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# Effects of Main Chemical Compounds in Cooking Oil Fume Condensates (COFCs) on Growth of Salvinia natans (L.) All.: I. Dodecane

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# ABSTRACT

There is growing concern about the impact of cooking oil fume condensates (COFCs) on the environment, including on the growth of plants. COFCs contain a wide range of chemical constituents which are closely related to the temperature of cooking oil. Among these components, hydrocarbons are believed to be among the most toxic. Dodecane is one of the principal hydrocarbons implicated. The paper deals with the experiments carried out in laboratory to clarify the toxic effects of COFCs, adding various amounts of dodecane to aquatic solutions in dishes containing the floating aquatic plant *Salvinia natans*. Inhibitory effects on the vegetative growth of *S. natans*, i.e. on the development of leaves and buds are reported in the experiments. The production of new leaves is significantly inhibited. The percent inhibition of growth rate (%) in the number of leaves produced is significantly correlated with the concentration of dodecane.  $LC_{50}$  values on day 4 and day 16 after treatment are 190 mg/L and 181 mg/L respectively. Dodecane has no effect on the pH values of test solutions. It is concluded that dodecane has significant effects on the vegetative growth of aquatic plants. *S. natans* is sensitive to dodecane and might be useful as an indicator of dodecane and thus COFCs pollution.

## INTRODUCTION

Oil and grease are serious hazardous contaminators for both terrestrial and aquatic ecosystems (Martin Jr. et al. 1991, Binark et al. 2000, Bucas & Saliot 2002, Khan et al. 2004, Matsui et al. 2005, Alonso-Alvarez et al. 2007, Gawad et al. 2008, Rajakovi-Ognjanovi et al. 2008, Zhang et al. 2009). Once oil and grease are discharged intentionally or unconsciously into environments, they will significantly influence the organisms living there (Salanitro et al. 1997, Asselin et al. 2008). Oil and grease pollutants in environment may arise from different anthropogenic sources, including crude oil spill (Diapoulis & Koussouris 1989, Lemiere et al. 2005), industrial effluents (Pandy et al. 2003, der Merwe et al. 2005, Cui et al. 2008) and domestic wastes (Matsui et al. 2005, Yakimov et al. 2007, Asselin et al. 2008). Oil and grease are easy to be oxidated into peroxides and produce aldehydes, ketones and aliphatic hydrocarbons at high temperatures (Wang et al. 2000). Chemical compounds contained in oil and grease may have different effects on ecosystems. Some of them may act on organisms directly (Yakimov et al. 2007), whereas others can influence organisms indirectly through food webs (Moore et al. 1997, Lemiere et al. 2005). Nonpolar components in oil and grease may enter cells through the lipid bilayer of the plasma membrane and alter structure and functions of organelles (Ikawa 2004). Hydrocarbons are one of the important components in oils. They are considered as the most threatening hazardous pollutants from oils due to their persistence in environments as well as their mutagenic and carcinogenic properties (Lemiere et al. 2005). They can not only inhibit seed germination and reduce plant growth directly (Chaineau et al. 1997), but also influence some organisms through food chain indirectly. For example, mussels contaminated by oil (especially polycyclic aromatic hydrocarbons, i.e. PAHs) can damage predators' DNA (Lemiere et al. 2005). Hydrocarbons are stable in environments and their negative influences to environments will be persistent for a long time (Ji 1993).

Cooking oil fume condensates (COFCs) are one of the sources of oil and grease pollutants. They are detrimental not only to human health (Shields et al. 1995, Chiang et al. 1997, Ko et al. 1997, Metayer et al. 2002, Feng et al. 2003, Dung et al. 2006), but also to atmosphere (Miao et al. 2005) and other organisms (Ye et al. 2001, Jiang et al. 2009). After being heated at high temperatures, cooking oil will produce lots of secondary chemical constituents which are important hazardous materials to environments (Miao et al. 2005). Among these constituents, hydrocarbons are very important because of their great amount and diversity (Liu et al. 2002).

In our earlier paper, we have analysed the effects of COFCs on the vegetative growth of *Salvinia natans*, an aquatic fern sensitive to COFCs. The results suggested that COFCs could accelerate the death of *S. natans*, and there

was an obvious correlation between COFCs effects and the concentration and the exposure time (Jiang et al. 2009). In order to further understand that which constituents in COFCs are the main factors influencing the growth of *S. natans*, the effects of dodecane on the vegetative growth were studied by using static toxicity testing method. In this paper, we try to answer the question whether dodecane has any effect on the mortality, vegetative organs, and relative growth rate of *S. natans*.

#### MATERIALS AND METHODS

**Collection of** *S. natans* **and treatment by dodecane:** *S. natans* is found widely in China, especially in east and south parts (Li 1998). The experiment was conducted during June to August, the best growth period of *S. natans* in the region.

The plant materials for experiment were collected from a pond in the countryside of Yangzhou city. Two hundred twenty healthy individuals of *S. natans* with similar size (four pairs of the latest floating leaves, similar lengths and fresh weights) were chosen and treated according to the method in our earlier report (Jiang et al. 2009). Each individual was assigned randomly to one of the 11 groups (one control and ten treatments) and was cultivated in a plastic pot ( $\emptyset$ 12 cm and 10 cm high) containing the prepared test solutions.

Dodecane was diluted in distilled water to prepare test solutions with the following concentrations: 0 mg/L (Control), 10 mg/L (C1), 50 mg/L (C2), 100 mg/L (C3), 120 mg/L (C4), 140 mg/L (C5), 160 mg/L (C6), 180 mg/L (C7), 200 mg/L (C8), 220 mg/L (C9), and 240 mg/L (C10). The pots were placed in a greenhouse (the temperature was  $30 \pm 2^{\circ}$ C and the light was provided by metal halide bulbs for 12 h/d).

The following parameters were recorded on the day 4, 6, 8, 10, 13, and 16: mortality, number of new leaves, number of apical and axillary buds, number of leaves turning yellow. The pH value of test solution was also measured.

**The analysis of data:** Probit analysis in SPSS 16.0 was used to determine  $LC_{50}$  of dodecane to *S. natans* for given exposure times (day 4 and day 16) according to Zambrano and Carballeira (1999). The following equation was used to calculate the relative growth rate (*RGR*) of each individual (OECD 2006):

$$RGR = \frac{\ln N_1 - \ln N_0}{\Delta t}$$

Where  $N_0$  and  $N_1$  were the total number of leaves at the beginning and end of the experiment respectively, 't' was the experimental period (day).

% *I* (percent inhibition of growth rate) was determined by the following equation (OECD 2006):

$$\%I = \frac{(RGRc - RGRt)}{RGRc} \times 100$$

Where *RGRc* and *RGRt* were the *RGR* of the control and treatment groups respectively.

One-way analysis of variance (ANOVA) was used to determine the differences among different treatments with SPSS 16.0. All variables were tested for normality and homogeneity of variances. The differences were statistically significant at p < 0.05.

#### RESULTS

Mortality of S. natans under the treatments of dodecane: During the whole experiment, dead individuals appeared in every group except the control. The mortality was also closely related to the treatment time. 86.59% (71) of the total death (82) occurred before day 4, and only 13.41% of the total death happened during the last 12 days of the experiment (Table 1). However, the mortality of S. natans showed a significant positive linear correlation with the concentrations of dodecane both on day 4 and day 16 (Day 4: y = 0.0028x-0.0393,  $R^2 = 0.93$ , p < 0.001; Day 16: y = 0.0025x + 0.0548,  $R^2 = 0.83$ , p < 0.001). LC<sub>50</sub> values of dodecane for *S. natans* based on probit analysis were 190 mg/L and 181 mg/L on day 4 and day 16 respectively. The lowest observed effect concentration (LOEC) based on mortality at the end of the experiment was 10 mg/L. These results showed that the lethal effect of dodecane on S. natans was acute and correlated positively with its concentration.

Effects of dodecane on the leaves of S. natans: At the beginning of the experiment, each individual of S. natans had 8 leaves (i.e. old leaves) left. The new leaves have grown as the experiment forwarded. The percentage of plants with old leaves turning yellow changed obviously during the experiment (Table 2). There were significant differences between the numbers of old leaves turning yellow in control and experimental groups except C1 on day 4 (df = 148, F = 6.376, p < 0.001). In C2-C10, there were over 50% individuals, which had old leaves turning yellow before day 4. The percentage of old leaves turning yellow was positively related to the concentration of dodecane ( $R^2 = 0.935$ , F = 130.128, p < 0.001). Except C1 (90%) and C2 (85%), all individuals in control and other experimental groups were observed with old leaves turned yellow on day 10. These results showed that dodecane could accelerate old leaves of S. natans turning yellow at the early stage more significantly. The higher the concentration of dodecane, the more significant its effect on turning old leaves yellow.

The mean numbers of new leaves in control and experimental groups increased steadily along with the treatment

time (Fig. 1). However, the mean number of new leaves in control was significantly higher than that in the experimental groups (df = 65, F = 8.367, p < 0.001), and higher the concentration of dodecane, the lower the number of new leaves. Presence of dodecane would limit the occurrences of new leaves and this effect correlated positively to the concentration. The percentages of individuals with new leaves turning yellow in different groups are given in Table 3. In the treatment groups, new leaves turned yellow obviously earlier than in control. Plants in control showed leaves turning yellow only from day 10 onward, whereas in the experimental groups except C1, some individuals showed new leaves turning yellow already before day 4. The results also suggested that dodecane could accelerate new leaves of S. natans turning yellow, and its effect correlated positively to the concentration. The RGRs in different groups based on the number of leaves are shown in Fig. 2. The RGR was much higher in control than in the experimental groups. There was significant linear correlation between the % I and the concentrations of dodecane ( $R^2 = 0.837$ , F = 41.106, p < 0.001) (Fig. 3). The results showed that dodecane significantly limited leaf occurrences of S. natans, the percent inhibition of growth rate in leaf numbers was significantly correlative to the concentration of dodecane.

Effects of dodecane on the buds of S. natans: The number of apical buds indicates the number of branches. Each individual of S. natans had only one apical bud and no branch at the beginning of the experiment. After being treated by dodecane, the number of apical buds (including apical buds of branches) changed greatly. The mean number of apical buds in control was significantly higher than that in the experimental groups during the experiment. At the earlier stage, there were significant differences of the numbers of axillary buds between control and the experimental groups. At the end of the experiment, the numbers of axillary buds in control were bigger significantly than that in C4-C10 (Table 4). Based on the results, it can be concluded that dodecane had a significant effect on the development of branches of S. natans. High concentration of dodecane (C4-C10) had an obvious effect on the growth of apical buds, while the effect of low concentration would weaken along with the treatment time.

The numbers of axillary buds were also influenced by dodecane. There were significant differences of the numbers of axillary buds between control and C4-C10 during the whole experiment (Table 5). High concentration of dodecane (C4-C10) had an obvious effect on the growth of axillary buds.

The changes of pH values in test solutions during the experiment: pH values of test solutions in different experi-

Table 1: Mortality of *S. natans* in different experimental groups during the experiment.

Experimental group	Day 4	Day 6	Day 8	Day 10	Day 13	Day 16
Control	0%	0%	0%	0%	0%	0%
C1	0%	0%	5%	10%	15%	15%
C2	10%	15%	20%	20%	25%	25%
C3	10%	10%	10%	10%	10%	10%
C4	40%	45%	45%	45%	45%	45%
C5	30%	30%	30%	30%	35%	35%
C6	40%	40%	40%	40%	45%	50%
C7	45%	45%	45%	45%	45%	45%
C8	60%	60%	60%	60%	65%	65%
C9	55%	55%	55%	55%	55%	55%

Table 2: The percentages of individuals of *S. natans* with old leaves turning yellow in different experimental groups during the experiment.

Experimental group	Day 4	Day 6	Day 8	Day 10	Day 13
Control	0	0	0	100%	100%
C1	0	0	10%	90%	100%
C2	50%	55%	60%	85%	100%
C3	65%	70%	75%	100%	100%
C4	90%	95%	95%	100%	100%
C5	75%	85%	85%	100%	100%
C6	80%	90%	90%	100%	100%
C7	80%	85%	100%	100%	100%
C8	85%	95%	100%	100%	100%
C9	100%	100%	100%	100%	100%
C10	100%	100%	100%	100%	100%

Table 3: The percentages of individuals of *S. natans* with new leaves turning yellow in different experimental groups during the experiment.

Experimental group	Day 4	Day 6	Day 8	Day 10	Day 13	Day 16
Control	0	0	0	5%	65%	70%
C1	0	0	5%	15%	20%	40%
C2	10%	30%	35%	35%	45%	70%
C3	25%	30%	30%	35%	40%	55%
C4	50%	55%	65%	70%	80%	85%
C5	45%	65%	70%	70%	80%	95%
C6	50%	60%	60%	65%	75%	100%
C7	45%	60%	70%	70%	80%	95%
C8	60%	60%	65%	75%	80%	95%
C9	70%	70%	75%	85%	90%	100%
C10	65%	70%	75%	85%	95%	100%

mental groups had similar change trends along with the treatment time, i.e. pH values increased continually before day 10 and decreased slightly after day 10 (Fig. 4). There was no difference of pH values among the experimental groups during the experiment ( $F_{10, 76} = 0.718$ , p = 0.705). This result suggested that the presence of dodecane had no effect on pH values of test solutions. It was different from that of COFCs, so there were potentially other chemical components in

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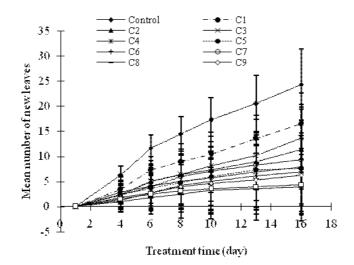


Fig. 1: The changes of the mean numbers of new leaves in different experimental groups along with treatment time (n = 20, mean  $\pm$  SD).

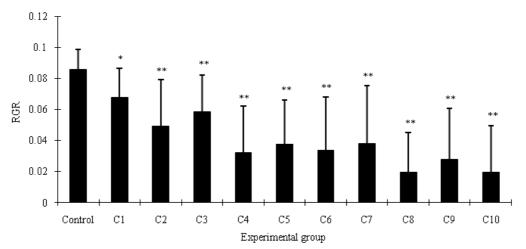


Fig. 2: *RGR* (mean  $\pm$  SD) of different experimental groups based on the total number of leaves. \*indicates that there are differences between the control and the experimental group (p < 0.05). \*\*indicates that there are significant differences between control and the experimental group (p < 0.01).

Table 5: Mean number of axillary buds of S. natans in different experimental groups during the experiment.

Experimental group	Day 4	Day 6	Day 8	Day 10	Day 13	Day 16
Control	2.15±0.489	2.55±0.686	2.9±0.852	2.9±0.852	2.9±0.788	3.05±0.686
C1	1.75±0.444 *	2.1±0.718 *	2.25±0.851 *	2.35±0.813 ns	2.45±0.826 ns	2.6±0.94 ns
C2	1.5±0.513 **	1.9±0.641 **	2.1±0.788 **	1.9±0.788 **	2.1±0.788 **	2.45±1.05 *
C3	1.7±0.47 **	1.7±0.657 **	2.05±0.826 **	2±0.725 **	2.4±0.821 ns	2.4±0.94 *
C4	1.35±0.489 **	1.55±0.605 **	1.7±0.657 **	1.6±0.681 **	1.55±0.686 **	1.7±0.979 **
C5	1.55±0.605 **	1.55±0.759 **	1.8±0.894 **	1.95±1.05 **	1.85±0.988 **	1.7±0.865 **
C6	1.55±0.605 **	1.8±0.894 **	1.85±0.988 **	1.95±1.191 **	1.75±0.91 **	1.85±1.226 **
C7	1.25±0.639 **	1.55±0.826 **	1.85±1.089 **	1.9±1.294 **	1.9±1.21 **	1.95±1.191 **
C8	1.25±0.444 **	1.25±0.444 **	1.25±0.444 **	1.35±0.587 **	1.35±0.587 **	1.3±0.571 **
C9	1.4±0.681 **	1.35±0.671 **	1.45±0.759 **	1.5±0.761 **	1.4±0.754 **	1.45±0.759 **
C10	1.35±0.587 **	1.3±0.571 **	1.4±0.754 **	1.45±0.887 **	1.25±0.639 **	1.25±0.639 **

ns = not significant (i.e., p > 0.05). \*p < 0.05; \*\*p < 0.01.

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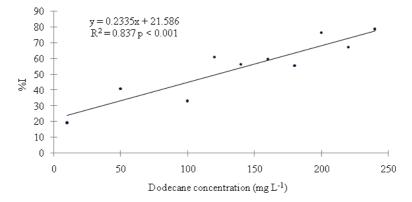


Fig. 3: The relationship between % I and dodecane concentrations at end of the experiment.

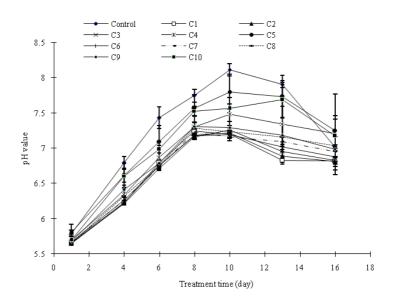


Fig. 4: The pH values of test solutions in different experimental groups along with the treatment time (days).

Table 5: Mean number of axillary buds of *S. natans* in different experimental groups during the experiment.

Experimental group	Day 4	Day 6	Day 8	Day 10	Day 13	Day 16
Control	2.7±0.979	2.85±1.04	2.95±0.759	2.95±0.945	2.95±0.826	3.1±0.718
C1	2.35±0.813 ns	2.6±0.940 ns	2.8±0.951 ns	2.75±0.967 ns	2.65±1.040 ns	2.55±1.317 ns
C2	1.95±1.234*	2.35±1.309 ns	2.4±1.429 ns	2.4±1.353 ns	2.4±1.353 ns	2.4±1.429 ns
C3	2.15±0.988 ns	2.55±1.234 ns	2.45±1.099 ns	2.3±1.031 ns	2.5±1.192 ns	2.15±1.268*
C4	1.5±1.395**	1.65±1.424**	1.75±1.552**	1.7±1.560**	1.75±1.551**	1.5±1.433**
C5	1.65±1.268**	1.55±1.191 **	1.45±1.146**	1.4±1.095**	1.45±1.191**	1.45±1.191**
C6	1.5±1.357**	1.45±1.538**	1.3±1.455**	1.4±1.602**	1.45±1.648**	1.35±1.531**
C7	1.5±1.504**	1.55±1.701**	1.55±1.731**	1.6±1.818**	1.45±1.572**	1.35±1.461**
C8	0.7±0.979**	0.7±1.08**	0.6±0.940**	0.65±1.137**	0.45±0.826**	0.45±887**
C9	0.9±1.120**	0.85±1.09**	0.85±1.089**	0.8±1.005**	0.75±0.967**	0.65±0.875**
C10	0.55±0.945**	0.6±1.046**	0.55±0.999**	0.5±0.889**	0.5±0.889**	0.45±0.826**

ns = not significant (i.e., p > 0.05). \*p < 0.05; \*\*p < 0.01.

COFCs, which could influence pH values of test solutions.

## DISCUSSION

Vegetative reproduction is very common in *S. natans* and other *Salvinia* species (Coelho et al. 2000, Jampeetong & Brix, 2009). It is sensitive to high  $NH_4^+$  levels (Jampeetong & Brix 2009) and COFCs (Jiang et al. 2009). These results clearly show that dodecane, one of the main components of COFCs, has a negative effect on the test organism, *S. natans*. Dodecane limited the development of new leaves and buds, accelerated leaves turning yellow and caused an increase in mortality. The lethal effect of dodecane on *S. natans* was acute and correlated positively with its concentration.

The influence of COFCs on algae and plants has been shown before. Uptake of hydrocarbons could disrupt algae's cellular metabolism (Lobban & Harrison 1997), influence plants by entering the lipophilic layer of cell membrane and disrupting its spacing (Zambrano & Carballeira 1999). In our earlier study, we found that  $LC_{50}$  of COFCs for *S. natans* was about 801 mg/L on day 4 after treatment. Here, we report  $LC_{50}$  of dodecane for *S. natans* on day 4 is about 190 mg/L. It seems that dodecane is more harmful to *S. natans* than COFCs in general.

Dodecane belongs to hydrocarbons, which are one of the main components of COFCs and other oils (Liu et al. 2002, Swati et al. 2008). Its molecular weight and relative density are 170.38 and 0.7487 respectively. It is difficult to be dissolved in water and will form an 'oil' film covering the surface of water when it enters aquatic ecosystems. As a low boiling point hydrocarbon, dodecane is volatilizable. Zambrano & Carballeira (1999) found that part of petroleum hydrocarbons with low density and boiling point was very toxic and could reduce the potential photochemical efficiency of photosystem II and photosynthesis rate.

This may have been the case here: as a free-floating fern, the floating leaves and buds of *S. natans* contact dodecane directly, while roots, i.e. submerged leaves do not. Floating leaves of *S. natans* play an important role in its photosynthesis (Li 1998, Jiang et al. 2009). Although we did not measure photosynthetic activity directly, we hypothesize that dodecane may influence the vegetative growth of *S. natans* mainly through influencing its leaves, and then affecting its photosynthesis.

We conclude that *S. natans* is a suitable test organism to assess the inhibitory effects of dodecane and probably also of other constituents of COFCs. Furthermore, it makes sense to measure not only mortality, but also the effects on vegetative organs. As we showed, the number of new leaves decreased under treatment by dodecane. The number (or area size) of floating leaves during the treatment by toxic agents is a useful index which can reflect well the effects of toxic materials on plants (Zambrano & Carballeira, 1999, Jiang et al. 2009).

The effects of dodecane on vegetative growth and mortality of *S. natans* were also dependent on exposure time. In our experiment, mortality was highest during the first 4 days. Possibly, the concentrations of dodecane in the experimental pots decreased over time for its volatility. pH values did not change with respect to dodecane concentration, whereas in our earlier study the presence of COFCs significantly influenced the pH values of test solutions (Jiang et al. 2009). This effect may be caused by other chemical components in COFCs, and not by dodecane.

#### CONCLUSION

Based on the discussion above, it is easy to conclude that dodecane is one of the main chemical constituents in COFCs which has a great acute effect on the vegetative growth of *S. natans*. It may influence *S. natans* through turning leaves yellow, limiting buds growth, and causing the death of individuals. Yellow leaves will decrease the photosynthesis rate of *S. natans*. Dodecane may influence the vegetative growth of *S. natans* through disrupting cellular metabolism. Because *S. natans* is also sensitive to dodecane, it can be used as an indicator of dodecane pollution.

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