lindane, an organochlorine insecticide on the limb regeneration capacity of the penaeid prawn, Penaeus monodon. Because of their wide spread usage, phosphamidon and lindane often find their way into the aquatic environment (Couch 1979) and are toxic to several crustaceans (Reddy et al. 1986; Reddy & Rao 1986). P. monodon forms one of the commercially important cultivable species of India and is considered to be a sensitive indicator of marine or estuarine pollution (Butler 1966).

MATERIALS AND METHODS

Prawns were collected from Buckingham Canal near Kavali seacoast (14°5'E and 80°5'N) Andhra Pradesh, India. Intermolt individuals of 10 ± 1 g in weight were transferred to large glass aquaria containing seawater and acclimatized to the laboratory conditions for 1 week at constant salinity of 15 ± 1 ppt, pH 7.1 ± 0.2, and temperature of 23 ± 2°C. Continuous aeration was provided. The prawns were fed ad libitum with oil cake powder. The medium was changed daily to remove scattered food particles and excreta. Technical grade phosphamidon (92% w/v; 0,0-dimethyl-0(1-methyl-2-chloro-2-diethyl-carbomoyl-vinyl) phosphate; CIBA-GEIGY, Bombay) and lindane (98%, Hexachlorocyclohexane; Pesticides India, Udaipur) were used as test

chemicals. LC_{50} values for 96 h were found to be 14 mg/L and 10^{-2} x 10^{-2} mg/L in static bioassay system for phosphamidon and lindane, respectively (Reddy & Rao 1986). The standard stock solution and appropriate working solutions were prepared as described earlier (Reddy & Rao 1987; 1988; Murthy et al. 1985). Prawns were divided into ten groups of 50 each. The left fifth periopod was removed from each prawn with a finetipped forceps. One of the groups served as control while the others were exposed to 0.1, 0.2, 0.5, 1 and 2 mg/L of phosphamidon and 10×10^{-3}, 10×10^{-4}, 75×10^{-5} and 50×10^{-5} mg/L of lindane, respectively from, the day of limb removal until the prawns completed at least one ecdysis or until the termination of the experiment (30 days). These prawns were examined microscopically in order to assess the progress in the limb regeneration and advances in the molt cycle. The regeneration index R-value was calculated by following the method of Bliss (1956).

$$\text{R-value} = \frac{\text{Length of the limb bud}}{\text{Carapace length}} \times 100$$

RESULTS AND DISCUSSION

The R values calculated from the limb bud measurements made following the limb removal (Fig. 1 & 2) illustrate the effect of phosphamidon (organophosphate insecticide) and lindane (organochlorine insecticide) on limb regeneration in the penaeid prawn, P. monodon. Both phosphamidon and lindane were able to inhibit the limb regeneration in a dose-dependent manner. At the concentrations tested, these insecticides did not alter the duration of the intermolt and premolt stages of the prawn. Generally the developing limb bud tissues, which have little protective covering during the early phases of development, and the newly molted prawns, which have a relatively thin cuticle and highly permeable epithelia, were more sensitive to these insecticides in the medium than were intermolt prawns which have a relatively thicker cuticle and less permeable epithelia. Sodium pentachlorophenate inhibits the early phases of limb regeneration (wound healing, mitosis and early dedifferentiation) in the grass shrimp, Palaemonetes pugio (Rao et al. 1978; 1979). The present study shows that both phosphamidon and lindane inhibit limb regeneration in the prawn in a dose-dependent manner (Fig. 1 & 2). Since both phosphamidon and lindane were able to inhibit limb regeneration without causing alterations in the duration of the intermolt cycle, it would appear likely that they exert their effects directly on

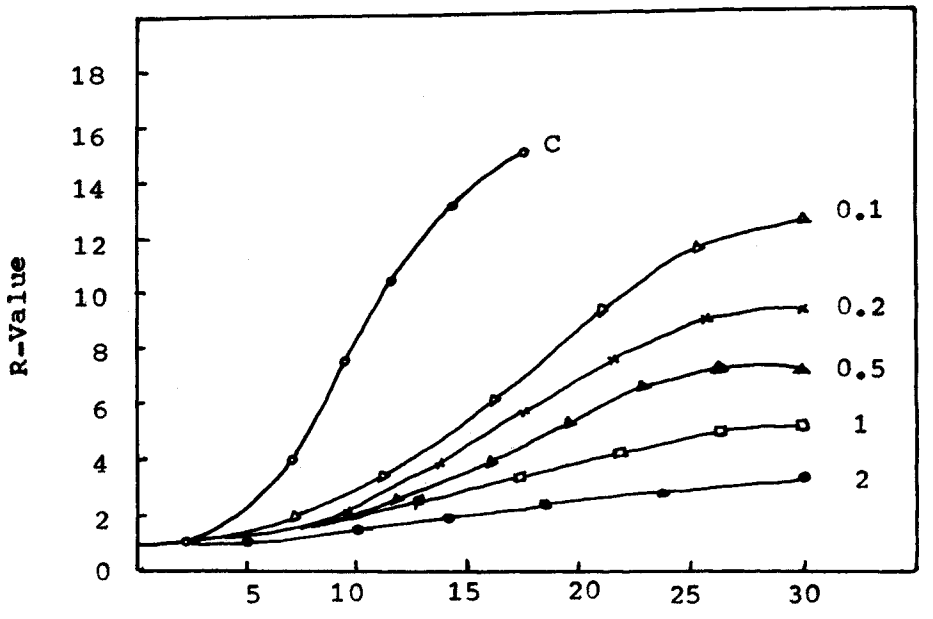

Figure 1. Effect of phosphamidon (mg/L) on limb regeneration of Penaeus monodon.

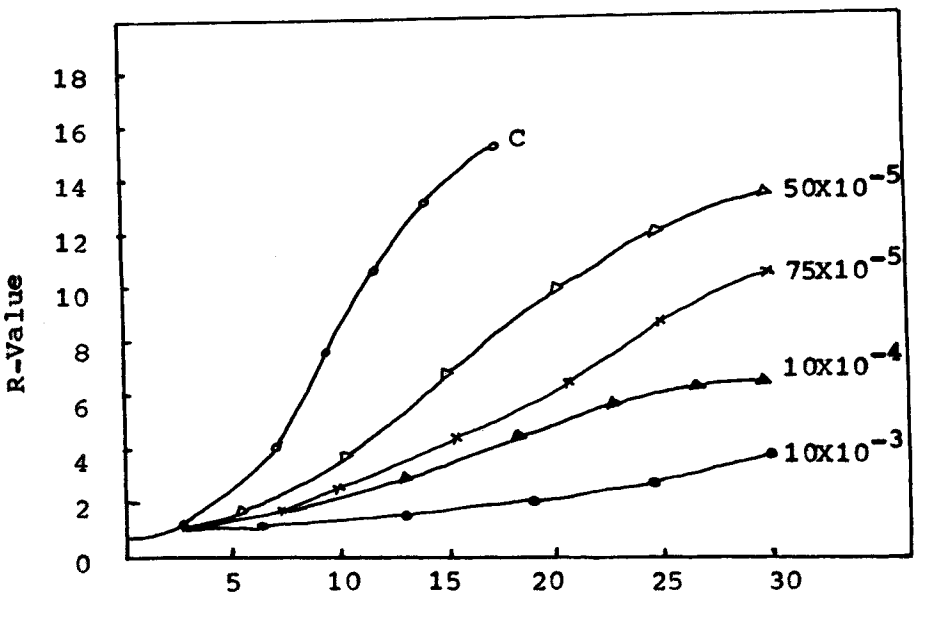

Figure 2. Effect of lindane (mg/L) on limb regeneration of Penaeus monodon.

the target tissue, i.e., developing limb bud rather than acting indirectly via the neuroendocrine system of the prawn. Similar kind of observations were also reported that aquatic pollutants like DDT (Weis & Mantel 1977) and heavy metals (Weis 1976; 1977; Weis & Weis 1979) seem to influence the regeneration as well as molting in fiddler crabs. Sumithion, an organophosphorous insecticide known to exert its effects not only on the developing limb bud but also on the neuroendocrine system of the fresh water field crab, Oziotelphusa senex senex (Reddy et al. 1983). A concentration of 2 mg/L of phosphamidon and 10 x 10^{-3} mg/L of lindane cause a complete inhibition of regeneration and also delay in the initiation of limb bud regeneration was observed. Similar kind of observations were also made by Rao et al. (1978) in the shrimp, Palaemonetes pugio after exposure to pentachlorophenol.

The present investigation demonstrates that the phosphamidon and lindane, irrespective of their possessing different prosthetic group and mode of action, cause delay in the onset of limb bud regeneration in prawns. The crustacean limb bud regeneration is a sensitive parameter which not only can be useful in monitoring the toxic responses of aquatic biota to different chemical pollutants, but also provides fundamental data with regarding to the growth rate of these crustaceans without sacrificing the animals.

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