Bull Environ Contam Toxicol (2009) 83:537–541
DOI 10.1007/s00128-009-9836-z

Inhibition of the Growth of *Alexandrium tamarense* by Algicidal Substances in Chinese Fir (*Cunninghamia lanceolata*)

Wei-Dong Yang · Jie-Sheng Liu · Hong-Ye Li · Xin-Lian Zhang · Yu-Zao Qi

Received: 11 November 2007 / Accepted: 9 July 2009 / Published online: 28 July 2009

Abstract The wood sawdust from Chinese fir (*Cunninghamia lanceolata*) exhibited stronger inhibition on the growth of *Alexandrium tamarense* than those from alder (*Alnus cremastogyne*), pine (*Pinus massoniana*), birch (*Betula alnoides*) and sapele (*Entandrophragma cylindricum*). The water extract, acetone-water extract and essential oil from fir sawdust were all shown to inhibit the growth of *A. tamarense*. The inhibition of fir essential oil was the strongest among all the above wood sources while the half effective concentration was only 0.65 mg/L. These results suggested that the fir essential oil may play an important role in the algicidal effect of Chinese fir.

Keywords HABs · *Alexandrium tamarense* · Chinese fir · Essential oil

In recent years, harmful algal blooms (HABs) have been apparently increasing in China and other countries, causing a quite negative impact on ecosystems, human health, tourism and economic development. Therefore, it is urgently needed to investigate how to control HABs effectively. However, progress is still slow in developing effective and economic controlling strategies (Anderson 1997). Many studies on controlling or inhibiting the growth of HABs species have been conducted. For example, copper sulfate and other alternatives, such as clay, macrophyte, zooplankton, virus, and bacteria, have been shown to prevent the occurrence of HABs (Nagayama et al. 2003; Yu et al. 1994). However, these methods are either too expensive to implement or non-specific.

Allelopathic effects are natural phenomena involving the functions of the secondary metabolites produced by plants, algae, bacteria and fungi, to negatively influence the growth and development of neighboring plants (Legrand et al. 2003). It appears promising to use allelopathy to control HABs due to its low cost, effectiveness and environmental friendliness. Chinese fir (*Cunninghamia lanceolata*) is a fast-growing tree and widely distributed in Southern China. It is a major type of trees in forest and commercial species in China. However, the wood sawdust from Chinese fir is often abandoned. Huang et al. (2002) demonstrated that Chinese fir produced some allelochemicals, which had negative effects on the growth of other plants, even itself. Yang et al. (2005) reported that asepsis and rude fir wood sawdust had a great effect on the growth of *Alexandrium tamarense*, and the extract from fir wood sawdust could inhibit the growth of *A. tamarense*, too. Although the microalgae sinking effect of fir wood sawdust occur, some algicidal substances such as phenols and other allelochemicals from fir might be primarily responsible for the inhibition. Overwhelming evidences indicated that the phenols play an important role in the allelopathic effect of the fir and they were reported to have a significant inhibition on the growth of algae (Nakai et al. 2001).

Species of *Alexandrium* are the most harmful alga of all HABs, which caused disastrous damages to the fishery and aquaculture industry, such as abalone breeding, prawn and caged fish cultures (Horner et al. 1997). *Alexandrium* spp. produces deadly paralytic shellfish poisoning toxins (PST), which can be accumulated in the shellfish and passed on to humans. The main objective of this study is to investigate the algicidal substances in Chinese fir that inhibit *A. tamarense* and explore an algicide to control HABs with high efficiency and low toxicity and cost.

W.-D. Yang (✉) · J.-S. Liu · H.-Y. Li · X.-L. Zhang · Y.-Z. Qi
Department of Biotechnology, Jinan University,
Guangzhou 510632, China
e-mail: tywd@jnu.edu.cn

Materials and Methods

The Chinese fir and other wood sawdusts were collected from a carpentry factory at Jinan University, Guangzhou, P. R. China. The wood sawdusts were stored at room temperature till use. *A. tamarense* was obtained from the Environment Science Research Centre, Xiamen University, P. R. China.

A. tamarense was grown as batch cultures in Erlenmeyer flasks containing *K* medium filter-sterilized through 0.2 μm Millipore filters. Algal cultures were incubated at 20 ± 1°C under Artificial Climate Incubator of 200 μmol m^{-2} s^{-1} cool-white fluorescent tubes under a 12/12 h light/dark cycle. Cell density was determined by counting microalgae cells under an inverted microscope.

Algal cultures were grown in triplicate for each wood sawdust by adding 0.02 g of the sawdust to 100 mL of algal culture. The initial cell density was 1.83×10^6 cells/L. Flasks were incubated as described above for 72 h and agitated constantly. An aliquot of 0.1 mL of each culture was sampled daily at a set time for counting microalgae cells. The culture without sawdust was used as control.

Water extract was prepared by soaking 5 g of fir wood sawdust for 20 days in 500 mL of deionized water at room temperature, followed by filtration. The resulting filtrate was enriched with inorganic nutrients to the required concentrations in the *K* medium. Fifty mL of the reconstituted medium was added into 100 mL of algal culture at cell density of 1.62×10^6 cells/L, whereas 50 mL of regular *K* medium was added into the control flask.

Total phenol contents in different wood sawdusts were measured by the Prussian blue assay (Budini et al. 1980). Relative contents of total phenol could be compared with optical density.

The essential oils from wood sawdusts of Chinese fir, *Betula alnoides*, *Alnus cremastogyne*, *Entandrophragma cylindricum* and *Pinus massoniana* were isolated by steam distillation. All the essential oils were dissolved in acetone at 25 g/L as stock solutions. To evaluate the effect of Chinese fir essential oil on the growth of algae, the Chinese fir essential oil was added into 100 mL of algal cultures at an initial cell density of 5.09×10^6 cells/L to obtain final concentrations of 0.5, 1.25 and 2.0 mg/L, respectively, and the growth of algae was monitored. To compare the algicidal activities of essential oils from different wood sawdusts, each essential oil was added into 100 mL of algal culture at 4.50×10^6 cells/L to a final concentration of 0.5 mg/L.

The GC-MS analyses of the water extract, acetone/water extract and essential oil from Chinese fir wood sawdust were carried out on a HP6890GC/5973MS (Sabulal et al. 2006) under the following conditions: transfer line 280°C, oven temperature programme 80–220°C (10°C min^{-1}), carrier gas He 1.0 mL min^{-1}, mass spectra: electron impact (EI^+) mode 70 eV, ion source temperature 230°C. Individual components were identified by NIST 2.0/EPA/HIH database matching. Relative percentages of individual components in the oil were calculated from their peak areas in the total ion chromatogram obtained from GC-MS experiment.

Results and Discussion

Figure 1 shows the effects of different wood sawdusts on the growth of *A. tamarense*. The cell density in culture supplemented with wood sawdust from *P. massoniana* remained nearly unchanged during 72 h incubation, while that in culture supplemented with fir sawdust decreased. The cell density in culture supplemented with sawdust of *A. cremastogyne* increased exponentially same as that in the control. The increases in cell density in the cultures supplemented with sawdust of *B. alnoides* or *E. cylindricum* were less than that in the control 48 h after addition of sawdust. These indicate that not all sources of wood sawdust can inhibit the growth of *A. tamarense,* and wood sawdust of fir shows the strongest algicidal activity among all the wood sawdusts tested.

The total phenol relative contents in wood sawdusts varied significantly between the species (Fig. 2). They did not correlate well with algicidal activities of the sawdusts. For example, fir sawdust showed the strongest algicidal activity with a moderate level of phenols whereas sawdust of *B. alnoides* contained the highest level of phenols but with a moderate activity. These indicate that phenols may not be the predominant allelochemicals in the Chinese fir sawdust.

The strong inhibitory activity of the water extract from wood sawdust of Chinese fir on the growth of *A. tamarense* is clearly demonstrated in Fig. 3. All cultures containing the water extract experienced a constant drop in cell density during 3 days' incubation, whereas the control culture grew exponentially, suggesting that the water extract contains

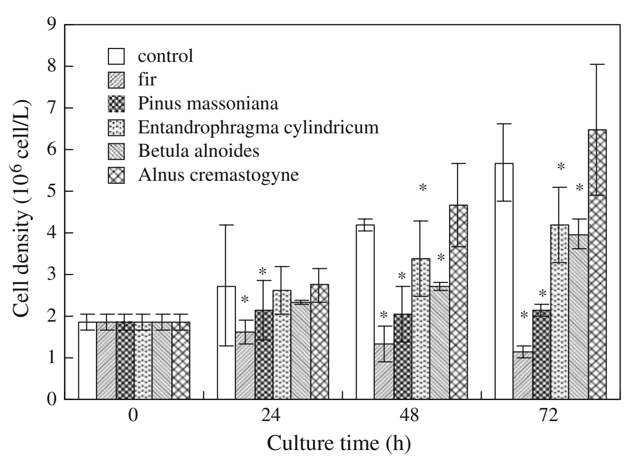

Fig. 1 Effects of different wood sawdust on the growth of *A. tamarense*. * Means $p < 0.05$, compared with control

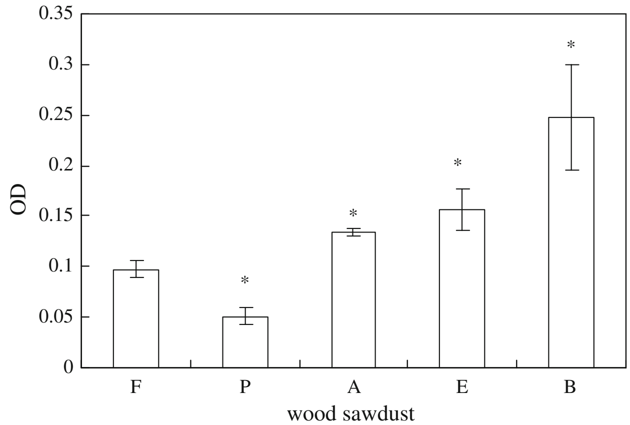

Fig. 2 Comparison in contents of total phenols between different wood sawdust. F, Chinese fir; P, *P. massoniana*; A, *A. cremastogyne*; B, *B. alnoides*; E, *E. cylindricum*. * Means $p < 0.05$, compared with control

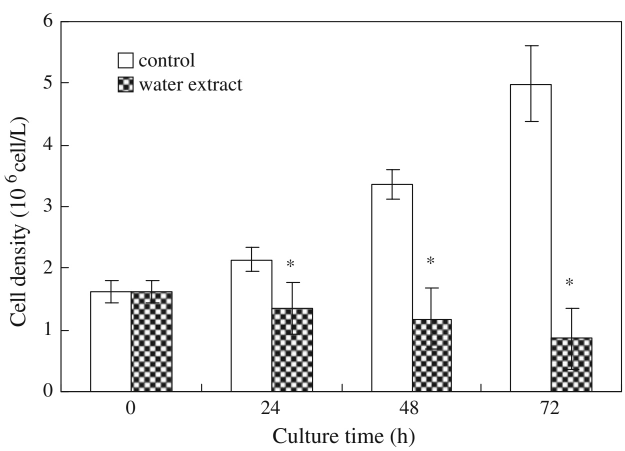

Fig. 3 Inhibition of the growth of *A. tamarense* by water extract from sawdust of Chinese fir. * Means $p < 0.05$, compared with the control

substances inhibiting the growth of *A. tamarense*, and such substances can be extracted from the fir sawdust with water.

In addition to phenols, the other most common allelochemicals are terpenoids. Many studies have shown that terpenes of essential oil have strong allelopathic effects (Scrivanti et al. 2003; Angelini et al. 2003). In order to further understand the allelopathic property of the Chinese fir, we evaluated algicidal activities of essential oils from different wood. The effects of fir essential oil on *A. tamarense* are shown in Fig. 4. Similar to the extracts, though the algal cell density was very high, fir essential oil exhibited significant inhibition on the growth of *A. tamarense* in a dose-dependent manner. The inhibition increased with increasing concentrations of the essential oil. The half effective concentration of essential oil was only about 0.65 mg/L.

The effects of the essential oils from various wood sawdusts on the growth of *A. tamarense* were similar to the

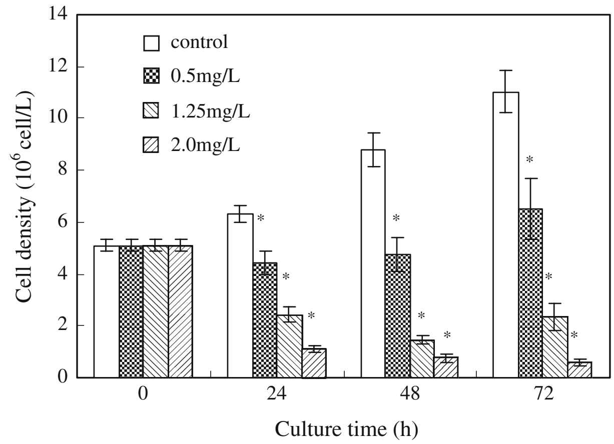

Fig. 4 Inhibition of the growth of *A. tamarense* by essential oil from Chinese fir. * Means $p < 0.05$, compared with the control

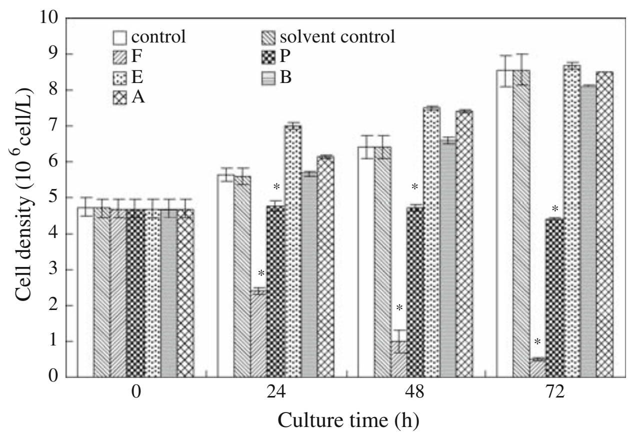

Fig. 5 Effects of essential oils in various wood sawdust on the growth of *A. tamarense*. F, Chinese fir; P, *P. massoniana*; A, *A. cremastogyne*; B, *B. alnoides*; E, *E. cylindricum*. * Means $p < 0.05$, compared with the control

corresponding wood sawdusts (Fig. 5). The cell density in culture supplemented with Chinese fir oil decreased, whereas the cell density in culture supplemented with the essential oil from *P. massoniana* hardly changed during 72 h incubation, and the cell density in cultures supplied with other essential oils increased as control. These suggest that the fir essential oil might be responsible for the inhibition of fir sawdust on the growth of *A. tamarense*.

The acetone–water extract from Chinese fir sawdust was reported to show significant algicidal activity (Yang et al. 2005), so its chemical compositions were analyzed in this study. Many common chemical components were identified by GC-MS in essential oil, acetone-water extract and water extract from Chinese fir (Table 1). But the amount of compounds identified in each sample was different; the major constituents were terpenes in all three samples prepared from Chinese fir. α-Cedrol and α-terpineol were found in the essential oil, β-eudesmol, borneol and α-terpineol in

Table 1 Identified components in wood sawdust of Chinese fir (%)

Essential oil		Extract of acetone–water		Filtrate	
Components	Content	Components	Content	Components	Content
α-Thujone	0.135	Acetic acid	4.320	α-Cedrene	1.872
Acetic acid	0.358	L-Camphor	4.737	T-Muurolol	1.542
L-Camphor	1.035	2-Fenchanol	4.306	Valencene	0.390
Benzaldehyde	0.070	Camphene hydrate	1.026	α-Cedrol	82.653
3-Pinanone	0.065	Terpinen-4-ol	1.701	Viridiflorol	0.887
2-Fenchanol	1.626	Acetophenone	0.634	Borneoll	0.758
Camphene hydrate	0.253	α-Terpineol	7.981	Labdien-8-ol	1.106
Terpinen-4-ol	0.461	Borneol	15.518	Manool	5.417
Benihinal	0.061	Berbenone	1.997	α-Terpineol	5.376
Pinocarveol	0.099	Myrtenol	0.945		
Acetophenone	0.276	Exo-2-hydroxycineole	0.851		
Camphol	0.125	Benzothiazole	3.860		
α-Terpineol	23.379	Germacrene B	1.369		
Borneol	6.020	Cis-m-menthan-8-ol-6-one	1.999		
Berbenone	0.354	T-Muurolol	4.010		
α-Phellandrene	0.048	Viridiflorol	5.228		
Myrtenol	0.290	Butyl hexadecanoate	3.018		
Hexanoic acid	0.633	α-Eudesmol	3.114		
p-Cymen-8-ol	0.561	Butyl stearate	2.539		
Benzylalcohol	0.127	α-Bisabolol	5.997		
Heptanoic acid	0.198	β-Eudesmol	24.851		
Benzothiazole	1.99				
Phenol	0.275				
Octanoic acid	0.299				
α-Cedrol	55.160				
Germacrene B	0.416				
Nonanoic acid	0.436				
Valencene	0.481				
T-Muurolol	0.963				
Viridiflorol	2.306				
2-Methylthio- benzothiazole	0.384				
Manool	0.526				
14-Labdien-8-ol	0.599				

acetone-water extract, and α-cedrol in the water extract. Certain major terpenes in acetone-extract (α-terpineol) and water extract (α-cedrol) were also major ones in the essential oil, suggesting the possible role of terpenes from the wood sawdust in the algal growth inhibition.

Many plant species contain essential oils with allelochemical properties (Gog et al. 2005) and many hypotheses have been put forward to understand the mechanisms of their biological activities (Scrivanti et al. 2003; Nishida et al. 2005; Zunino and Zygadlo 2004). However, broad diversity in the constituents of essential oil impedes revealment of any common molecular mechanism of biological activities, which also led to some difficulties in exploring the algicidal mechanisms of essential oils. Our results showed that Chinese fir essential oil could inhibit algae proliferation and make algae lose motility, shed, swell and lyse. Due to the lipophilic property of the essential oil, possibly it could enter the cell, induce membrane lipid peroxidation and increase membrane permeability. However, the mechanism of inhibition of Chinese fir essential oil on HABs species is still unclear and further study is needed.

In conclusion, we demonstrated that some allelochemicals were responsible for the algicidal effect of the Chinese fir, in addition to the algal sinking effect of fir sawdust. Due to the universality and low utilization of the fir, it is feasible that the fir essential oil could be a potential candidate for HABs control. However, it is difficult to commercially isolate the essential oil from the fir, thus further research should be carried out in future.

Acknowledgments This work was supported by National Natural Science Foundation of China (U0733006), Project of Environmental Protection Bureau of Guangzhou and Science and Technology Project of Guangdong Province in China (2004B20501007).

References

Anderson DM (1997) Turning back the harmful red tide. Nature 388:513–514

Angelini LG, Carpanese G, Cioni PL, Morelli I, Macchia M, Flamini G (2003) Essential oils from *Mediterranean Lamiaceae* as weed germination inhibitors. J Agric Food Chem 51:6158–6164

Budini R, Tonelli D, Girotti S (1980) Analysis of total phenols using the prussian blue method. J Agric Food Chem 28:1236–1238

Gog L, Berenbaum MR, DeLucia EH, Zangerl AR (2005) Autotoxic effects of essential oils on photosynthesis in parsley, parsnip, and rough lemon. Chemoecology 15:115–119

Horner RA, Garrison DL, Plumley FG (1997) Harmful algal blooms and red tide problems on the U.S. west coast. Limnol Oceanogr 42:1076–1088

Huang ZQ, Haig T, Wang SL, Han SJ (2002) Autotoxicity of Chinese fir on seed germination and seedling growth. Allelopathy J 9: 187–193

Legrand C, Rengefors K, Fistarol GO, Graneli E (2003) Allelopathy in phytoplankton: biochemical, ecological and evolutionary aspects. Phycologia 42:406–419

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

Nakai S, Inoue Y, Hosomi M (2001) Algae growth inhibition effects and inducement modes by plant-producing phenols. Wat Res 35: 1855–1859

Nishida N, Tamotsu S, Nagata N, Saito C, Sakai A (2005) Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. J Chem Ecol 31:1187–1203

Sabulal B, Dan M, Pradeep NS, Valsamma RK, George V (2006) Composition and antimicrobial activity of essential oil from the fruits of *Amomum cannicarpum*. Acta Pharm 56:473–480

Scrivanti LR, Zunino MP, Zygadlo JA (2003) *Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. Biochem System Ecol 31:563–572

Yang WD, Zhang XL, Liu JS, Gao J, Zhang P (2005) Inhibitory effect and sinking behaviour of wood meals from Chinese fir on *Alexandrium tamarense* in cultures. Acta Hydrobiol Sin 29:211–219

Yu ZM, Zou JZ, Ma XN (1994) Application of clays to removal of red tide organisms I. Coagulation of red tide organisms with clays. Chin J Ocean Limn 12:193–200

Zunino MP, Zygadlo JA (2004) Effect of monoterpenes on lipid oxidation in maize. Planta 219:303–309