# INHIBITORY EFFECT AND SINKING BEHAVIOUR OF WOOD MEALS FROM CHINA FIR ON *ALEXANDRIUM TAMARENSE* IN CULTURES

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**Abstract**: In recent years, there had been an apparent increase in the occurrence of harmful algal blooms (HABs) throughout the world. Many researchers focused on the methods to control HABs, and many approaches had been found, but seldom be applied in practice. The method to control HABs, which was economical, effective and friendly to environment, was prerequisite and the exploration of such method was getting a spotlight in HABs field. In this paper, effects of the wood meals from China fir on the growth of *Alexandrium tamarense* were assessed in laboratory. The inhibitory effect of the extracts from fir wood meals and sinking behaviour of the wood meals on microalga was observed, and possible mechanism was put forward. The results showed that fir wood meals whether autoclaved or not had negative impact on *A. tamarense*. The growth of *A. tamarense* was inhibited by 0.04% w/v of fir wood meals, with the cell density some 20-fold lower than the control. In addition, the extracts containing polyphenols from fir wood meal was found to inhibit the growth of *A. tamarense*. Sinking action of fir wood meals on microalga did occurred. These results suggested that fir wood meals may be a potential candidate for HABs control. Some algicidal bioactive substances such as polyphenols and sinking action of fir wood meals were responsible for that.

**Key words**: Red tide; China fir; *Alexandrium tamarense*
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Harmful algal blooms (HABs) were not new phenomena, which were recorded in the Bible and in the fossil record. However, in recent years HABs were becoming an increase global problem without solution or alternatives to control them[1, 2]. HABs had a major impact on ecosystem, human health and economic development[3, 4]. Therefore, it was very important to find an economical, effective and nonpolluted method of controlling HABs. In order to reduce HABs damage and remove HABs-causing algae, scientists and funding agencies had been slow to investigate possible control strategy[4]. Since copper sulfate was applied to prevent HAB, several materials had been also shown to be useful alternatives, for example, clay, macrophytes, zooplankton, viruses, and bacterial[4—9]. It was regretted that they was deemed too expensive or (and) non-specific. Members of the *Alexandrium* genus were one of the most feared of all HABs, because they produced deadly paralytic shellfish poisoning (PSP) that was accumulated within the shellfish and people could die after consumption of toxic shellfish[10, 11].

China fir (*Cunninghamia lanceolata*) was fast-growing and high-yielding tree and widely distributed in Southern China. The species was major forest type as well as major commercial tree species in China. However, the wood meals from fir was often abandoned as garbage in most cases. Many papers demonstrated that China fir produced some allelochemicals, which had negative impact on the growth of other plants, even itself[12, 13]. However, there seemed to be no report about the allelopathic effects of China fir on algae. The main objective of this study was to explore the possibility that the wood meals from fir was used as a potential candidate to control HABs, through the investigation of influence of fir wood meals on *A. tamarense* in culture.

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## 1 Materials and methods

**1.1 Samples** *Alexandrium tamarense* (strain ATHK), were obtained from the Environment Science Research Centre, Xiamen University, P. R. China. The alga cultures were incubated at 20±1 ℃, and 4000 lux 12 h: 12 h illumination from cool-white fluoresent lamps, in k media prepared with sterile-filtered man-made seawaters (salinity 33‰) with 0.2μm filtration units. *A. tamarense* were counted using a 0.1 ml counting chamber under an inverted microscope[14].

China fir (*Cunninghamia lanceolata*) wood meals were collected from a carpentry factory of Jinan University in Guangzhou, China. After being filtered, the wood meals were stored in term of size at room temperature until required.

**1.2 Inhibitory effect of China fir wood meals on *A. tamarense*** Wood meals (90-μm-filtered, autoclaved or not, 0.04g) were added to 250ml conical flasks containing 100 ml algae suspension. The initial cell density was $1.92\times10^3$ cells·$ml^{-1}$. The flasks were incubated as described above for up to 3 days and agitated daily. 0.1ml of the cultures was sampled at a set time each day after agitated enough, for counting microalga cells under an XSZ-D2 inverted microscope. The tests were carried out in triplicate, and the culture without any addition was used as a control.

**1.3 Sinking behaviour of China fir wood meals on *A. tamarense*** Sinking behaviour of China fir wood meals on *A. tamarense* was observed as described below. For group A, 0.04 g (dry weight, 90μm-filtered) wood meal and 100 ml of fresh man-made seawater enriched k-medium were added into 250 ml beakers containing 100 ml cultures, and then agitated enough. For group B and C, 0.04 g of fir wood meals were suspended in 100 ml of fresh man-made seawater enriched k-medium for 30 or 60 minutes, respectively. 100ml filtrate obtained from the resulting slurry was transfered to 250ml beakers containing 100 ml cultures. 0.1ml aliquots were collected in 2 cm depth under the surface of the cultures (not agitated), and cell density was counted using an inverted microscope after 30, 60, 90 and 120 min, respectively. The comparison between the change in cell density induced by fir wood meals and that by the extracts was made to learn the sinking behaviour of the wood meals. The tests were carried out in triplicate, and the culture received a 100ml dose of fresh man-made seawater enriched k-medium only was used as a control.

**1.4 Algicidal activities of the extracts from fir wood meals** Crude extracts containing polyphenols from fir wood meals were obtained according to the method described by Andreas Schieber et al[15]. Breifly, 10g of frozen fir wood meals was added into 200 ml acetone/water (80: 20, v/v) containing ascorbic acid (1g/L) and agitated for 48 hours in a rotary shaker at room temperature. The extracts were evaporated to 20% volume (40ml, without acetone) in rotary vacuum evaporator. The algicidal effect of the extracts of fir wood meals was evaluated by the addition of 1, 2, 3ml extracts to flasks containing 100ml of *A. tamarense* in cultures. The procedure and culture condition was the same as that described in 2.2.

**1.5 Statistic analysis** Statistical significance of difference between the two groups was determined by student's *t* test. *P* value of $<0.05$ was considered as significant.

## 2 Results and discussion

### 2.1 The effect of fir wood meals on the growth of *A. tamarense*

The presence of fir wood meals had very significant inhibition on the growth of *A. tamarense* (Fig.1). Algal growth in the control showed a sharp increase in the cell density over the 3-day experiment, with the cell density reaching around $6\times10^6$ cells/L. In contrast, algal growth in the cultures treated with fir wood meals revealed a sig-

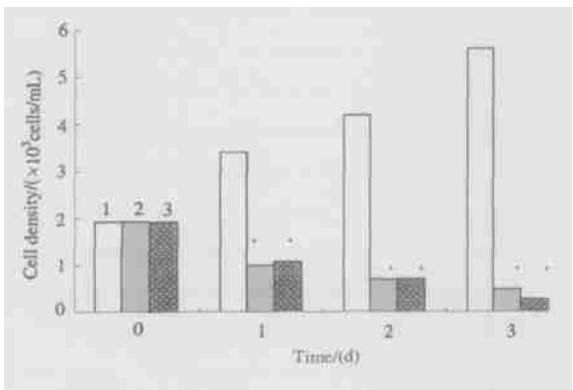


Fig. 1 Inhibition by fir wood meals on the growth of *A. tamarense*, initial cell density, $1.92\times10^6$ cells/L, * means $P<0.05$, compared with the control. 1, control; 2, fir wood meals; 3, fir wood meals (abiotic).

nificant decrease, the cell density down to $3\times 10^5$ cells/L. In addition, Algal growth was always inhibited by wood meals of fir whether autoclaved or not. The results indicated that fir wood meals could remarkably inhibited the growth of alga, the algicidal activity might be attributed to the possible allelochemicals in fir wood meals and sinking effect of the wood meals, but not potential bacteria from the wood meals.

### 2.2 Sinking behaviour of China fir wood meals on *A. tamarense*

Fig. 2 demonstrated that impact of fir wood meals and the extracts from it on *A. tamarense*. Some decreases in cell density of *A. tamarense* were observed in the assays except for the control during 120 min experiment, whereas the decrease in cell density of *A. tamarense* induced by the extracts from fir wood meals was less than that by fir wood meals itself. The results indicated fir wood meals had certain sinking action on microalga, which maybe play an important role in inhibitory effect of fir wood meals on the growth of *A. tamarense*. In addition, significant decrease ($P<0.05$) in cell density of *A. tamarense* was also observed in group B and C after 4 days (data not showed), the inhibition of the extracts suggested that chemical substances from the wood meals might involve in the mechanism of the wood meals on microalga, too.

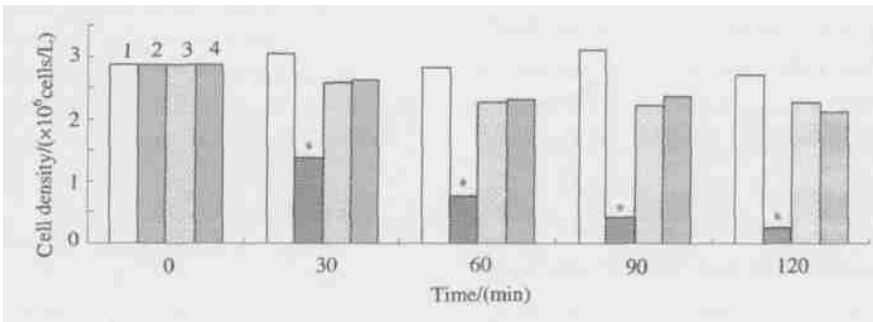


Fig. 2 Changes in *A. tamarense* cell density during 120 min after fir wood meals or filtrate added. initial cell density, $2.87\times 10^6$cells/L, * means $P<0.05$, compared with the control. 1, control; 2, group A; 3, group B; 4, group C.

### 2.3 Effect of the extract of fir wood meal on the growth of *A. tamarense*

In Fig. 3, algicidal effects of the extracts of fir wood meals on *A. tamarense* were demonstrated. Though the algal cell density was very high, the extracts had significant inhibition on the growth of *A. tamarense* while a sharp increase in the cell density was found in the control. The results indicated that polyphenols from the wood meals could inhibit the growth of *A. tamarense*, which was consistent with previous studies that suggested that allelochemicals such as polyphenols from fir could influence the growth of plants by affecting membrane system, hormone level, minerals uptake, photosynthesis, and respiration[16]. It could be predict that polyphenols from the wood meals maybe one of causes for the inhibitory effect of wood meals on micoalga.

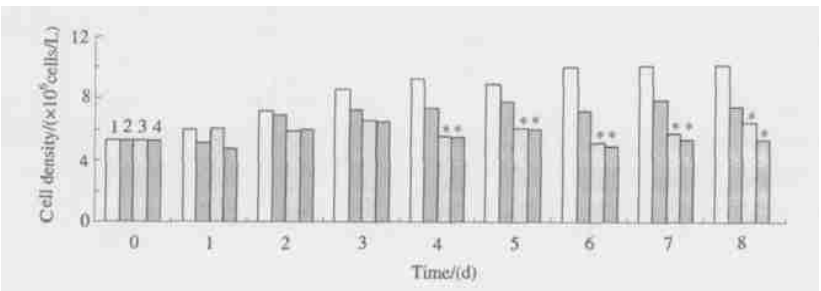


Fig. 3 Inhibition of the extracts of fir wood meals on the growth of *A. tamarense*, initial cell density, $5.31\times 10^6$cells/L, * means $P<0.05$ compared with the control. 1, control; 2, extracts(1ml); 3, extracts(2ml); 4, extracts(3ml).

During the algicidal effect assays, the change in shape of *A. tamarense* was observed under inverted microscope. Most of the algal cells were single which was immotile in the treatment, while there were mainly cellular chains of 2-4 cells per chain and being highly motiled in the control. The results indicated that algicidal bioactive substances from fir could remarkably suppressed the cell division and motility. Taking account of the results mentioned above, it was reasonable that polyphenols and sinking action of fir wood meals may be responsible for this algicidal effect. The nature of the inhibition was currently being examined in an effort to increase the efficiency of the treatment.

China fir (*Cunninghamia lanceolata*) was fast-growing and high-yielding plant, and widely distributed in Southern China. What's more, the wood meals from fir were often abandoned as byproducts in most cases. Collection of abundant cheap wood meals represented an easy and economical means of potential HAB control. In this study it had been demonstrated that China fir wood meals could effectively inhibit the growth of *A. tamarense*. These suggested that the wood meals from fir may be a potential candidate for HAB control; polyphenols and sinking action of the wood meals may be responsible for the algicidal effect. Taking account of the availability in wood meals from fir and remarkable algicidal effect on HAB alga, isolation and identification of the allelopathic substances against HAB algae in fir wood meals was significant and should be addressed in the future.

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# 杉木粉对塔玛亚历山大藻生长的抑制作用

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摘要: 近年来, 世界范围内赤潮(HABs)发生的频次有明显增长的趋势。尽管已发展了多种控制赤潮的方法, 但真正能推广应用的方法寥寥无几, 寻找新的、高效、经济、无污染的赤潮防治方法仍然是赤潮领域研究的热点。本文研究了杉木粉对赤潮藻——塔玛亚历山大藻(*Alexandrium tamarense*)生长的影响, 并对其机制进行了初步探讨。结果显示, 杉木粉能明显抑制塔玛亚历山大藻的生长, 杉木粉对藻细胞有明显的沉降作用, 其多酚提取物具有显著的抑藻作用。这些结果提示, 杉木粉可能是一种潜在的控制赤潮藻生长的新材料, 其抑藻机制可能与杉木粉中的多酚类活性物质和杉木粉对藻的沉降作用有关。

关键词: 赤潮; 杉木; 塔玛亚历山大藻