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LARVICIDAL ACTIVITY OF SOME MARINE MACROPHYTES AGAINST *ARTEMIA SALINA*

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Petroleum ether (PE) and chloroform (C) soluble fractions of methanolic extracts of nine different macrophytes viz. mangroves (*Derris heptaphyla*, *Salvadora persica*, *Sonneratia caseolaris* and *Clerodendrum inerme*), seaweeds (*Acanthophora muscoides* and *Microdictyon pseudohapteron*, seagrasses (*Halophila ovalis* and *Syringodium isoetifolium* and lichens (*Umbilicaria aprine*) were tested for larvicidal activity using *Artemia salina* nauplii. The LC50 values for the PE-fraction of *M. pseudohapteron* and *A muscoides* and C-fraction of *S. isoetifolium* were found to be 11.75, 42.5 and 41.5 ppm respectively. Varying LC50 values below 100 ppm were shown by the C-fraction of *U. aprine* (53 ppm), *M. pseudohapteron* (72 ppm), *S. casseolaris* (77 ppm), *S. persica* (84.37 ppm) and *C. inerme* (98 ppm). All the remaining fractions were effective at levels between 100 and 500 ppm. The PE-fraction of *S. isoetifolium* was ineffective even at 500 ppm.

Key Words : marine macrophytes; *Artemia salina*; bioassay

INTRODUCTION

Marine plants have long been recognized as producers of biologically active substances. Potential activity of some marine plants like mangroves, seaweeds, seagrasses and lichen have been reported from both India and elsewhere (Naqvi *et al.*, 1980; Bernard and Clement 1983; Bernard and Pesando 1989; Kamat *et al.*, 1991; Sridhar and Vidyavathi 1991; Thangam and Kathiresan 1991; Vidyavathi and Sridhar 1991; Ballesteros *et al.*, 1992; Mizukoshi *et al.*, 1992; Premnathan *et al.*, 1992; Cannel 1993; Bilorenzo *et al.*, 1993).

As a part of the ongoing program to search for potentially active metabolites from marine plants, petroleum ether and chloroform soluble fractions of methanolic extracts of nine marine macrophytes, collected from different locations were screened for toxicity studies. The aim of this study was to increase our knowledge of the toxicity of marine macrophytes, including species not previously tested, but widely distributed along the Indian coast. Brine shrimp *Artemia salina* was used for bioassay testing.

MATERIALS AND METHODS

Extraction of marine macrophytes and preparation of experimental solutions:

Samples were collected from different locations as shown in Table 1. They were washed free from extraneous matter, air dried in shade, powdered and subjected to methanolic extraction using cold percolation and vacuum evaporation. The extracts were partitioned with petroleum ether and chloroform successively using a separating funnel to obtain the petroleum ether fraction (PE-f) and chloroform fraction (C-f) after vacuum evaporation of the solvents.

Experimental solutions of different concentrations viz. 10, 20, 50, 100, 200 and 500ppm were prepared by redissolving known weight of the fractions in minimum volume of methanol (0.1 to 0.2ml) (Price *et al.*, 1974). Dilutions were made using filtered seawater (FSW) by vigorous stirring. In addition, solutions were also prepared with methanol and FSW as control to the experimental set.

Hatching of cysts and collection of nauplii :

Cysts of *Artemia salina* were hatched in a 250ml Erlenmeyer flask containing seawater (35‰) at room temperature. Aeration was provided gently from the bottom of the tube. Newly hatched nauplii of the 1st instar were sucked out after 18-24 hrs by thin capillary glass tube and transferred into glass petri dishes containing extracts of known concentration.

Preparation for laboratory bioassay :

Study with *Artemia* nauplii consisted of 24h bioassay test each with

six concentrations ranging from 10 to 500ppm, triplicated with 10 nauplii each. Separate controls were also run with 10 nauplii each. At the end of the experimental period the number of mobile and dead larvae in each Petri dish were checked using a hand lens. Nauplii were considered dead if they were lying immobile at the bottom of the petridish.

Statistical analysis :

The data derived from the test series is treated by the Reed-Muench method (Isphen and Feigl 1970; Miya et al., 1973). The dose that will kill 50% of the animal is obtained by plotting the number of accumulated alive and accumulated dead on the same axis (Isphen and Feigl, 1970). The point where the two curves cross is the dosage which is taken as the lethal concentration expressed as LC₅₀. The Standard Error, SE LC₅₀ was calculated using the formula.

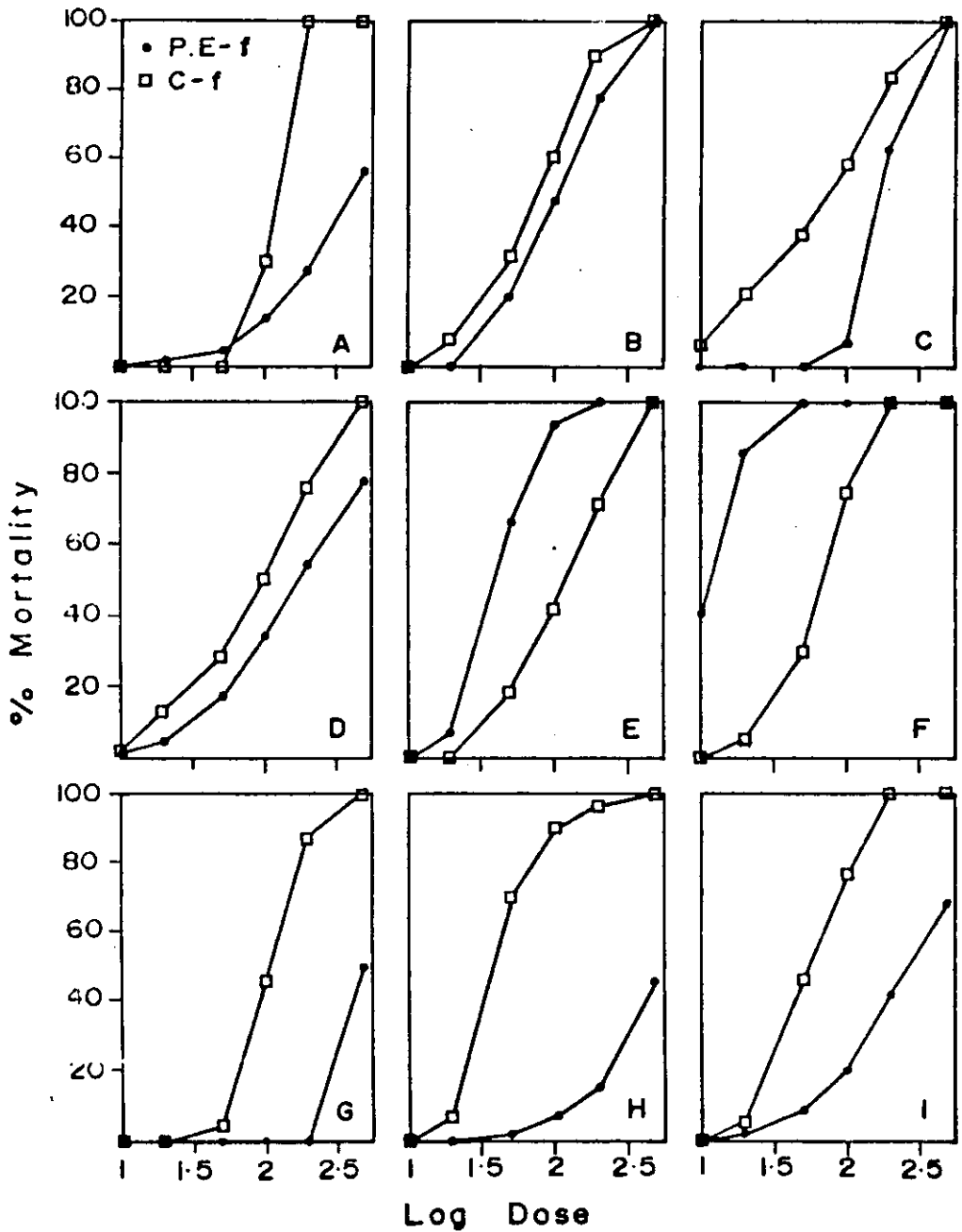
$$SE LC_{50} = \sqrt{(0.79h R/n)}$$

- where h = average of the interval between dosages (log dose)
R = interquartile range (LC 75 to LC 25) from the cumulative percentages (obtained from the plot of percentage mortality versus log dose)
n = number of test animals (average)

RESULTS

The percent mortality caused by varying concentrations of marine plant extracts taken as log dose against *Artemia* nauplii is shown in Fig 1. The LC₅₀ values and the standard error (SE) measurement of LC₅₀ is given in Table 1.

It is observed that in most cases (mangroves, seagrass and lichen), the chloroform fractions showed higher larvicidal activity against *Artemia* nauplii as compared to the petroleum ether fractions. However, in the case of seaweeds the PE-fractions of *A. muscoides* and *M. pseudohapteron* showed comparatively higher larvicidal activity than the C-fraction. From the E present data it is observed that the PE-fraction of *M. pseudohapteron*



Percent mortality was plotted against concentration (Log dose) for the petroleum ether and chloroform fractions of marine macrophytes viz. A- *Derris heptaphyla*, B- *Salvadora persica*, C- *Sonneratia caseolaris*, D- *Clerodendrum inerme*, E- *Acanthophora muscoides*, F- *Microdictyon pseudohapteron*, G- *Halophila ovalis*, H- *Syringodium isoetifolium*, I- *Umbilicaria aprina*.

TABLE 1: Larvicidal activity of Petroleum ether (PE-f) and Chloroform fractions (C-f) of 9 marine extracts against *Artemia salina*

Name	LC50 (ppm)		SE LD50	
	PE-f	C-f	PE-f	C-f
<i>D. heptaphyla</i> (Goa)	462.5	156.25	0.136	0.081
<i>S. persica</i> (Goa)	106.0	84.37	0.115	0.115
<i>S. caseolaris</i> (Goa)	181.2	77.00	0.109	0.146
<i>C. inerme</i> (Goa)	193.7	98.00	0.146	0.136
<i>A. muscoides</i> (Gujarat)	42.5	125.00	0.090	0.123
<i>M. pseudohapteron</i> (Gujarat)	11.75	72.00	0.098	0.099
<i>H. ovalis</i> (Tuticorin)	500.0	119.00	0.103	0.096
<i>S. isoetifolium</i> (Tuticorin)	-	41.50	-	0.103
<i>U. aprine</i> (Antarctica)	275.0	53.00	0.130	0.115

PE-fraction of *S. isoetifolium* did not cause LC50 even at 500 ppm.

showed excellent activity with LC50 values of 11.75 ppm. This is followed by C-fraction of *S. isoetifolium* (41.5 ppm) and PE-fraction of *A. muscoides* (42.5 ppm). C-fractions of *U. aprine* (53 ppm), *M. pseudohapteron* (72 ppm), *S. caseolaris* (77 ppm), *S. persica* (84.37 ppm), *C. inerme* (98 ppm) were effective between 53 and 98 ppm. Among the extracts studied the following were effective at concentration between 106 and 500 ppm. PE-fraction of *S. persica* (106 ppm); C-fractions of *H. ovalis* (119 ppm) *A. muscoides* (125 ppm) and *D. heptaphyla* (156.25 ppm); PE-fraction of *S. caseolaris* (181.25 ppm), *C. inerme* (193.75 ppm), *U. aprine* (275 ppm), *D. heptaphyla* (462.5 ppm) and *H. ovalis* (500 ppm). However, in the case of PE-fraction of *S. isoetifolium* 50% mortality was not evident even at 500 ppm.

DISCUSSION

Artemia salina nauplii was used as a reference organism to study the larvicidal activity of marine macrophytes for the following reasons: i) easy year round availability of the dry *Artemia* cysts. ii) It is a suitable test species to evaluate the relative toxicity of a broad range of chemical compounds. iii) *Artemia* does not belong to the group of most sensitive marine organisms. iv) The early larval stages of *Artemia*, which can survive

for a few days without feeding, is most suited for acute toxicity tests and finally, v) the first instar stage is less sensitive than the later instars (Vanhaecke *et al.*, 1981; Teng 1993). Although it may be argued that no single organism or testing protocol fulfills all the criteria to determine the toxicity of materials, *Artemia's* role is one of being a reference organism in assays, as much as a predictor of chemical effects of species in the marine environment (Persoone & Wells, 1987). According to Abernethy *et al.* (1986), it was possible to predict lethal toxicity of a chemical or formulation from *Artemia* data.

Michael *et al.*, (1956), was the first to report the use of brine shrimp as a test organism. Since then there have been several reports based on the use of this organism for environmental studies (Zillioux *et al.*, 1973; Price *et al.*, 1974; Sorgeloos *et al.*, 1978), screening for natural toxins (Harwig and Scott 1971; Reiss 1972) and as a natural screening for bioactive substances in plant extracts (Meyer *et al.*, 1982). It has been proposed as a standard test by Vanhaecke and Persoone (1982).

In the present study methanol was used as a solvent to redissolve known weight of the fractions as it is known to be least toxic (LC 10,000ppm) compared to other organic solvents (Price *et al.*, 1974).

According to Vanhaecke *et al.* (1981), the LC50 values for *Artemia* nauplii using sodium lauryl sulphate as reference toxicant were between the range of 13.3 to 19.9 ppm. Thus, the activity of PE-fraction of *M. pseudohapteron*, showing LC50 values of 11.75 ppm is comparable to any effective toxicant. The other fractions exhibiting 50% lethality below 50ppm were C-fraction of seagrass *S. isoetifolium* (41.5 ppm) and PE-fraction of seaweed *A. muscoides* (42.5 ppm). Although not much is known regarding the toxicity of these particular species, in general, Hornsey and Hide (1974) has reported activity to be wide spread throughout Chlorophyceae, Phaeophyceae and Rhodophyceae. According to Caccamese *et al.* (1985), the activity was concentrated almost entirely in Rhodomelaceae as compared to the other groups. In the present study also, *A. muscoides*, which belongs to Rhodomelaceae showed good larvicidal activity (LC 50 = 42.5 ppm). This is in agreement with the observations of Caccamese *et al.*, (1985). No information is available in the literature on toxicity of *M.*

pseudohapteron and *S. isoetifolium*. To our knowledge the present report is the first on the activity of these macrophytes on *Artemia salina*.

As in the case of mangroves, C-fractions of seagrass and lichens were also more potent than the PE-fractions. Earlier studies have revealed that *Zostera marina*, a seagrass, possessed high larvicidal activity (Harris, 1946; Todd *et al.*, 1993). However, unlike the mangroves, seagrass and lichens, PE-fraction of seaweeds showed better activity against the test organisms used.

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