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BIOPOTENTIALS OF SEAWEEDS COLLECTED FROM SOUTHWEST COAST OF INDIA

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Key words: bioactive compounds, seaweed extract, brine shrimp bioassay, nematicidal, ichthyotoxic, larvicidal activity.

ABSTRACT

Southwest coast of India is a unique marine habitat infested with diverse seaweeds. Therefore, the present study was initiated to explore bioactive potential of major seaweeds. Thirteen seaweeds belonging to three groups were collected from the coast was extracted in methanol:dichloromethane (1:1) and tested for different range of bioactivities including brine shrimp cytotoxicity, larvicidal, ichthyotoxic and nematicidal activities. It was found that out of 13 seaweeds extracts, *Dictyota dichotoma* and *Hypnea pannosa* showed lethal effect on root knot nematode *Meloidogyne javanica.* All 13 tested seaweeds invariably showed varied range of ichthyotoxicity particularly *D. dichotoma, Valoniopsis pachynema* and *Acrosiphonia orientalis* showed highest toxicity. Of the 13 seaweed extracts studied, *A. orientalis*, *Padina tetrastromatica* and *Centeroceras clavulatum* were most effective against second instar mosquito larvae but the activity of *P. tetrastromatica* was not restored in the third instar bioassay. *A. orientalis* exhibited a wide range of activities including brine shrimp cytotoxicity, antifeedant and larvicidal activity. The finding envisages that crude extracts of all seaweeds contained synergistic bioactivities which can be used for the production of potential biopesticides and novel pharmaceutical leads.

I. INTRODUCTION

Seaweeds are the extraordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae [10]. Approximately 841 species of marine algae found in both inter-tidal and deep water regions of the Indian coast [33]. A post-tsunami survey conducted along the peninsular coast India evidenced that the collection site of the present study (Kollam coast) flourished with highest diversity of seaweeds [28]. The tsunami affected peninsular coast of India flourished with 15 species of seaweeds belonging to 14 genera of Rhodophyta, 15 species to 9 genera of Phaeophyta and 15 species to 8 genera of Chlorophyta. India ranks first among all countries bordering the Indian Ocean ahead of Australia and South Africa in the number of recorded specific and intraspecific seaweed taxa [40]. These vast varieties of seaweeds were found to possess useful untapped biochemical compounds, which might be a potential source of drug leads in the future [23]. Until now more than 2400 marine natural products have been isolated from seaweeds of subtropical and tropical populations [31, 12]. These natural products, are known as secondary metabolites which posses a broad range of ecological interactions in marine life [21].

The inhibitory substances biosynthesized by the seaweeds were noted as early as in 1917 [18]. The first observation regarding antibiotic activities of seaweeds was reported by Pratt *et al.* [37]. Recent findings evidenced that seaweeds contained antibacterial [50], antiviral [43, 15], antifungal [47, 2], cytotoxic [47] and larvicidal potentials [48]. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthyotoxicity in lower form of animals [45]. In this background, the present study was initiated to explore the bioactive potential of major seaweeds infested along southwest coast of India as a potential source of marine bioprospecting.

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II. MATERIALS AND METHODS

Seaweeds belonging to chlorophyta, phaeophyta and rhodophyta were collected in different season (April to September 2007) during the lowest tide of chart datum from the seaweed infested locations along southwest coast of India, Kollam (08º 54' N & 76º 38' E) and Varkala (08º 28' N & 76º 55'

E) area. The study area, southwest coast comprising of numerous sandy beaches and irregularly distributed rocky substratum interspersed with sandy intertidal pools inhabited with a wide variety of marine algae [28]. The algae, which infested exclusively on the intertidal rocky and other substratum, were selected for the collection in order to avoid other algal contamination. These algae were collected using a metal scraper. Immediately after collection, they were washed in fresh seawater to remove the epiphytes, sand and other extraneous matter. After draining off the water, the algae were wiped with a blotting sheet and air-dried under shade. After completing the shade drying process, they were cut into small pieces and dried in an oven at 45°C. Completely dried material was weighed and ground finely in a mechanical grinder.

1. Extraction of Crude Bioactives

For extraction of crude bioactives, 500 g of finely powdered algal material was refluxed three times in a 5 liter capacity round bottom flask in a water bath at 65ºC for about 6 h using dichloromethane: methanol (1:1) as a binary azeotropic solvent [42]. The extracts were filtered and concentrated to recover the excess solvents in another distillation system. The concentrated extract (about 100ml) was again filtered through a Whatman no. 1 filter paper fitted with a Buchner funnel using suction pressure. Finally, it was reduced to thick oily natured crude extract in a rotary vacuum evaporator (Yamato) at 40°C, collected in airtight plastic vials and stored in the refrigerator for further activity studies.

2. Brine Shrimp Assay

About 0.1 g of *Artemia salina* cysts was aerated in 1 L capacity glass cylinder (jar) containing filtered seawater. The air stone was placed in the bottom of the jar to ensure complete hydration of the cysts. After 24 h, the newly hatched free-swimming pink-coloured nauplii were harvested from the bottom outlet. As the cyst capsules floated on the surface, this collection method ensured pure harvest of nauplii. The freshly hatched free-swimming nauplii were used for the bioassay. The assay system was prepared with 2 ml of filtered seawater containing chosen concentration of extract in cavity blocks (Embryo cup). Parallel vehicle control (using 2 % methanol) and negative control (without vehicle) wells also kept. In each, 20 nauplii were transferred and the setup was allowed to remain for 24 h, under constant illumination. After 24 h, the dead nauplii were counted with a hand lens. Based on the percent mortality, the LD_{50} of the test compound was determined using probit scale [55].

3. In Vitro Nematicidal Activity

Nematicidal activity was evaluated using juvenile nematodes of *Meloidogyne javanica.* Assay system was prepared with 2 ml Milli Q water containing different concentrations of seaweed extracts (1, 2, 3 and 4 mg/ml) in glass tubes. 10 juveniles of *M. javanica* were transferred in test, positive (with 2% methanol) and negative (without vehicle) control tubes. Each concentration had three replicates. Mortality was observed under a zoom streomicroscope after 24 h of exposure. The mortality percentage was converted into probit scale to determine the LD_{50} values.

4. Ichthyotoxic Activity of Seaweeds

Fingerlings (1.5-2.0 cm) of marine acclimated marine acclimated *Danio aequipinnatus* were used for evaluating the ichthyotoxic potential. Five fingerlings were introduced in each experimental and control glass bowls containing 1000 ml seawater dissolved with chosen concentration of the extract. Immediate reflex changes and mortality were observed continuously for six hours and at 1 h interval for the next 12 h. After 24 h of exposure, the number of dead and live fish was counted. The acute toxicological reflexes were observed and recorded.

5. Larvicidal Activity of Seaweeds

The persistence of larvicidal activity of various extracts was tested against the urban mosquito *Culex quinquefasiatus* using standard bioassay protocol. Eggs of *C. quinquefasiatus* were obtained from drainage system. Eggs were placed in clean water and kept at room temperature for hatching. Larval development was monitored for seven days. The second and third stage larvae were collected at the tip of a pasture pipette and placed in cotton bud to remove excess water and transferred to the test vial (ten/vial). The larval mortality was observed using various concentrations of seaweed extracts (100, 200, 300, 400 & 500 µg/ml) including positive (with 2% methanol) and negative controls (without vehicle). The number of larvae surviving after 24 h of exposure was recorded. All the experiments were repeated six times to validate the findings statistically.

III. RESULTS AND DISCUSSION

1. Organisms and Seasonality

Seaweeds belong to Chlorophyta (6 species), Phaeophyta (3 species) and Rhodophyta (4 species) were identified after recent classification [44]. The species of division chlorophyta are collected during post monsoon season (July to September) and are distributed in the rocky outcrops of intertidal zone. Among the six members of chlorophyta, *Ulva fasciata*, *A. orientalis*, *Chaetomorpha antennina* and *Enteromorpha intestanalis* were dominated in this area. While the samples of phaeophyta were distributed widely in subtidal zone and their abundance was observed during November to April. *D. dichotoma* was the dominant brown alga infested along the coast. The red algae were observed in different habitats of upper intertidal, intertidal and subtidal zones during April onwards. Extensive bed of *Gracilaria corticata* was found even in off-season. Plants of *C. clavulatum* were quite common in this period. The dry weight and wet weight ratio varied according to the biogeographical factors and its extraction yield differ from species to species of different algal groups [26]. Data for wet weight, dry weight and yield per gram of seaweeds were presented in Table 1.

Table 1. Extract yield of seaweeds collected from southwest coast.

Division	Species	Wet wt. (g)	Dry wt. (g)	Yield (g/dry wt.)
Chlorophyta	*Valoniopsis pachynema* (G. Martens) Børgesen	4300	648	0.0629
	Chaetomorpha antennina (Bory de Saint-Vincent) Kützing	3080	719	0.088
	Enteromorpha intestinalis (Linnaeus) Nees var. *crispa* (Roth) Greville	2800	861	0.11
	Acrosiphonia orientalis (J. Agardh) P. Silva.	5600	1240	0.082
	Ulva fasciata Delile	3400	558	0.053
	Caulerpa racemosa (Forsskål) J. Agardh	5900	813	0.09
Phaeophyta	*Dictyota dichotoma* (Hudson) Lamouroux.	6000	593	0.1904
	Padina tetrastromatica Hauck	6000	780	0.085
	Chnoospora bicanaliculata V. Krishnamurthy & Thomas	4400	568	0.07
Rhodophyta	*Gracilaria corticata* (J. Agardh) J. Agardh	7500	1186	0.248
	Hypnea pannosa J. Agardh	5800	528	0.027
	Centroceras clavulatum (C. Agardh) Montagne	4500	751	0.039
	Cheilosporum spectabile Harvey ex Grunow	3000	1670	0.029

2. Brine Shrimp Cytotoxicity of Seaweeds

Of the 13 seaweed extracts examined for brine shrimp cytotoxicity, *A. orientalis* was found to be highly effective (LD 50 = 125.8 µg/ml) whereas *V. pachynema, E. intestanalis*, and *Caulerpa racemosa* showed considerable action and their LD50 value was below 250 µg/ml and for other extracts LD 50 value occurred above 250 µg/ml. (Table 2). Brine shrimp cytotoxicity test has been used as a bioassay for a variety of toxic substances [30] and it can be extrapolated for cell-line toxicity and anti tumour activity. Cytotoxic property by plant material is due to the presence of antitumour compounds [3]. Many of the secondary metabolites biosynthesized by the marine plants are well known for there cytotoxic property. Fucoidans exhibit antitumour, anticancer, antimetastatic and fibrinolytic properties in mice [7]. Religa *et al.* [38] reported that fucoidans also reduces cell proliferation. Stypoldione, isolated from *Stypodium sp.* was a potent cytotoxic metabolite, which halts mitotic spindle formation [16, 25]. Palermo *et al.* [34] reported a compound, Condriamide A from *Chondria sp*. exhibited cytotoxicity towards human nasopharyngeal and colorectan cancer cells. Terpenes from seaweeds displayed wide spectrum of cytotoxic and antitumour activities [53, 9]. Caulerpenyne from *Caulerpa sp.* showed its bioactivity against human cell lines and having anticancer, antitumour and antiproliferating properties [14, 35, 4]. Urones *et al.* [51] acknowledged two compound showing antitumour properties isolated from *Cystophora sp.* are meroterpenes and usneoidone. The present findings suggested that *A. orientalis* could be explored for prospecting novel anticancer leads.

3. Nematicidal Activity

It was found that out of 13 seaweeds extracts D. dichotoma and H. pannosa had lethal effects and killed 50% of nematodes when their LD50 value were 1.23 and 1.25 mg/ml respectively, whereas the extracts of C. antennina, C. racemosa, Cheilosporum spectabile and V. pachynema showed LD50 value above 2 mg/ml (Table 3). Root knot nematode are distributed worldwide, infecting wide range of plants including major crops and caused considerable economic loss [41]. Throughout of the world, root knot nematode causes an average crop loss of 8 to 11% [27]. There are many early reports showing application of seaweed extracts can reduce the nematode infestation in plants [8, 57, 58]. Presence of cytokinins in seaweed extracts may be responsible for nematicidal activity [13]. According to Wu *et al.* [58] betaines of seaweed extracts can suppress the growth of nematodes. Terpenoid compounds in seaweeds known to have nematicidal activity [1]. Domoic acid isolated from Digenea simplex and Chondria armata displayed anthelminthic activity [22]. Considering the emerging issues pertaining to the use of chemical pesticides, suitable alternative resources and ecofriendly perspective are an urgent need of sustainable agriculture. The present finding brings out new insight in the development of ecofriendly biopesticides for the management of root-knot nematodes.

4. Antifeedant Activity of Seaweeds

In the present study, all 13 tested seaweeds showed varied range of ichthyotoxicity. Highest toxicity was observed in *D. dichotoma, V. pachynema* and *A. orientalis*. Among these, *D.*

Table 2. Brine shrimp cytotoxicity of seaweeds.

Seaweeds	Mortality (%) in various concentrations			
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml
V. pachynema	8 ± 2.03	49 ± 3.6	96.66 ± 5.77	100 ± 0.0
C. antennina	0	27.6±3.2	91.66 ± 2.88	100 ± 0.0
E. intestanalis	6.1±2.4	43±4.42	100 ± 0.0	100 ± 0.0
A. orientalis	14.33 ± 4.5	100 ± 0.0	100 ± 0.0	100 ± 0.0
U. fasciata	5.8±2.1	32.4±6.3	84.33 ± 4.04	100 ± 0.0
C. racemosa	7±1.6	38.6±3.7	100 0.0	100 ± 0.0
D. dichotoma	0	20±1.54	89.33 ± 3.05	100 ± 0.0
P. tetrastromatica	0	14.33 ± 4.04	85.66 ±5.5	100 ± 0.0
C. bicanaliculata	0	16±4.21	53 ± 3.21	100 ± 0.0
G. corticata	0	26 ± 6.0	67.66 ± 6.8	100 ± 0.0
H. pannosa	0	25.33 ± 5.03	86 ± 4.0	100 ± 0.0
C. clavulatum	0	22.1±2.6	95.66 ± 4.04	100 ± 0.0
C. spectabile	0	11.2±3.8	40 ± 5.0	100 ± 0.0
Control +	0	0	0	0
Control -	0	0	0	0

Mean ± SD ***n* = 6 experiments**

Table 3. Nematicidal activity of seaweeds.

Seaweeds	Mortality (%) in various concentrations			
	1 mg/ml	2 mg/ml	3 mg/ml	4 mg/ml
V. pachynema	8 ± 3.87	31 ± 8.54	60 ± 3.60	100 ± 0.0
C. antennina	12 ± 6.08	32.33 ± 9.29	100 ± 0.0	100 ± 0.0
E. intestanalis	26 ± 4.13	70.3 ± 9.5	100 ± 0.0	100 ± 0.0
A.. orientalis	18 ± 1.24	80.33 ± 1.52	100 ± 0.0	100 ± 0.0
U. fasciata	14.3± 3.14	75.33 ± 4.5	86 ± 2.31	100 ± 0.0
C. racemosa	15 ± 4.52	44.33 ± 6.02	88 ± 5.42	100 ± 0.0
D. dichotoma	36.4 ± 5.13	100 ± 0.0	100 ± 0.0	100 ± 0.0
P. tetrastromatica	10 ± 3.14	60 ± 5.29	66 ± 1.08	100 ± 0.0
C. bicanaliculata	28 ± 2.8	56 ± 6.17	76 ± 3.91	100 ± 0.0
G. corticata	21.2 ± 1.38	58.66 ± 8.08	100 ± 0.0	100 ± 0.0
H. pannosa	38.7 ± 6.07	100 ± 0.0	100 ± 0.0	100 ± 0.0
C. clavulatum	14.2 ± 5.27	56 ± 5.29	92 ± 2.98	100 ± 0.0
C. spectabile	6.2 ± 1.24	43 ± 6.08	91 ± 1.78	100 ± 0.0
Control +	0	0	0	0
Control -	0	0	0	0

Mean ± SD ***n* = 6 experiment**

dichotoma was considered as extremely toxic since 100% mortality was observed even at lowest test concentration (50 µg/ml). *D. dichotoma* has been early reported to have antifeedant activity [52]. This extreme toxicity could be attributed to the existence of complex mixtures of terpenoids, acetogenins and aromatic terpenes synthesized by marine algae Dictotales [11, 39]. Some of these compounds are effective deterrents, preventing the algal consumption [20]. In the present study, *E. intestinalis, H, pannosa, C. racemosa* and *C. spectabile* were moderately toxic and all other five species showed low toxicity (Table 4). It was found that all the extracts impart same sort of behavioral changes. In the initial stages, fingerlings showed rapid movement with high stress and surface gasping, slow swimming while in advance stages showed occasional paralysis and inclined to the bottom in an inverted posture (Table 5).

Antipredation is an index of ecological potential against herbivory. Seaweeds were considered as one of the highly selective feed of herbivorous fishes. Herbivory is a prominent threat in the cultivation of seaweeds in open sea. Marine algae are well known for their chemical defense mechanism against herbivory [36]. According to Norris and Fenical [32] bioactive natural product of seaweeds are evolved due to high grazing pressure of herbivory. Van *et al.* [54] reported that substantial spatial variation in concentrations of metabolites thought to have antifeedant properties. The potent secondary metabolites in seaweeds and their lower consumption by hebivory are an index of algal-herbivory interactions [46, 19]. The chemical defense mechanism of the algae can be potentially useful to humans in the form of natural antibiotics and UV-sunscreens [45]. Based on the present findings it could be inferred that seaweed, D. *dichotoma* can reduce the grazing pressure by herbivorous fishes and this antifeedant defense mechanism makes it a potential candidate for the development of eco-friendly ichthyocides.

5. Larvicidal Activity

Data obtained from the larvicidal activity of seaweed extracts at the end of 24 h suggested that second instar larvae were highly sensitive than third instar. Of the 13 seaweed extracts studied *A. orientalis*, *P. tetrastromatica* and *C. clavulatum* were most effective against second instar larvae and having LD_{50} value of 91, 96 and 97 µg/ml respectively (Table 6). However, a lower level of activity was observed in Chnoospora *bicanaliculata*, *D. dichotoma*, *C. spectabile* and *G. corticata* (LD_{50} value 245, 226, 223 and 220 µg/ml). In third instar larvicidal assay, secondary metabolities of *A. orientalis*, *C. racemosa* and *C. clavulatum* were found to be highly lethal (LD_{50} value 158, 194 and 199 µg/ml) whereas *G. corticata* and *C. bicanaliculata* having less lethality (LD_{50} value 446 and 389 µg/ml). The present study revealed that *A. orientalis* contained potential mosquito control principles and can be used for the development of biocontrol tactics.

Mosquito are the vector for large number of human pathogens than any other group of arthropods [17, 5]. Their uncontrollable breeding is posing a serious threat to the modern humanity. Every year more than 500 million people become severely affected with malaria. Many plant derived natural compounds were tested for mosquito control [6]. Early report envisages that marine plant extracts contain promising larvicidal activity [48,

Table 4. Ichthyotoxicity of seaweeds.

Seaweeds	Mortality (%) in various concentrations							Time (h)* for 100% mortality	Toxicity
	25µg/ml	50µg/ml	100µg/ml	150µg/ml	200µg/ml	250µg/ml	300µg/ml		
V. pachynema	0	47.2±2.23	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	2	HT
C. antennina	0	0	0	0	23±2.76	55.3±1.8	100±0.0	6	LT
E. intestanalis	0	0	0	20.3±4.5	80±7.2	100±0.0	100±0.0	3	MT
A. orientalis	0	65 ± 5.6	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	2	HT
U. fasciata	0	0	0	10.5±4.11	43±1.3	100±0.0	100±0.0	6	LT
C. racemosa	0	43±2.33	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	3	MT
D. dichotoma	40±6.11	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	2	HT
P. tetrastromatica	0	0	22.3±3.17	100±0.0	100±0.0	100±0.0	100±0.0	6	LT
C. bicanaliculata	0	0	0	0	0	27±5.9	100±0.0	6	LT
G. corticata	0	0	0	0	10.6±2.4	41.26±1.9	100±0.0	6	LT
H. pannosa	0	0	0	16.12±2.1	30±1.5	100±0.0	100±0.0	3	MT
C. clavulatum	0	0	0	0	24±8.5	37±1.2	100±0.0	6	LT
C. spectabile	0	31±7.2	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	3	MT
Control +	0	0	0	0	0	0	0	-	-

***Onset time of 100% mortality at lowest lethal concentration.**
HT=highly toxic, MT=medium toxic, LT=low toxic.

Table 5. Behavioral changes observed in *D. aequipinnatus* after algal extract treatment.

Stages	Behavioral changes
Stage 1	Rapid movement with high stress Surface gasping
Stage 2	Slow swimming Violent movement of gill chambers
Stage 3	Loose of equilibrium Occasional paralysis
Stage 4	Inverted posture and mortality

49]. Watanabe *et al.* [56] found that polyhalogenated monoterpenes, aplysiaterpenoid A and telfairine isolated from Plocamium *sp.* showed insecticidal activity against mosquito larvae. Ishibashi *et al.* [24] reported the extracts of Aglaia elliptifolia showed insecticidal activity. Application of larvicides in their breeding places is a successful way to control its density.

In the present study, the green alga *A. orientalis* was highly bioactive seaweed among the tested taxa. Seaweeds are taxonomically heterogeneous group of organisms, which is commonly classified into three divisions according to their primary or secondary photosynthetic pigments. There is considerable ecological and physiological variation among different species within these three divisions, including production of bioactive secondary metabolites that may function to deter herbivory [29]. Epiphytic bacterial assemblages of seaweeds tested for bioactivity indicated that the green alga *A. orientalis* harboured antagonistic heterotrophic biofilm (epiphytic) bacterium *Marinobacter sp.* EB5 (data not shown). Therefore the highest bioactivity associated with the green alga *A. orientalis* may be due to two possible reasons/ phenomenon (i) the epiphytic antagonistic producer *Marinobacter sp.* EB5 might have sequestrated antibacterial substances on the surface biofilm layer which ultimately increase of bioactivity profile of *A. orientalis* or (ii) the host seaweed itself might produce antibacterial substances against fouling/predatory pressure in the habitat.

IV. CONCLUSION

From the present study, it can be concluded that the potency of the marine alga *A. orientalis* was very high which exhibited a wide range of activity in brine shrimp cytotoxicity, antifeedant and larvicidal bioassays whereas the extracts of *D. dichotoma* showed significant activity against herbivorous fishes and nematodes. The extracts of *H. pannosa* was effective to nematodes and second instar larvae. Therefore, the seaweed *H. pannosa* might be a potential source for developing ecologically significant bioactive compounds including biodegradable pesticides, and biopharmaceuticals.

Table 6. Larvicidal potential of seaweeds against second and third instar larvae of *Culex* sp.

Seaweeds	Mortality (%) in various concentrations									
	100µg/ml		200µg/ml		300µg/ml		400µg/ml		500µg/ml	
	2 instar	3 instar	2 instar	3 instar	2 instar	3 instar	2instar	3 instar	2 instar	3 instar
V. pachynema	28.1 ± 5.03	0	100 ± 0.0	0	100 ± 0.0	25.23 ± 8.12	100 ± 0.0	75.2 ± 2.31	100 ± 0.0	100 ± 0.0
C. antennina	47.5 ± 1.83	0	100 ± 0.0	0	100 ± 0.0	0	100 ± 0.0	69.6 ± 4.04	100 ± 0.0	100 ± 0.0
E. intestanalis	53.4 ± 2.17	0	100 ± 0.0	46.7 ± 4.83	100 ± 0.0	88.8 ± 1.13	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
A. orientalis	62.6 ± 3.8	0	100 ± 0.0	81.8 ± 4.18	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100± 0.0
U. fasciata	38.2 ± 6.07	0	100 ± 0.0	0	100 ± 0.0	37.3 ± 4.32	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
C. racemosa	33.02 ± 1.23	21.2 ± 3.2	96.01 ± 2.75	57.14 ± 2.8	100 ± 0.0	67.6 ± 4.63	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
D. dichotoma	0	0	36.09 ± 8.62	13.2 ± 2.31	100 ± 0.0	35.12 ± 4.03	100 ± 0.0	81.3 ± 1.26	100 ± 0.0	100 ± 0.0
P. tetrastromatica	57.3 ± 3.18	0	100 ± 0.0	16.6 ± 3.84	100 ± 0.0	75.5 ± 5.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
C. bicanaliculata	0	0	28.65 ± 5.87	0	83.43 ± 4.87	18 ± 1.2	100 ± 0.0	62.5 ± 6.12	100 ± 0.0	100 ± 0.0
G. corticata	0	0	23.7 ± 4.87	0	100 ± 0.0	7.2 ± 2.5	100 ± 0.0	29.6 ± 3.78	100 ± 0.0	100± 0.0
H. pannosa	43.4 ± 6.03	6.2 ± 2.12	100 ± 0.0	33.33 ± 6.25	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
C. clavulatum	58.3 ± 4.91	23.2 ± 3.4	100 ± 0.0	66.6 ± 3.96	100 ± 0.0	83.4 ± 3.16	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
C. spectabile	43.1 ± 8.31	0	100 ± 0.0	33.4 ± 6.25	100 ± 0.0	50.4 ± 1.56	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Control +	0	0	0	0	0	0	0	0	0	0
Control -	0	0	0	0	0	0	0	0	0	0

Mean ± SD *n* =6 experiment

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