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ORIGINAL RESEARCH ARTICLE

Inhibitory effects of the brown macroalga *Turbinaria ornata* on cyst germination and progeny cells of five harmful dinoflagellate species

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Abstract This study investigates the inhibitory effects of thalli and their extracts of the macroalga *Turbinaria ornata* on the germination of dinoflagellate cysts, previously isolated from Red Sea surface sediments. The experiments were conducted on cysts of five harmful dinoflagellate species including *Alexandrium catenella*, *Cochlodinium polykrikos*, *Dinophysis accuminata*, *Prorocentrum cordatum* and *Scrippsiella trochoidea*. The results showed neither macroalgal thalli nor their extracts had direct impact on the cyst germination of all species. Instead, these macroalgal materials remarkably affected the germling viability and culturability of progeny cells of these cysts. Dry macroalgal thalli exhibited stronger inhibitory effects on germling viability and cell culturability (IC_{50}= 0.235—0.543, 0.385—1.43 mg mL^{-1}, respectively) than fresh thalli (IC_{50}=2.201—4.716, 2.17—7.18 mg mL^{-1}, respectively). The macroalgal ethanol extract was approximately 2-5 times more effective (IC_{50} = 0.012—0.047 and 0.024—0.089 mg mL^{-1}, respectively) than aqueous extract (IC_{50} = 0.04—0.1 and 0.054—0.207 mg mL^{-1}, respectively) against the germling viability and vegetative progeny cells of all cyst species. Among different species, *A. catenella* and *C. polykrikos* germlings were more sensitive to macroalgal thalli and their extracts than those of *S. trochoidea*, *P. cordatum* and *D. acuminata*.

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Meanwhile, progeny cells of *A. catenella* exhibited the highest sensitivity to all macroalgal materials. Our results suggest that the use of *T. ornata* may be a promising strategy for inhibiting the division of progeny cells of dinoflagellate cysts and impairing the recurrence of HABs in confined coastal areas.

1. Introduction

Dinoflagellates are one of the phytoplankton groups that contribute to primary production in marine and freshwater environments (Bravo and Figueroa, 2014). However, many species can form harmful algal blooms (HABs) and produce toxins that represent a threat to aquaculture, fisheries and public health (Lewitus et al., 2012; Mohamed and Mosaad, 2007; Mohamed and Al-Shehri, 2012; Mohamed, 2018). The life cycle of many dinoflagellates encompasses an asexual vegetative phase, which reproduces by binary fission, and a sexual phase reproducing by fusion of haploid gametes (Pfiester and Anderson, 1987). The zygote may return to the vegetative stage or transform into a resting cyst, which sinks into sea bottom to sustain unfavorable conditions and remains viable in sediments for about 5—10 years (Anderson et al., 1995; Figueroa et al., 2007) or longer (up to 1 00 years) for cysts of some *Alexandrium* species (Miyazono et al., 2012). Upon the return of favorable conditions, these cysts can germinate and re-establish plankton cells that reproduce and form algal blooms in the sea water (Sun et al., 2007). Generally, cyst germination is regulated by external factors such as temperature, light, oxygen levels, and by physiological traits e.g., the dormancy period (Kremp et al., 2001). Dinoflagellate cysts thus constitute a seed source (i.e., initial inoculum) of plankton populations (Sun et al., 2007) and reflect the bloom location in subsequent seasons (Anderson et al., 2014). Climate change and warming would also enhance the germination of dinoflagellate cysts (Brosnahan et al., 2020). Therefore, the inhibition of cyst germination would restrict the development of extensive coastal blooms. Although several studies have reported the inhibitory properties of macroalgae on the growth of motile vegetative cells of HAB species through the release of algicidal allelochemicals (Ben Gharbia et al., 2017; Jin et al., 2003; Wang et al., 2007), no study has been made on the inhibitory effects of macroalgae on the cyst germination of dinoflagellate species. Therefore, the novelty of our study lies in the investigation of potential impact of macroalgae on early development stages (i.e., cyst germination, germling viability and culturability of progeny cells) of harmful dinoflagellates.

Turbinaria ornata is one of the most conspicuous macroalgal species that grows along the Red Sea coasts of Saudi Arabia. *T. ornata* has been reported to exhibit antifouling activities, particularly against microalgae (Salama et al., 2018). Therefore, our study aimed to assess the ability of this macroalga to inhibit the cyst germination and progeny cells of different dinoflagellate cysts collected from the same location, to be used as environmentally benign bioagent of controlling HABs formation.

2. Material and methods

2.1. Macroalgae and dinoflagellate cysts

Turbinaria ornata (Turner) J. Agardh used for this study was collected from Al-Shouqyq region on the east coast of the Red Sea in Saudi Arabia (19°65′N, 42°18′E), and identified according to Guiry and Guiry (2011). The algal specimens were extensively rinsed with sterile filtered (0.2 μm) seawater and rubbed with a fine brush to remove debris and epiphytes. Thereafter, macroalgal thalli were treated with a mixture of 30% ethanol and 1% sodium hypochlorite for 10 min to kill and eliminate attached bacteria and microalgae without damaging algal cells (Kientz et al 2011). Cleaned macroalgal thalli were then dried in an oven at 37°C for 48 hours. Dried thalli were cut into small pieces, then ground into a fine powder, and stored in darkness until further use. Germination experiments were carried out on cysts in re-suspended slurries of natural sediments, that were previously collected from the Red Sea (Mohamed and Al-Sheri, 2011), and stored in 10 ml vials that were tightly sealed and wrapped with aluminum foil and kept at 4°C to prevent germination. The experiments were performed on cysts of five potentially toxic dinoflagellate species including *A. catenella*, *C. polykrikos*, *S. trochoidea*, *P. cordatum*, *D. acuminata* (Mohamed, 2018).

2.2. Inhibitory effects of fresh and dry thalli of *T. ornata* on cyst germination

Different amounts of washed fresh thalli of *T. ornata* (1.0, 2.5, 5, 10 and 20 mg mL^{-1}) were added to glass culture tubes. Each tube was particularized for a single cyst species and contained 100 cyst individuals and 10 ml sterile filtered sea water. For the effect of dry thalli, the dry powder was added at 0.1, 0.5, 1, 5, and 10 mg mL^{-1} to glass tubes each containing 100 cysts of each species and 10 ml sterile filtered sea water. Concentrations of fresh and dry thalli used in our experiments were chosen based on concentrations of other macroalgae used in previous studies (Fresh thalli: 1—16 mg mL^{-1}; Dry thalli: 0.15—2.4 mg mL^{-1}) and exhibited algicidal activity against harmful dinoflagellates (Sun et al. 2016). However, the concentrations 20 mg fresh thalli mL^{-1} and 10 mg dry thalli mL^{-1} were used to determine the inhibition effect at higher concentrations. The tubes were incubated at 15°C using a 12:12 h light: dark cycle provided by cool white illumination tubes at 80 μmol m^{-2} s^{-1}. These germination conditions were earlier reported as the optimum for the germination of our dinoflagellate cysts (Mohamed and Al-Shehri, 2011). Cysts were mon-

itored every 2 days for germination and growth for a maximum of one month. After the time-course of germination, the tubes were shaken gently to loosen cyst deposits on the bottom and distribute flagellate cells and cysts evenly. Three 1-mL subsamples were then taken from each tube and poured into a Sedgewick-Rafter chamber for counting ungerminated cysts by Zeiss light microscopy according to Genovesi et al. (2009). The percentage of cyst germination was calculated by dividing the number of germinated cysts (initial number of cysts − number of ungerminated cysts) by the initial number of cysts and then multiplying the product by 100. Meanwhile, each germling cell was isolated by pipetting immediately after germination and inoculated into a new 10 mL glass tube containing F/2 medium and the same concentration of macroalgal thalli or its extracts used in each treatment. Tubes were then incubated at the same conditions described above. After 7 days, the number of motile vegetative cells was determined in all tubes. The percentage of viable germlings was estimated as the number of cells observed alive after excystment divided by total germinated cysts × 100. The percentage of culturable cells was expressed as the number of tubes with living motile cells divided by total viable germlings, and then multiplying the product by 100.

2.3. Inhibitory effects of *T. ornata* aqueous and ethanolic extracts on cyst germination

Aqueous and ethanol extracts of *T. ornata* were prepared by grinding 5 g of dry thalli in sterile distilled water or 95% ethanol, respectively. The slurry was left on a shaker at room temperature for 24 hours, and then centrifuged at 6000 rpm at 15°C for 15 min. and the supernatant was filtered through 0.22 μm polycarbonate filter. The supernatant of ethanol extract was evaporated to remove the organic solvent and the aqueous phase was adjusted to a final concentration of 10 mg mL^{-1} with sterile distilled water. Aqueous and ethanol extracts of *T. ornata* were added at 0.01, 0.05, 0.1, 0.5, and 1 mg mL^{-1} to glass culture tubes, each particularized for a single cyst species and containing 100 cysts and 10 ml sterile filtered sea water. These extract concentrations were selected based on concentrations of other macroalgae assessed in previous studies (0.01—0.96 mg mL^{-1}) and showed strong inhibition against harmful microalgae (Sun et al., 2016). The tubes were incubated at the same conditions mentioned above in the experiments of effects of fresh and dry thalli. Inhibitory effects of aqueous and ethanol extracts on cyst germination, germling viability and progeny cells were assessed by the same methods described above. In all experiments, tubes containing 100 cysts and 10 ml sterile filtered sea water (i.e., without macroalgal thalli or extract) were used as control. Each experiment was run in triplicate.

2.4. GC-MS analysis of the macroalgal extract

The GC-MS analysis was carried out for the ethanol extract of *T. ornata*, as it showed the strongest inhibitory activity against germling and vegetative progeny cells of dinoflagellate cysts. The GC-MS analysis was done using GC-MS THERMO GC-TRACE ULTRA Version-5.0. The standard non-polar capillary column, with dimensions of 30 m × 25 mm × 0.25 μm was used and the injection volume was one microliter. The extract was diluted in ethanol and injected in the split mode. Helium was used as a carrier gas with a flow rate of 1 ml min^{-1}. The GC oven temperature was programmed from 80°C to 260°C at a rate of 5°C min^{-1}. Chemical compounds were identified using spectrum database NIST 11 software installed in GC-MS instrument. The relative % amount was calculated by matching its peak area to the total areas.

2.5. Statistical analysis

All data were expressed as mean± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test in order to assess the significant differences in cyst germination, germling and progeny cells viability between control and treatments, and as well as among different species using the software SPSS (version 16.0). Differences were considered to be significant at $P < 0.05$. Computations of median inhibitory concentration (IC_{50}) of a data-set obtained from our study were performed using Finney's method of probit analysis with SPSS computer statistical software. The IC_{50} value is derived by fitting a regression equation arithmetically.

3. Results

Results of the experiments of the inhibitory effects of the brown macroalga *T. ornata* on the percentages of cyst germination, germling viability and progeny cells of five dinoflagellate species are presented in Figures 1—4. The first germinated cysts were observed on day 15 in both control and treatment groups. For all cyst species, the cyst germination percentage did not differ significantly between control and treatments ($P > 0.05$). However, the addition of macroalgal materials to experimental tubes markedly influenced the germling viability (i.e., number of viable germling cells observed alive after germination) and their culturability (i.e., the ability of progeny cells to divide and give rise to motile vegetative cells). These effects varied significantly ($P < 0.05$) between macroalgal materials (i.e., fresh thalli, dry thalli, aqueous extract and ethanol extract) for a single cyst species.

3.1. Effects of fresh and dry macroalgal thalli on cyst germination

Incubation of dinoflagellate cysts with different amounts of fresh macroalgal thalli suppressed the viability of germlings of all five cyst species used in this study. The percentage of viable germlings decreased (from 81% to 4%) with the increase of the amount of fresh algal thalli, and the viability of these germlings was completely lost at a concentration of 20 mg mL^{-1} for all cyst species (Figure 1A—E). Results also showed that lower concentrations (1, 2.5, 5 and 10 mg mL^{-1}) of fresh algal thalli exhibited marked mortality in progeny cells (i.e. culturability) of all cyst species, and all

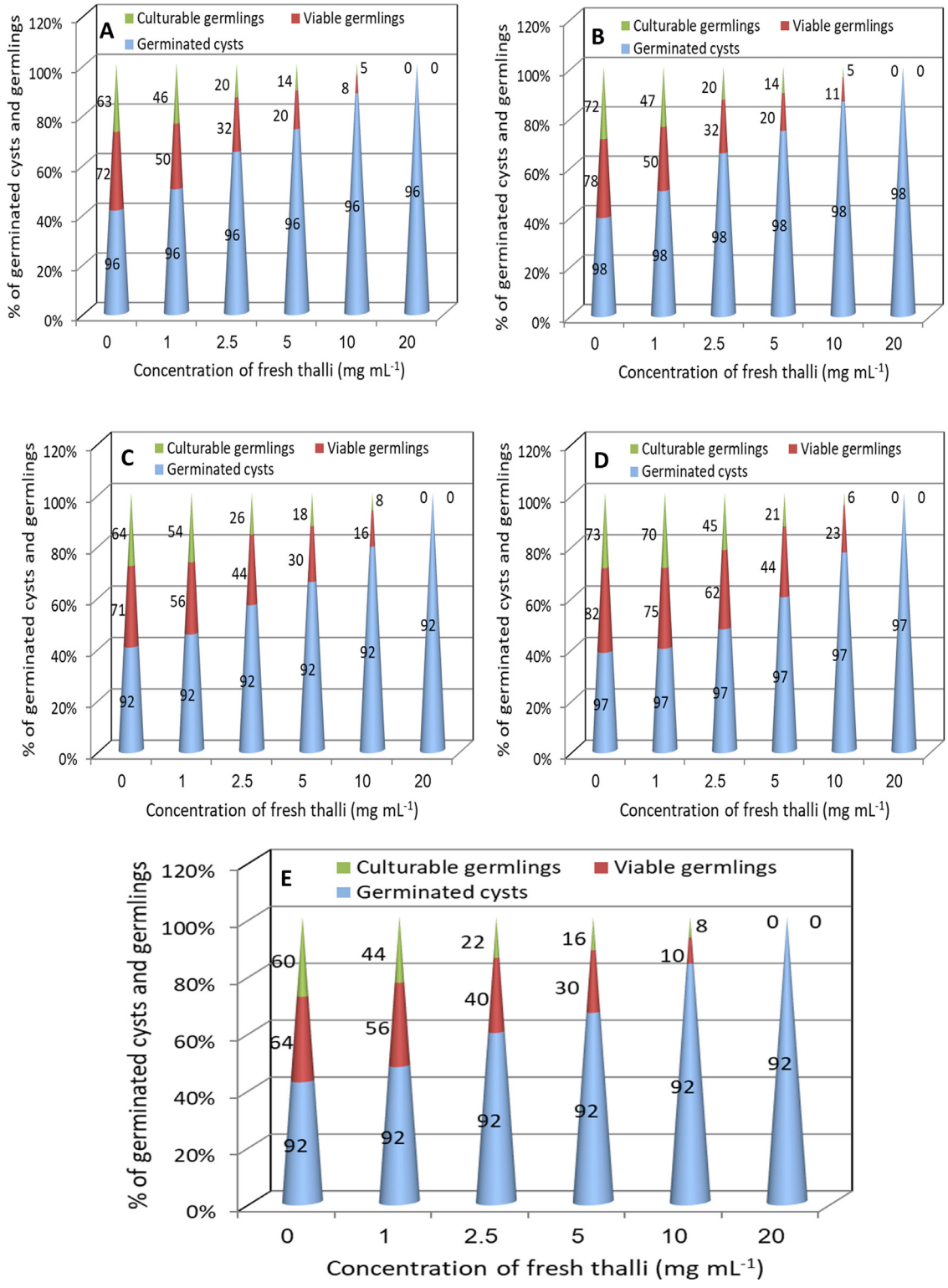

Figure 1 Inhibitory effects of fresh thalli of *Turbinaria ornata* on cyst germination, and viability and culturability of germlings of HAB species cysts (A) *Alexandrium catenella*, (B) *Scrippsiella trochoidea*, (C) *Cochlodinium polykrikos*, (D) *Prorocentrum cordatum*, and (E) *Dinophysis acuminata*. Data points are means ±SD (n = 3).

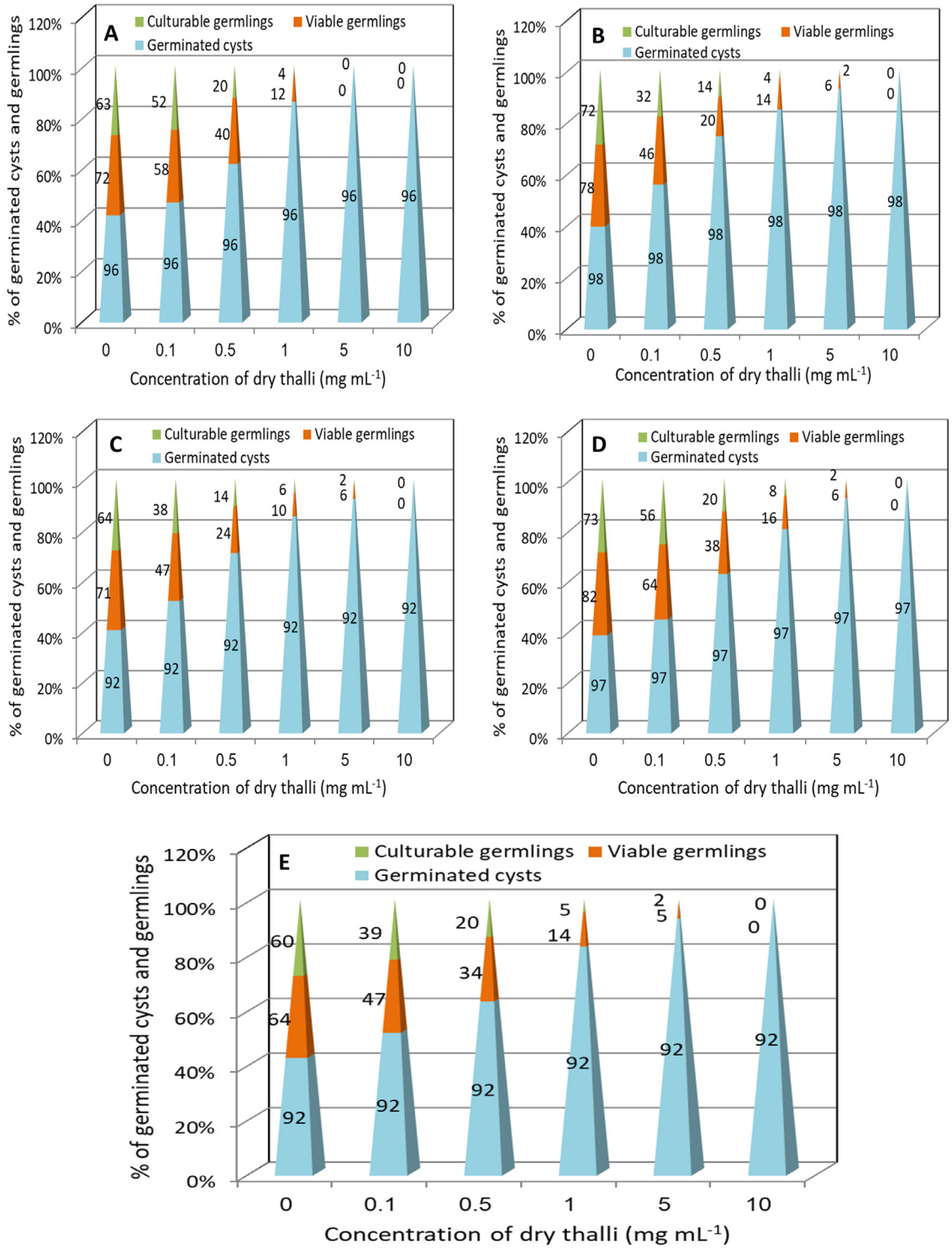

Figure 2 Inhibitory effects of dry thalli of *Turbinaria ornata* on cyst germination, and viability and culturability of germlings of HAB species cysts (A) *Alexandrium catenella*, (B) *Scrippsiella trochoidea*, (C) *Cochlodinium polykrikos*, (D) *Prorocentrum cordatum*, and (E) *Dinophysis acuminata*. Data points are means ±SD (n = 3).

progeny cells had died at the highest inoculation mass (20 mg mL^{-1}) of fresh thalli (Figure 1A—E). The dried macroalgal thalli also had inhibitory effects on viable germlings of different cyst species, but with stronger inhibition ($P < 0.05$) than that of fresh thalli. The inhibitory effect of dry thalli on the viability of cyst germlings varied with the amount of dry macroalga ($P < 0.05$). This viability was strongly suppressed by the highest two concentrations of dry thalli (5 and 10 mg mL^{-1}), with no germlings survived (Figure 2A—E). On the other hand, lower concentrations of dry thalli (0.1, 0.5, and 1 mg mL^{-1}) caused a remarkable reduction in the percentage of viable germlings of all cyst species, but with-

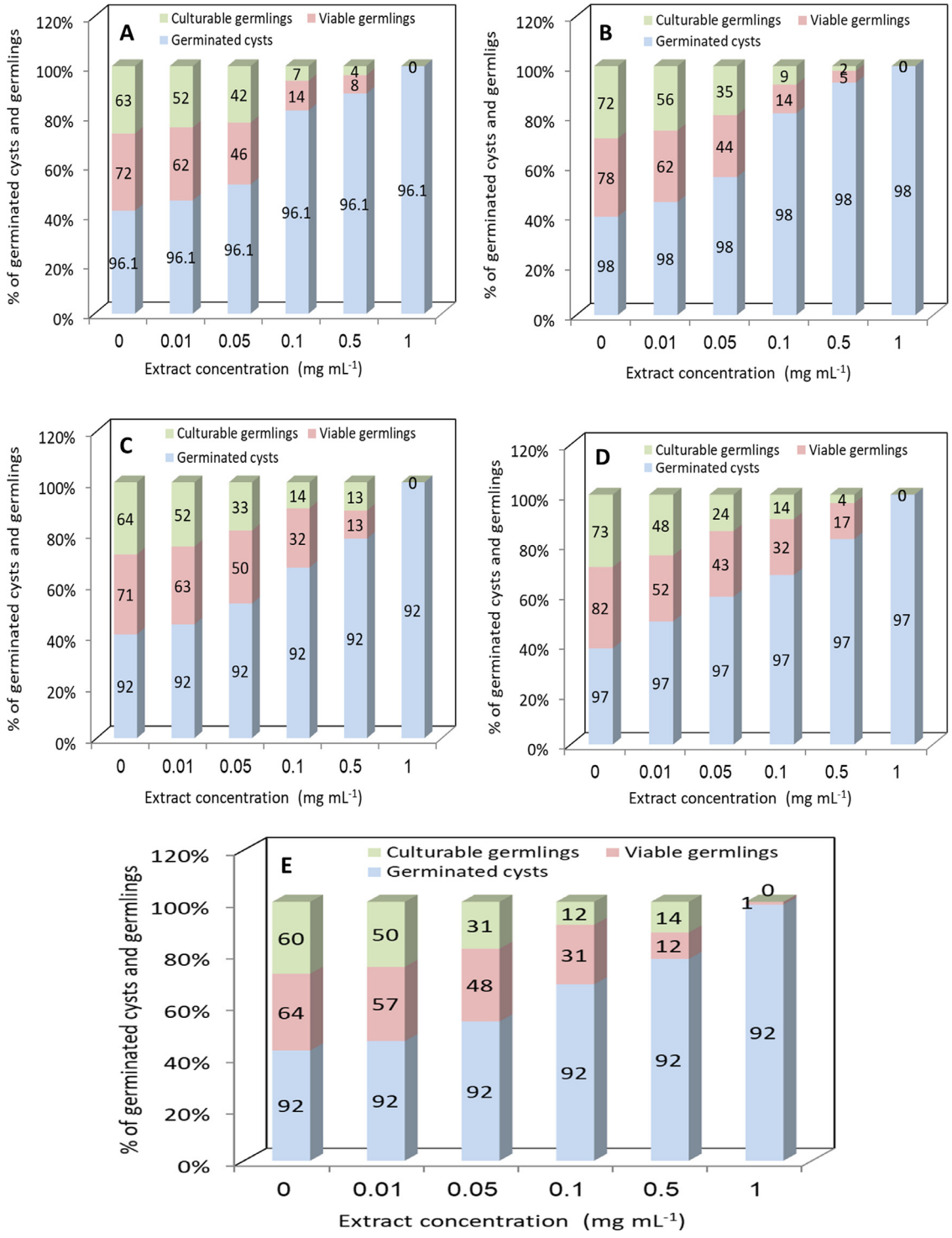

Figure 3 Inhibitory effects of aqueous extract of *Turbinaria ornata* on cyst germination, and viability and culturability of germlings of HAB species cysts (A) *Alexandrium catenella*, (B) *Scrippsiella trochoidea*, (C) *Cochlodinium polykrikos*, (D) *Prorocentrum cordatum*, and (E) *Dinophysis acuminata*. Data points are means ±SD (n = 3).

out complete death. The culturability of progeny cells of all cyst species was also influenced by dry macroalgal material, where it decreased gradually (from 73 to 1%) with the increase of dry macroalgal thalli and died at the two highest dosages (5 and 10 mg mL^{-1}) (Figure 2A–E).

3.2. Effect of macroalgal extracts on cyst germination

The addition of aqueous or ethanol extract of *T. ornata* to cyst cultures caused a significant reduction in the percent-

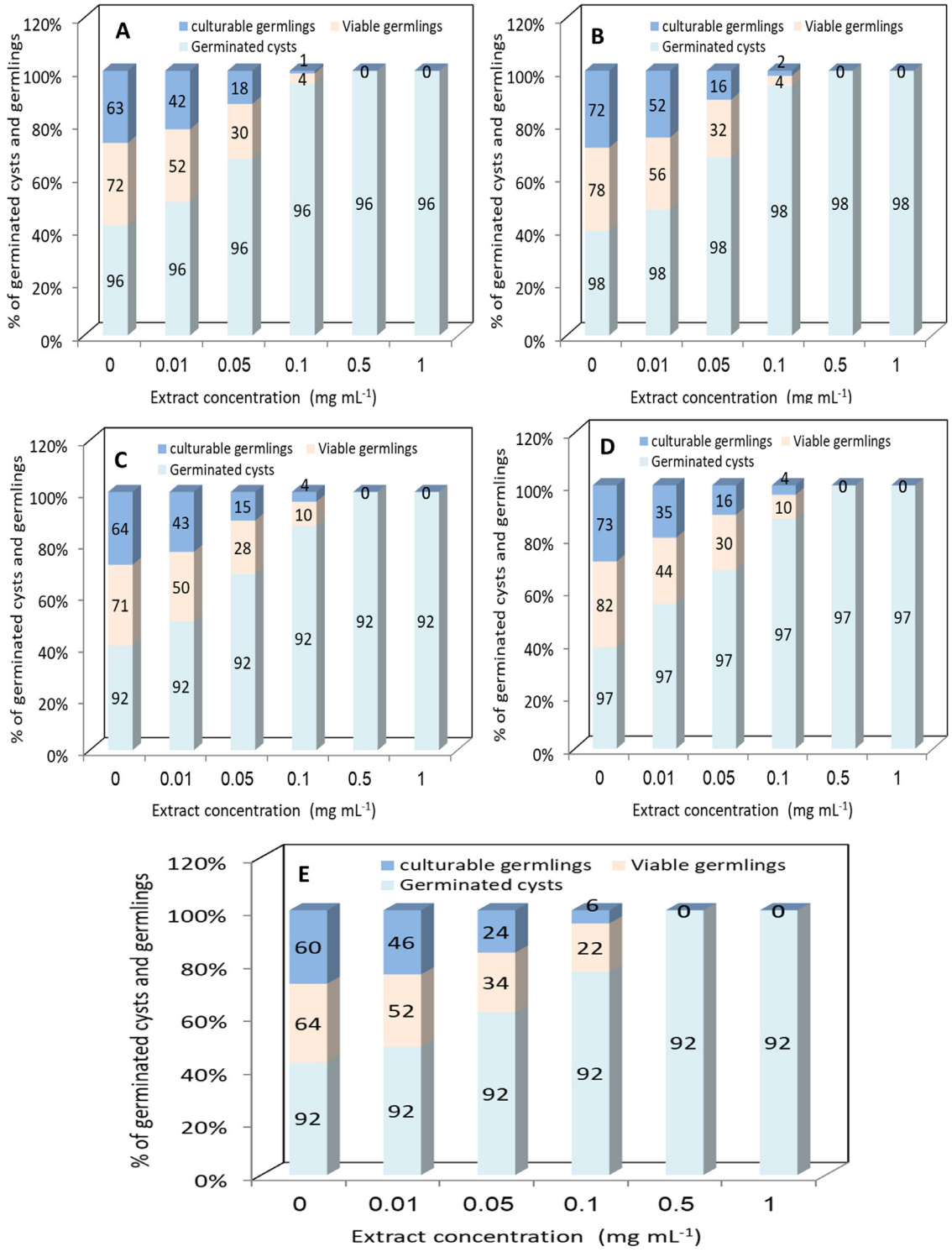

Figure 4 Inhibitory effects of ethanol extract of *Turbinaria ornata* on cyst germination, and viability and culturability of germlings of HAB species cysts (A) *Alexandrium catenella*, (B) *Scrippsiella trochoidea*, (C) *Cochlodinium polykrikos*, (D) *Prorocentrum cordatum*, and (E) *Dinophysis acuminata*. Data points are means ±SD (n = 3).

age of viable germlings of all cyst species. The germling viability percentage decreased markedly ($P < 0.05$) with the concentration of aqueous or ethanol extract. Aqueous extract of a concentration of 1 mg mL^{-1} resulted in the death of germlings of all cyst species (Figure 3A—E), while the lethal effect of ethanol extract on germling viability was observed at a concentration of 0.5 mg mL^{-1} (Figure 4A—E). Similarly, the culturability of progeny cells of different cyst species was suppressed by both aqueous and ethanol extracts. By increasing the concentration of either aqueous

Table 1 Normalized IC_{50} values (based on Probit analysis) of fresh and dry thalli, and aqueous and ethanolic extracts of *Turbinaria ornata* against germling viability.

Cyst species	IC_{50} (mg mL^{-1})			
	Fresh thalli	Dry thalli	Aqueous extract	Ethanolic extract
Gonyaulacales				
Alexandrium catenella	2.237±0.4	0.403±0.1	0.05±0.004	0.028±0.003
Gimnodinales				
Cochlodinium polykrikos	2.201±0.5	0.083±0.02	0.042±0.001	0.026±0.002
Peridiniales				
Scrippsiella trochoidea	2.936±0.8	0.235±0.05	0.1±0.02	0.022±0.004
Prorocentrales				
Prorocentrum cordatum	4.716±1	0.543±0.07	0.04±0.005	0.012±0.001
Dinophysiales				
Dinophysis acuminata	3.161±0.7	0.314±0.08	0.101±0.02	0.047±0.003

Table 2 Normalized IC_{50} values (based on Probit analysis) of fresh and dry thalli, and aqueous and ethanol extracts of *Turbinaria ornata* against culturability of germling cells.

Cyst species	IC_{50} (mg mL^{-1})			
	Fresh thalli	Dry thalli	Aqueous extract	Ethanolic extract
Gonyaulacales				
Alexandrium catenella	2.17±0.7	0.385±0.8	0.054±0.001	0.024±0.003
Gimnodinales				
Cochlodinium polykrikos	7.18±1.5	1.07±0.2	0.107±0.003	0.076±0.004
Peridiniales				
Scrippsiella trochoidea	7.17±1.7	1.11±0.3	0.144±0.02	0.086±0.003
Prorocentrales				
Prorocentrum cordatum	7.3±2	1.43±0.3	0.119±0.03	0.082±0.003
Dinophysiales				
Dinophysis acuminata	7.9±2.1	1.22±0.2	0.128±0.04	0.089±0.004

or ethanol extract, a concomitant decrease ($P < 0.05$) was observed in the percentage of cell culturability (Figures 3A–E and 4A–E). Similar to germling viability, the cell culturability of all cyst species was completely lost at 1 mg mL^{-1} aqueous extract (Figure 3A–E) compared to 0.5 mg mL^{-1} ethanol extract (Figure 4A–E).

3.3. Comparison of inhibitory effects based on IC_{50} values

IC_{50} values of inhibitory effects of macroalgal thalli and its extracts against germlings and progeny cells of dinoflagellate cysts were compared in Tables 1, 2. The IC_{50} values of dry macroalgal thalli (0.235–0.543 mg mL^{-1}) for germling viability inhibition were significantly lower ($P < 0.05$) than corresponding IC_{50} values (2.201–4.716 mg mL^{-1}) of fresh thalli (Table 1). Similarly, the IC_{50} values of ethanol extract ($IC_{50} = 0.04–0.1$ mg mL^{-1}) towards germling viability were lower ($P < 0.05$) than IC_{50} values (0.04–0.1 mg mL^{-1}) of aqueous extract (Table 1). Regarding the effect on progeny cells survival (i.e., culturability), the IC_{50} values for dry thalli (0.385–1.43 mg mL^{-1}) were about 4–5 folds greater ($P < 0.05$) than that those of fresh thalli (2.17–7.18 mg mL^{-1}) (Table 2). The IC_{50} values of ethanol extract (0.024–0.089 mg mL^{-1}) against progeny cells of all cyst species were about 2 times lower ($P < 0.05$) than those of aqueous extract (0.054–0.107 mg mL^{-1}) (Table 2). At the level of cyst species, the ANOVA analysis showed that the IC_{50} values of fresh and dry thalli, and aqueous and ethanol extracts towards germling viability (Table 1) and culturability of progeny cells (Table 2) varied significantly ($P < 0.05$) among different species. However, Tukey's test revealed no significant differences in these IC_{50} values between some cyst species. In this respect, no significant difference in IC_{50} values of fresh thalli was found between *A. catenella* and *C. polykrikos* germlings (P=0.9), nor between *S. trochoidea* and *D. acuminata* cysts (P=0.08). The highest IC_{50} value of fresh thalli (4.716 mg mL^{-1}) was reported in *P. cordatum* germlings, and the lowest (2.201 mg mL^{-1}) was in *C. polykrikos* germlings (Table 1). Also, the IC_{50} values of dry macroalgal thalli, did not significantly vary between *A. catenella* and *P. cordatum* (P=0.2) according to Tukey's test. The lowest IC_{50} (0.083 mg mL^{-1}) of dry thalli was incurred in *C. polykrikos* germlings, and the highest (0.543 mg mL^{-1}) was in *P. cordatum* germlings (Table 1). For the inhibitory effect of macroalgal aqueous extract, Tukey's test revealed no significant variation in IC_{50} values either among *A. catenella*, *C. polykrikos* and

Table 3 GC-MS analysis of ethanol extract of *Turbinaria ornata*.

Peak No.	Retention time (min)	Compound	Chemical formula	Molecular mass	Peak area (%)
1	16.66	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	4.34
2	19.78	Benzo(k)fluoranthene	$C_{20}H_{12}$	252.30	4.74
3	23.25	Bufencarb-2	$C_{13}H_{19}NO_2$	221.30	8.81
4	25.23	Heptachlor	$C_{10}H_5Cl_7$	373.31	25.8
5	26.18	Kresoxim-Methyl	$C_{18}H_{19}NO_4$	313.34	6.78
6	28.83	n-Tetradecane	$C_{14}H_{30}$	198.38	9.31
7	29.02	Isopropyl isothiocyanate	C_4H_7NS	101.17	9.16
8	29.38	Di-n-octylphthalate	$C_{24}H_{38}O_4$	390.55	6.97
9	34.93	Vanillylmandelic acid	$C_9H_{10}O_5$	198.17	1.87
10	36.91	Tetramethrin-1	$C_{19}H_{25}NO_4$	331.40	2.46
11	37.92	Acetamiprid	$C_{10}H_{11}ClN_4$	222.67	3.21
12	42.09	Eicosapentaenoic acid (EPA)	$C_{20}H_{30}O_2$	302.45	3.77
13	44.19	Heptanal	$C_7H_{14}O$	114.18	6.78
14	45.12	Humulene epoxide III	$C_{15}H_{24}O$	220.35	6

P. cordatum germlings (P=0.4), or between *S. trochoidea* and *D. acuminata* germlings (P=0.8). The highest IC_{50} (0.1 mg mL^{-1}) was reported for *C. polykrikos* and *D. acuminata* germlings, while the lowest (0.04 mg mL^{-1}) was recorded for *P. cordatum* germlings. For ethanol extract, the IC_{50} values did not exhibit any significant difference (P=0.9) among *A. catenella, C. polykrikos*, *S. trochoidea* germlings. The highest IC_{50} (0.047 mg mL^{-1}) was reported for *D. acuminata* germlings, and the lowest (0.012 mg mL^{-1}) was reported for *P. cordatum* germlings (Table 1). Regarding progeny cells culturability, the results revealed no significant differences in the IC_{50} values of all macroalgal materials (P= 0.2—0.9) among five cyst species (*A. catenella, C. polykrikos*, *S. trochoidea*, *P. cordatum* and *D. acuminata*) (Table 2). The lowest IC_{50} values of all algal materials against cell culturability were incurred in *A. catenella*, whereas the highest values were recorded in *D. acuminata* (Table 2).

3.4. GC-MS analysis of *T. ornata* extract

The GC- MS analysis of crude ethanol extract of *T. ornata* revealed the presence of 14 peaks of volatile compounds. The name of these compounds, their molecular mass, molecular formula and percentages were presented in Table 3. The extract contained potential algicidal substances including the halogenated aromatic compound-heptachlor (25.8%), the saturated fatty acid-n-hexadecanoic (4.34%), the unsaturated fatty acid-eicosapentaenoic acid (3.77%), the ester-di-n-octylphthalate (6.97%) and a monocyclic sesquiterpene-humulene epoxide—III (6%).

4. Discussion

This study clearly demonstrated that the macroalga *T. ornata* did not affect dinoflagellate cyst germination directly, but rather inhibited the viability of germling cells so that they became unable to produce motile vegetative cells (i.e. impaired the successful recurrence of new planktonic populations). Our results showed that all cysts of tested species could successfully germinate in both control and treatments (93—99%), but not all germinated cysts could give rise to viable germlings even in control cultures (73%). This agrees with the concept that the cysts would be able to germinate but the germling cells would not be able to divide (Genovesi et al., 2009; Vahtera et al., 2014). Several studies showed that cyst germination and germling viability are largely associated with external environmental factors including temperature, light and oxygen availability (Brosnahan et al., 2020; Genovesi et al., 2009; Vahtera et al., 2014). Regarding the contribution of biotic factors to dinoflagellate cysts, our study is the first to explore the biological interaction between macroalgae and cyst germination. In this study, despite there was no significant effect on cyst germination, the percentages of viable germlings and progeny cells culturability of all cyst species were significantly reduced when exposed to *T. ornata* thalli or its extracts compared to control. This indicates that active substances of this macroalga could inhibit metabolic processes that govern the division and growth of germlings and progeny cells, with no effect on the enzymes involved in the cyst germination. Therefore, vegetative progeny cells survival after excystment is considered as a key factor in the process of dinoflagellate bloom initiation (Mardones et al., 2016), and hence the inhibition of germling cell viability and division of progeny cells of dinoflagellate cysts retards the recurrence of relevant species in the water column. Therefore, the viability of germling cells after excystment represents a bottleneck in the bloom initiation (Genovesi et al., 2009).

Based on IC_{50} values, dry thalli (IC_{50} = 0.235—0.543 mg mL^{-1}) of *T. ornata* were more effective than fresh thalli (IC_{50} = 2.201—4.716 mg mL^{-1}) against the germling viability and vegetative cell survival (IC_{50} = 0.385—1.43, 2.17—7.18 mg mL^{-1}, respectively) of all cyst species. This may be due to the addition of dry macroalgal material to experimental culture tubes as a large pulse so that initial concentrations of active substances were much higher than in fresh thalli. Additionally, the macroalgal ethanol extract was approximately 2—5 times more effective against the germling viability and vegetative motile cells of all cyst species (IC_{50} = 0.012—0.047 and 0.024—0.089 mg mL^{-1}, respectively) than aqueous extract (IC_{50} = 0.04—0.1 and

0.054—0.207 mg mL^{-1}, respectively). These results confirmed the evidence that alcoholic solvents like ethanol are more suitable than water in extracting active substances from plants (Emad et al., 2009; Sultana et al., 2009). On the other hand, *T. ornata* extracts exhibited strong inhibitory effects on the germling viability and progeny cells survival compared to fresh and dry thalli. This could be attributed to the low amounts of active substances released from dry or fresh thalli into cyst cultures, compared to high amounts extracted and concentrated from the algal material. This also indicates that allelochemicals are congregated more in macroalgal cells than being released into the surrounding environment (Ye and Zhang, 2013).

The results of this study also showed that the IC_{50} values required for the inhibition of germling viability are lower than those inhibiting the cell division and growth of motile vegetative cells (i.e. culturability). This indicates that germling cells are more sensitive towards the active substances of this macroalga than motile vegetative cells. Our experiments also demonstrated that *T. ornata* is a broad-spectrum macroalga, exerting an inhibitory effect on the germling viability and vegetative cells survival of different dinoflagellate cyst species. These results, therefore, support the findings of previous studies reporting the allelopathic inhibitory effects of marine macroalgae including green microalgae (*Ulva*) (Ben Gharbia et al., 2017), brown macroalgae (*Sargassum*) (Wang et al., 2007), and red macroalgae (*Gracilaria*, Pyropia) (Patil et al., 2020; Tang et al., 2015) against the growth of several HAB forming microalgae. However, in our study, the sensitivity of germling and vegetative progeny cells to macroalgal materials varied between some species. *A. catenella* and *C. polykrikos* germlings were more sensitive to macroalgal thalli and their extracts than *S. trochoidea*, *P. cordatum* and *D. acuminata* germlings. Whereas vegetative progeny cells of *A. catenella* were the most sensitive ones to all macroalgal materials tested. This indicates that *T. ornata* may affect germlings and progeny cells of dinoflagellate species at various extents. Previously, the brown alga *Ecklonia kurome* was found to inhibit the proliferation of several HAB microalgae, with higher susceptibility exhibited by *Karenia mikimotoi* and *C. polykrikoides* compared to lesser sensitivity reflected by *Chattonella antiqua* (Nagayama et al., 2003). Those authors attributed the inhibitory properties of *E. kurome* to the production of bioactive compounds, phlorotannins. In our study, the inhibitory properties of *T. ornata* against germling and vegetative progeny cells of dinoflagellate species may be due to the presence of a high percentage of heptachlor, which has been previously demonstrated to have antialgal effects (Zeng et al., 2018). Other bioactive compounds, found with low percentages in *T. ornata* extract including n-hexadecanoic, eicosapentaenoic acid, di-n-octylphthalate and the sesquiterpene-humulene epoxide -III, could also be involved in the inhibitory effects of this macroalga. These compounds have been found in earlier studies to exhibit algicidal activity against red tide microalgae. The saturated fatty acid, n-hexadecanoic isolated from marine green alga *Ulva intestinalis* exerted potent algicidal activity against *Heterosigma akashiwo and Prorocentrum micans* (Sun et al., 2016). Eicospentaenoic acid and di-n-octylphthalate isolated from of the red alga *Corallina pilulifera* showed strong algicidal activity against the toxic dinoflagellate, *C. polykrikoides* (Oh et al., 2010; Zerrifi et al., 2018). Sesquiterpenoids from the red alga, *Porphyra yezoensis* significantly inhibited two harmful red tide dinoflagellates, *K. mikimitoi* and *Prorocentrum donghaiense* (Sun et al., 2018). Nevertheless, isolation and identification of these active substances produced by *T. ornata*, and their inhibitory mechanisms on physiological processes of germlings and vegetative progeny cells certainly warrants further investigation for the purpose of controlling and mitigation of HABs.

5. Conclusions

Our results provide the first evidence that the brown macroalga *T. ornata* can suppress the germling viability and survival of vegetative progeny cells of dinoflagellate cysts after germination, and thereby could retard the recurrence of blooms of relevant species in the water column. The results revealed that the macroalgal dry powder and its ethanol extract were effectively strong against germling and vegetative progeny cells of dinoflagellate cysts. As macroalgae powders or extracts have been reported as environmentally benign means of potential HAB control (Jeong et al., 2000; Wang et al., 2007), these macroalgal materials of *T. ornata* could be applied to confined coastal areas to inhibit the growth and division of progeny cells after cyst excystment and restrict recurrence of HABs in these regions. In this respect, we pay attention that it is prudent to prevent the growth of harmful microalgae at early stages (i.e. germlings and progeny cells) rather than treatment of algal blooms with algicides that may exacerbate the problem through induction of the cell lysis and releasing tremendous amounts of algal toxins into the aquatic environment. However, prior to application, mesocosm experiments should be carried out to more accurately estimate the proportion of germlings and their progeny that reach the illuminated layers of the water column, in relation to the number of germinated cysts in the presence of macroalgal extracts or powders treatments under the natural environmental conditions. Further research is also required to test the negative effects of *T. ornata* on other organisms, particularly fish and bivalves. This assay could be carried out using oyster larvae as they have been found to be susceptible to pollutants and macroalgal extracts at low concentrations (His et al., 1999; Nelson and Gregg, 2013).

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Supplementary materials

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References

Anderson, D.M., Fukuyo, Y., Matsuoka, K., 1995. Cyst methodologies. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on harmful marine microalgae. IOC Manuals and Guides, no. 33. UNESCO, Paris, 229—245.

Anderson, D.M., Keafer, B.A., Kleindinst, J.L., McGillicuddy, D.J., Martin, J.L., Norton, K., Pilskaln, C.H., Smith, J.L., Sherwood, C.R., Butman, B., 2014. *Alexandrium catenella* cysts in the Gulf of Maine: long-term time series of abundance and distribution, and linkages to past and future blooms. Deep-Sea Res. Pt. II. 103, 6—26. https://doi.org/10.1016/j.dsr2.2013.10.002

Ben Gharbia, H., Kéfi-Daly Yahia, O., Cecchi, P., Masseret, E., Amzil, Z., Herve, F., Rovillon, G., Nouri, H., M'Rabet, C., Couet, D., Triki, H.Z., Laabir, M., 2017. New insights on the species-specific allelopathic interactions between macrophytes and marine HAB dinoflagellates. PLoS ONE 12, e0187963. https://doi.org/10.1371/journal.pone.0187963

Bravo, I., Figueroa, R.I., 2014. Towards an ecological understanding of dinoflagellate cyst functions. Microorganisms 2, 11—32. https://doi.org/10.3390/microorganisms2010011

Brosnahana, M.L., Fischerb, A.D., Lopezc, C.B., Stephanie, K., Moored, S.K., Andersona, D.M., 2020. Cyst-forming dinoflagellates in a warming climate. Harmful Algae 91, 101728. https://doi.org/10.1016/j.hal.2019.101728

Emad, M.A., Amna, S.K., Nazlina, I., 2009. Antibacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* against methicillin resistant *Staphylococcus aureus* (MRSA). Sci. Res. Essays. 4, 351—356.

Figueroa, R.I., Garces, E., Bravo, I., 2007. Comparative study of the life cycles of *Alexandrium tamutum* and *Alexandrium minutum* (Gonyaulacales, Dinophyceae) in culture. J. Phycol. 43, 1039—1053. https://doi.org/10.1111/j.1529-8817.2007.00393.x

Genovesi, B., Laabir, M., Masseret, E., Collos, Y., Vaquer, A., Grzebyk, D., 2009. Dormancy and germination features in resting cysts of *Alexandrium tamarense* species complex (Dinophyceae) can facilitate bloom formation in a shallow lagoon (Thau, southern France). J. Plankton Res. 31, 1209—1224. https://doi.org/10.1093/plankt/fbp066

Guiry, M.D., Guiry, G.M., 2011. *AlgaeBase*. World-wide electronic publication. National University of Ireland, Galway. http://www.algaebase.org

His, E., Beiras, R., Searman, M.N.L., 1999. The assessment of marine pollution: bioassays with bivalve embryos and larvae. Adv. Mar. Biol. 37, 1—178. https://doi.org/10.1016/S0065-2881(08)60428-9

Jeong, J.H., Jin, H.J., Sohn, C.H., Suh, K.H., Hong, Y.K., 2000. Algicidal activity of the seaweed *Corallina pilulifera* against red tide microalgae. J. Appl. Phycol. 12, 37—43. https://doi.org/10.1007/s10811-009-9478-x

Jin, Q., Dong, S., 2003. Comparative studies on the allelopathic effects of two different strains of *Ulva pertusa* on *Heterosigma akashiwo* and *Alexandrium tamarense*. J. Exp. Mar. Bio. Ecol. 293, 41—55. https://doi.org/10.1016/S0022-0981(03)00214-4

Kientz, B., Thabard, M., Cragg, S.M., Pope, J., Hellio, C., 2011. A new method for removing microflora from macroalgal surfaces: an important step for natural product discovery. Bot. Mar. 54, 457—469. https://doi.org/10.1515/BOT.2011.053

Kremp, A., 2001. Effects of cyst resuspension on germination and seeding of two bloom-forming dinoflagellates in the Baltic Sea. Mar. Ecol. Prog. Ser. 216, 57—66. https://doi.org/10.3354/meps216057

Lewitus, A.J., Horner, R.A., Caron, D.A., Garcia-Mendoza, E., Hickey, B.M., Hunter, M., Huppert, D.D., Kudela, R.M., Langlois, G.W., Largier, J.l., Lessarde, J., Ralonde, R., Rensel, J.E.J., Strutton, P.G., Trainer, V.I., 2012. Harmful algal blooms along the North American west coast region: history, trends, causes, and impacts. Harmful Algae 19, 133—159. https://doi.org/10.1016/j.hal.2012.06.009

Mardones, J.I., Bolch, C., Guzmán, L., Paredes, J., Varela, D., Hallegraeff, G.M., 2016. Role of resting cysts in Chilean *Alexandrium catenella* dinoflagellate blooms revisited. Harmful Algae 55, 238—249. https://doi.org/10.1016/j.hal.2016.03.020

Miyazono, A., Nagai, S., Kudo, I., Tanizawa, K., 2012. Viability of *Alexandrium tamarensis* cysts in the sediments of Funka bay, Hokkaido, Japan: over a hundred year survival times for cysts. Harmful Algae 16, 81—88. https://doi.org/10.1016/j.hal.2012.02.001

Mohamed, Z.A., 2018. Potentially harmful microalgae and algal blooms in the Red Sea: Current knowledge and research needs. Mar. Environ. Res. 140, 234—242. https://doi.org/10.1016/j.marenvres.2018.06.019

Mohamed, Z.A., Al-Shehri, A.M., 2011. Occurrence and germination of dinoflagellate cysts in surface sediments from the Red Sea off the coasts of Saudi Arabia. Oceanologia 53 (1), 121—136. https://doi.org/10.5697/oc.53-1.121

Mohamed, Z.A., Al-Shehri, A.M., 2012. The link between shrimp farm runoff and blooms of toxic *Heterosigma akashiwo* in the Red Sea coastal waters. Oceanologia 54 (2), 287—309. https://doi.org/10.5697/oc.54-2.287

Mohamed, Z.A., Mesaad, I., 2007. First report on *Noctiluca scintillans* blooms in the Red Sea off the coasts of Saudi Arabia: consequences of eutrophication. Oceanologia 49 (3), 337—351.

Nagayama, K., Shibata, T., Fujimoto, K., Honjo, T., Nakamura, T., 2003. Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae. Aquaculture 218, 601—611. https://doi.org/10.1016/S0044-8486(02)00255-7

Nelson, T.A., Gregg, B.C., 2013. Determination of EC_{50} for normal oyster larval development in extracts from bloom-forming green seaweeds. Nautilus 127, 156—159.

Oh, M.Y., Lee, S.B., Jin, D.H., Hong, Y.K., Jin, H.J., 2010. Isolation of algicidal compounds from the red Alga *Corallina pilulifera* against red tide microalgae. J. Appl. Phycol. 22, 453—458. https://doi.org/10.1007/s10811-009-9478-x

Patil, V., Abate, R., Wu, W., Zhang, J., Lin, H., Chen, C., Liang, J., Sun, L., Li, X., Li, Y., Gao, Y., 2020. Allelopathic inhibitory effect of the macroalga *Pyropia haitanensis* (Rhodophyta) on harmful bloom-forming *Pseudo-nitzschia* species. Mar. Pollut. Bull. 161, 111752. https://doi.org/10.1016/j.marpolbul.2020.111752

Pfiester, L.A., Anderson, D.M., 1987. Dinoflagellate life cycles and their environmental control. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Sci. Publ., 611—648.

Salama, A.J., Satheesh, S., Balqadi, A.A., 2018. Antifouling activities of methanolic extracts of three macroalgal species from the Red Sea. J. Appl. Phycol. 30, 1943—1953. https://doi.org/10.1007/s10811-017-1345-6

Sultana, B., Anwar, F., Ashraf, M., 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14, 2167—2180. https://doi.org/10.3390/molecules14062167

Sun, A., Li, C., Lan, D., Zhu, Y., 2007. Dinoflagellate Cysts Records from Core Samples of Modern Marine Sediment at the Luoyuan Bay Mouth. Mar. Sci. Bull. 9, 36—45.

Sun, X., Jin, H., Zhang, L., Hu, W., Li, Y., Xu, N., 2016. Screening and isolation of the algicidal compounds from marine green alga *Ulva intestinalis*. Chin. J. Oceanol. Limnol. 34, 781—788. https://doi.org/10.1007/s00343-016-4383-z

Sun, Y.Y., Xing, J.Z., Zhang, J.S., Zhou, W.J., Pu, Y.F., 2018. Sesquiterpenoids with antialgal activity against the common red tide microalgae from marine macroalga *Porphyra yezoensis*. Environ. Sci. Pollut. Res. 25, 7844—7859. https://doi.org/10.1007/s11356-017-0958-2

Tang, Y.Z., Kang, Y., Berry, D., Gobler, C.J., 2015. The ability of the red macroalga, *Porphyra purpurea* (Rhodophyceae) to

inhibit the proliferation of seven common harmful microalgae. J. Appl. Phycol. 27, 531—544. https://doi.org/10.1007/s10811-014-0338-y

Vahtera, E., Crespo, B.G., McGillicuddy Jr, D.J., Olli, K., Anderson, D.M., 2014. *Alexandrium fundyense* cyst viability and germling survival in light vs. dark at a constant low temperature. Deep-Sea Res. Pt. II. 103, 112—119. https://doi.org/10.1016/j.dsr2.2013.05.010

Ye, C., Zhang, M., 2013. Allelopathic effect of macroalga *Gracilaria tenuistipitata* (Rhodophyta) on the photosynthetic apparatus of red-tide causing microalga *Prorocentrum micans*. IERI Procedia 5, 209—215. https://doi.org/10.1016/j.ieri.2013.11.094

Wang, Z.-H., Qi, Y.Z., Yang, Y.F., 2007. Cyst formation: an important mechanism for the termination of *Scrippsiella trochoidea* (Dinophyceae) Bloom. J. Plankton Res. 29, 209—218. https://doi.org/10.1093/plankt/fbm008

Zeng, H., Fu, X., Liang, Y., Qin, L., Mo, L., 2018. Risk assessment of an organochlorine pesticide mixture in the surface waters of Qingshitan Reservoir in Southwest China. RSC Adv. 8, 17797—17805. https://doi.org/10.1039/c8ra01881b

Zerrifi, S.E.A., El Khalloufi, F., Oudra, B., Vasconcelos, V., 2018. Seaweed bioactive compounds against pathogens and microalgae: Potential uses on pharmacology and harmful algae bloom control. Mar. Drugs 16, 55. https://doi.org/10.3390/md16020055