

Ichthyotoxic propensities of some Indian marine green algae (Chlorophytes)

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Twenty three extracts of chlorophytes have been assayed for ichthyotoxicity against the common mosquito fish *Gambusia affinis*. The extracts generally have exhibited a wide range of effects. Of the twenty three species of chlorophytes, 35% exhibited ichthyotoxic properties causing mortality in fish and the remaining 65% were found to be nontoxic. Further, *Bryopsis plumosa* was highly toxic, *Caulerpa cupressoides*, *Bryopsis* sp., and *Halimeda gracilis* were moderately toxic and *Caulerpa fergusonii*, *C.racemosa*, *C.peltata* and *C.taxifolia* were found less toxic. These results suggest that some marine chlorophytes possess protective chemical defense mechanism against herbivorous fishes.

Various physical and biological factors determine the distribution and abundance of marine macroalgae. Macrophytes as stationary organisms defend themselves against the attack of other organisms in a variety of ways. The probability of a plant being fed upon depends on the chemistry and morphology of the seaweeds, in addition to its quality and quantity^{1,2}. Chemical defense via tissue toxicity represents one form of lethal deterrent in marine macrophytes and terrestrial plants, which can serve as protective trait against herbivores. Though the antibiotic substances in Indian marine plants have been studied in detail^{3,5}, most of the Indian marine flora remain unexplored for their ichthyotoxic propensity. In view of this the present study was carried out to evaluate certain Indian marine chlorophytes for the ichthyotoxic property.

Materials and Methods

The algae used in this study were collected from the intertidal rocky areas of Kovalam (8° 22' N; 76° 57' E), Vizhinjam (8° 22' N; 76° 55' E), Muttom (8° 7' 15" N; 77° 1' E), Kanyakumari (8° 4' 45" N; 77° 34' E), Tuticorin (9° 5' N; 78° 12' E) and Mandapam (9° 18' N; 79° 8' E). The algal samples comprised of 23 species belonging to 9 genera (*Ulva*, *Enteromorpha*, *Chaetomorpha*, *Cladophora*, *Bryopsis*, *Caulerpa*, *Avrainvillea*, *Halimeda* and *Valoniopsis*). Methanolic extracts were prepared using the following procedure. The collected algal species were washed with freshwater to remove the

associated shell pieces, sand grains and epiphytes and then air dried. The air dried seaweeds were dried in the oven at 40±2°C for 24 hours and powdered. About 1 kg of powdered material was soaked in methanol and kept for about 12 hours with proper stirring. This was filtered and concentrated to dryness using rotary evaporator.

Ichthyotoxicity tests have been conducted according to the procedure developed by Yamanouchi⁶ & Bakus and Thun⁷. Instead of aqueous extracts, methanolic extracts of seaweeds were used in the present study. Extracts (100 mg and 50 mg) were dissolved in 0.5ml of methanol and added in 200ml of aquarium water in the 500 ml beaker (ie.50 mg and 25 mg extract con/100 ml of water). The organism used to determine toxicity was the common mosquito fish *Gambusia affinis* (Vertebrata, Pisces), as this fish has been used successfully in previous toxicity studies^{8,9}. Six experimental sets were made for each concentration (50 mg and 25 mg) and five randomly collected test fish were added to each beaker. Two replicate controls (containing fish and aquarium water only) were utilised in conjunction with each test run of three to four seaweed extracts. The mortality rate (alive or dead) and behavioural patterns were recorded following the geometric time scale: 22, 45, 90, 180, 360 and 720 minutes. The behaviour of the fish were observed in greater detail following the procedure of Coll *et al.*⁹ as—orientation (normal, lateral roll, vertical roll, or both lateral and vertical

roll); movement (normal, hypoactive or hyperactive); fin activity (normal, hypoactive or hyperactive) and response to visual stimulus. A circular black object was moved rapidly towards the fish from above causing sudden shading (normal, no response, hypoactive or hyperactive).

Results

The seaweeds examined exhibited a wide range of toxicity (Table 1). Among the 23 species of chlorophytes 8 species (35%) exhibited ichthyotoxic property causing mortality in fish and the remaining 15 species (65%) were found nontoxic at 50 mg concentration (Table 1). *Bryopsis plumosa* was found to be highly toxic killing all the test organisms within 45 minutes; three species (*Bryopsis* sp., *Caulerpa cupressoides* and *Halimeda gracilis*) were moderately toxic, killing all the test fish within 180 minutes; four algal species (*Caulerpa fergusonii*, *C. racemosa*, *C. peltata* and

C. taxifolia) took 720 minutes to kill $\geq 10\%$ of the fish and were considered less toxic (Table 2).

At 25 mg concentration, the algal species such as *Bryopsis* sp., *B. plumosa* and *Halimeda gracilis* took 720 min to cause 100% mortality in fish. *Bryopsis* spp., were toxic to the test organism even at lower concentrations of 10 mg and 5 mg. While *Caulerpa cupressoides* and *C. taxifolia* were toxic only at 50 mg and 25 mg concentrations. Among the seven species of the family Caulerpaceae, one species was moderately toxic and four species were less toxic. In the family Bryopsidaceae, all the species were found toxic, while in the family Codiaceae only *Halimeda gracilis* was moderately toxic. More than 75% of the seaweeds were found to cause abnormal behaviour in *Gambusia affinis* and were considered harmful. Five species of the algae did not change behavioural patterns of fish (Table 3).

Discussion

In the marine environment, toxicity offers an

Table 1—Results of ichthyotoxicity assays with green algal extracts

	Green algal species (Chlorophytes)	Nontoxic	Highly toxic	Toxic Moderately toxic	Less toxic
1	<i>Ulva fasciata</i> Delile	—			
2	<i>Ulva lactuca</i> Linnaeus	—			
3	<i>Ulva reticulata</i> Forssk.	—			
4	<i>Enteromorpha clathrata</i> (Roth) J. Ag.	—			
5	<i>Enteromorpha intestinalis</i> (L.) Link	—			
6	<i>Enteromorpha linza</i> (L.) J. Ag.	—			
7	<i>Chaetomorpha antennina</i> (Bory) Kutez	—			
8	<i>Chaetomorpha indica</i> Kutez	—			
9	<i>Cladophora fascicularis</i> (Mertens) Kutez	—			
10	<i>Bryopsis plumosa</i> (Huds) Ag.	—	+		
11	<i>Bryopsis</i> sp.			+	
12	<i>Caulerpa cupressoides</i> (Vahl.) Ag.			+	
13	<i>Caulerpa fergusonii</i> Murr.				+
14	<i>Caulerpa racemosa</i> (Forssk.) V. Bosse				+
15	<i>Caulerpa peltata</i> Lamour				+
16	<i>Caulerpa taxifolia</i> (Vahl.) Ag.				+
17	<i>Caulerpa scalpelliformis</i> (R.Br.) V. Bosse	—			
18	<i>Caulerpa crassifolia</i> (Ag.) J. Ag.	—			
19	<i>Avrainvillea erecta</i> (Berk.) Gepp.	—			
20	<i>Halimeda maculoloba</i> Decaisne	—			
21	<i>Halimeda gracilis</i> Harv. et. J. Ag.			+	
22	<i>Halimeda tuna</i> (Ell. et. sol.) Lamour	—			
23	<i>Valoniopsis pachynema</i> (Martens) Boerger	—			

— Non toxic; + Toxic

Table 2 — Mortality percentage of *Gambusia affinis*, treated with toxic algal extracts at different concentrations and time intervals

Green algal species	Extract conc.	Mortality (%)					
		22 min	45 min	90 min	180 min	360 min	720 min
<i>Bryopsis plumosa</i>	50 mg	50	50				
	25 mg	—	—	10	20	40	30
	10 mg	—	—	—	—	20	10
	5 mg	—	—	—	—	—	20
<i>Bryopsis</i> sp.	50 mg	20	30	50			
	25 mg	—	10	20	10	30	30
	10 mg	—	—	—	—	—	20
	5 mg	—	—	—	—	—	10
<i>Caulerpa cupressoides</i>	50 mg	—	20	10	70		
	25 mg	—	—	—	—	—	20
<i>Caulerpa fergusonii</i>	50 mg	—	—	—	10	20	10
<i>Caulerpa racemosa</i>	50 mg	—	—	—	10	10	10
<i>Caulerpa peltata</i>	50 mg	—	—	10	20	—	10
<i>Caulerpa taxifolia</i>	50 mg	—	—	—	20	30	10
	25 mg	—	—	—	—	10	10
<i>Halimeda gracilis</i>	50 mg	—	50	50			
	25 mg	—	10	10	20	20	20

important adaptation for organisms otherwise defenseless against predation^{10,11}. Tropical marine plants generally produce noxious and odorous secondary metabolites^{12,13} and crude extracts of some marine plants are potentially lethal to the test fish¹⁴⁻¹⁶. Present investigation demonstrates that some of the chlorophytes are markedly toxic to fish. The extract of *Bryopsis pennata* significantly deterred feeding by the acanthurid fish¹⁷, while juvenile rabbit fish was not deterred by this extract¹⁸. Meyer *et al.*¹⁹ have isolated bryopsin from *B.pennata*, which can be attributed to the toxic nature of the *Bryopsis* spp.

Four species of *Caulerpa*, which were toxic during the present investigation lends further support to the earlier conclusion that certain green algae belonging to the genus *Caulerpa* are ichthyotoxic^{14,20}. Paul & Fenical¹⁴ stated that the toxic terpenoids present in these algae are responsible for the toxicity. Species of *Caulerpa* produce the metabolite caulerpin and caulerpenyne in variable concentrations^{21,22}. Caulerpenyne shows a wide spectrum of biological activities like antibiotic, cytotoxic, antineoplastic and ichthyotoxic²¹⁻²³. Paul & Fenical¹⁵ observed that the sesquiterpenoid caulerpenyne at the concentration between 40 to

50% of the organic extract in many species of *Caulerpa* shows strong toxic properties. The broad range of toxicity and behavioural pattern exhibited by *Gambusia affinis* while treating with the extracts of *Caulerpa* spp., may be due to the presence of caulerpin and caulerpenyne in varying concentrations. Extracts of *Halimeda copiosa*, *H.opuntia* and *H.tuna* are toxic to fish, stops cell division in sea urchin eggs and causes mortality in sea urchin sperm at lower extract concentrations^{16,24}. The compound halimeditrial produced by *Halimeda* sp., is antibiotic, inhibits cell division and ichthyotoxic²⁵. This may be the reason for the ichthyotoxic properties of *Halimeda gracilis* and the erratic behavioural changes observed in the test fish treated with the extracts of *H.macroloba* and *H.tuna* in the present investigation.

There is evidence to suggest that the plant terpenoids which are toxic to herbivores act in a dose dependant manner¹¹. In the present study, it was found that the toxicity levels of green seaweeds varied at different concentrations, showing higher mortality rate at higher concentrations.

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Table 3—Behavioural states in *Gambusia affinis* exposed to crude extracts of green algae (50 mg / 100 ml con.)

Green algal species	Behavioural pattern at different time intervals (Minutes)											
	Orientation				Movement				Fin activity			
	22	45	90	180	360	720	22	45	90	180	360	720
<i>Ulva fasciata</i>	N	N	N	N	N	N	N	N	N	N	H	H
<i>Ulva lactuca</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Ulva reticulata</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Enteromorpha clathrata</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Enteromorpha intestinalis</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Enteromorpha linza</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Chaetomorpha antennina</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Chaetomorpha indica</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Cladophora fascicularis</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Bryopsis plumosa</i>	L	W	N	N	N	N	N	N	N	N	N	N
<i>Bryopsis</i> sp.	N	L	L	L	N	N	N	N	N	N	N	N
<i>Caulerpa cupressoides</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Caulerpa fergusonii</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Caulerpa racemosa</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Caulerpa peltata</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Caulerpa taxifolia</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Caulerpa scalpelliformis</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Caulerpa crassifolia</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Avrainvillea erecta</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Halimeda macroloba</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Halimeda gracilis</i>	L	W	N	N	N	N	N	N	N	N	N	N
<i>Halimeda tuna</i>	N	N	N	N	N	N	N	N	N	N	N	N
<i>Valoniopsis pachynema</i>	N	N	N	N	N	N	N	N	N	N	N	N

N—normal, L—lateral, V—vertical, W—lateral and vertical, H—hypoactive, P—hyperactive, R—no response

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