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ORIGINAL ARTICLE

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Essential oil from the leaves of *Cryptomeria japonica* acts as a silverfish (*Lepisma saccharina*) repellent and insecticide

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Abstract This is the first article to report the evaluation of a natural product used as an antisilverfish agent. Silverfish (*Lepisma saccharina*), primitive wingless insects, feed on a variety of materials, including paper, cotton, starch, and cereals. They can be a problem in libraries and other places where books, documents, and papers are stored. In this pilot study, the essential oil from leaves of *Cryptomeria japonica* was investigated to test its properties as a silverfish repellent and insecticide. The results from a repellency bioassay show that the essential oil significantly repelled silverfish. The repellent activity was 80% at a dosage of 0.01 mg/cm^3. When silverfish were exposed to a concentration of 0.16 mg/cm^3 of essential oil, they were killed within 10 h. The chemical composition of essential oil, the emissions from a test chamber, and the residue left on filter papers previously soaked with the essential oil in a chamber were analyzed by gas chromatography-mass spectrometry. The components of the essential oil were found to be: elemol (18.22%), 16-kaurene (11.63%), 3-carene (9.66%), sabinene (9.37%), 4-terpineol (9.06%), β-eudesmol (5.70%), α-pinene (5.62%), and limonene (5.26%). Only some constituents of the essential oil compounds collected by solid-phase microextraction were found to be emitted in the test chamber. The main constituents were: 3-carene (21.03%), *p*-cymene (10.95%), limonene (9.49%), β-myrcene (9.39%), γ-terpinene (9.10%), α-terpinene (8.57%), and 4-terpineol (7.97%).

Key words Silverfish (*Lepisma saccharina*) · Repellency · Insecticide · *Cryptomeria japonica* · Essential oil

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Introduction

With heightened concern for environmental problems and human health, the search for readily biodegradable and environmentally friendly insecticides or pest-control agents is viewed with more and more interest among scientists. Common silverfish (*Lepisma saccharina*) are primitive insects that owe their survival to their secretive life in damp, cool places. They are pests, belonging to the order Thysanura. They eat foods and materials that are high in protein, sugar, and starch such as paper, the glue on wallpaper and bound books, cereals, and dried meats. They also damage some natural and synthetic fibers and may leave yellow stains, especially on linen.[1] Some entomologists claim that it should not be necessary to use pesticides to control silverfish and instead advocate controlling numbers by focusing on reducing humidity and on heating or freezing infested articles.[2] However, for many places it is impossible to keep the whole environment under low humidity, especially in an island climate such as exists in Taiwan. It is therefore imperative that alternative methods are employed to control these pests.

Essential oils from plants are valuable secondary metabolites already used as raw materials in many fields, including perfumes, cosmetics, phytotherapy, and nutrition.[3] As such they are potentially a good source of environmentally friendly insecticides. Recently many researchers have focused on using essential oils as anti insect reagents.[4–8] However, to our knowledge, there is no literature referring to the use of essential oil or other natural product against silverfish. With the safety of domestic application in mind, we selected the essential oil of *Cryptomeria japonica* to test against silverfish. *Cryptomeria-japonica*, which was originally grown in Japan, is one of the most important plantation coniferous tree species in Taiwan. More than 100 phytocompounds have been identified from *C. japonica* to date.[9–21] These compounds possess various bioactivities, including antifungal, termiticidal, antimite, antipathogenic, and snail repellency.[4] In this current study, the essential oil of *C. japonica* was found to be excellent silverfish repellent and insecticide; the results of our findings are presented herein.

Materials and methods

Plant materials

Fresh leaves from 35-year-old *Cryptomeria japonica* were collected from the experimental forest of National Taiwan University (June 2004) and a voucher specimen was deposited at the Herbarium of the Department of Forestry, National Chung-Hsing University.

Essential oil preparation

Leaves of *C. japonica* (200 g) were cut into the small pieces (2–3 cm), then subjected to water distillation in a Clevenge-type apparatus for 6 h, followed by determination of oil content (ml/kg) based on leaf dry weight. Essential oil was stored in sample vials after deoxygenation with N_2 prior to analysis by gas chromatography (GC) and GC-mass spectrometry (GC-MS).

Silverfish

Adult silverfish (*Lepisma saccharina*) were collected in the storeroom and basement of the Forestry Hall, National Chung Hsing University (Taichung, Taiwan) and identified by Professor Wen-Feng Hsiao (Department of Biological Resources, National Chiayi University). They were then reared in plastic containers ($15 \times 20 \times 10\,cm^3$), containing cellulose and mixed feed in a 1:1 ratio (composition of mixed feed: milk powder, oatmeal, yeast; 1:9:1) at 25° ± 3°C in 90% relative humidity in the dark.

Repellency bioassay

The degree to which the essential oil of *C. japonica* repelled silverfish was tested with a two-choice assay in a homemade acrylic apparatus shown in Fig. 1. The volume of the testing chambers, A, B, and C, were the same size ($14.8 \times 10 \times 10\,cm^3$; length × width × height). Ten minutes before 30 silverfish were placed in the central chamber B, $2 \times 2\,cm^2$ filter papers (Advantec #2) impregnated with different concentrations of essential oil or without essential oil were put into chamber C and chamber A, respectively. Thirty silverfish then moved from chamber B into either chamber C or chamber A and the repellency activity (%) was recorded after 2, 4, 18, 24, 36, and 48 h of exposure time. Repellency activity (%) = $[1 - (X/30)] \times 100$ where X = numbers of silverfish distributed in chamber C.

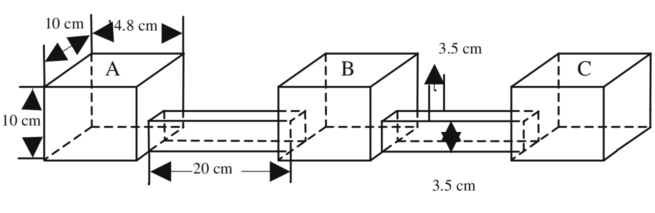

Fig. 1. Apparatus for the repellency assay against silverfish. Chamber A (*left*): filter paper only; chamber B (*central*): without filter paper; chamber C (*right*): filter paper impregnated with essential oil

Mortal activity test

To evaluate the insecticidal activity of essential oil from *C. japonica*, various amounts of essential oil were applied to filter papers ($2 \times 2\,cm^2$) that were then placed into Petri dishes (9 cm diameter × 1.0 cm height) that contained ten adult silverfish. A few drops of water were put onto the bottom edge of each of the dishes. The dishes were placed in a growth chamber maintained at 26°C and 90% relative humidity. The mortality of the silverfish was recorded periodically for up to 10 h, using the expression, mortality (%) = (number of dead silverfish/total number of test silverfish) × 100. Triplicate samples were made for each amount of essential oil sampled.

Extraction of volatile compounds by solid-phase microextraction

In order to monitor the emission compounds in chamber C (Fig. 1) when repellency assays were performed, a solid-phase microextraction (SPME) technique was used. An SPME holder and carboxen-polydimethylsiloxane (75 μm) fibers were obtained from Supelco (Bellefonte, PA, USA). Before use, SPME fibers were conditioned by heating in a hot injection port of a gas chromatogram (GC) at 250°C for 20 min in order to remove contaminants. Extractions for volatile compounds were performed directly by placing an SPME fiber into chamber C for 5 min during the repellency assays. After extraction, the SPME fiber was removed from the assay chamber and immediately inserted into the injection port of a GC using SPME-liner where thermal desorption occurred at 260°C for 30 s.

Extraction of residue compounds from filter papers

The filter papers that were impregnated with essential oil were extracted with 10 ml diethyl ether after the repellency assays. The extraction solution was then analyzed by GC-MS.

GC–MS analyses of essential oil

The compositions of essential oils and their emissions in the test chamber as well as the residue on the filter papers were analyzed by GC-MS (HP G1800A; Hewlett Packard, USA), equipped with a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific). The temperature program was as follows: 40°C for 1 min, then increased by $4°C\,min^{-1}$

to 260°C and held for 4 min. The other parameters was as follows: injection temperature, 270°C; ion source temperature, 280°C; EI, 70 eV; carrier gas, He at 1 ml/min; injection volume, 1 μl; spilt ratio, 1:50; and mass range, *m/z* 45–425. Quantification was obtained from percentage peak areas from the gas chromatogram. Identification of individual compounds was carried out using the Wiley/NBS Registry of Mass Spectral Database and NIST search and authentic reference compounds. Chromatographic results expressed as area percentages were calculated with a response factor of 1.0.

Results and discussion

To investigate the potential application of the essential oil of *Cryptomerai japonica* against silverfish, the fresh leaves of 35-year-old *C. japonica* were subjected to water distillation. The yield of essential oil was 24.6 ml/kg. Essential oils are complex mixtures comprised of various compounds. Each of these compounds contributes to the beneficial or adverse activity of the essential oil. For this reason, it is necessary to explore the complete composition of an essential oil when investigating the viability of a specific application.[22] GC-MS equipped with a capillary column is the most popular technique used to comprehend the chemical ingredients of an essential oil. Figure 2a and Table 1 shows the results of GC-MS analyses of leaf essential oil from *C. japonica*. Elemol (18.22%), 16-kaurene (11.63%), 3-carene (9.66%), sabinene (9.37%), 4-terpineol (9.06%), β-eudesmol (5.70%), α-pinene (5.62%), and limonene (5.26%) were the most abundant constituents. The percent-

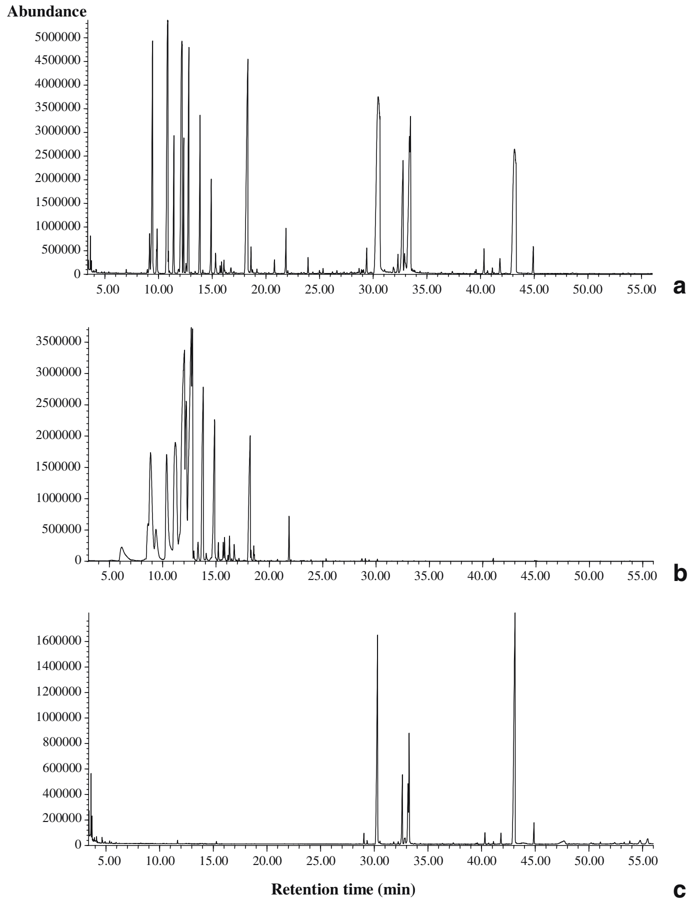

Fig. 2a–c. Total ion chromatograms of the essential oil of *Cryptomeria japonica* leaves. **a** Essential oil; **b** emission in the test chamber extracted by solid-phase microextraction; **c** diethyl ether extract from the residue in the filter paper

Table 1. Constituents of essential oil from leaves of *Cryptomeria japonica* and emissions in the test chamber and the residue on the filter paper

Compounds	Retention time (min)	Essential oil (%)	Emission in chamber[a] (%)	Residue on filter paper (%)
α-Thujene	9.08	0.79	0.89	–
α-Pinene	9.35	5.62	6.06	–
Camphene	9.79	0.96	1.29	–
Sabinene	10.81	9.37	4.72	–
β-Pinene	10.92	0.19	–[b]	–
β-Myrcene	11.40	2.59	9.39	–
3-Carene	12.16	9.66	21.03	–
α-Terpinene	12.32	1.90	8.57	–
p-Cymene	12.56	0.21	10.95	–
Limonene	12.79	5.26	9.49	–
γ-Terpinene	13.85	3.08	9.10	–
α-Terpinolene	14.90	1.58	5.65	–
Linalool	15.32	0.47	0.31	–
4-Terpineol	18.32	9.06	7.97	–
α-Terpineol	18.79	0.51	0.29	–
Bornyl acetate	21.88	0.76	1.10	–
δ-Cadinene	29.41	0.53	–	0.76
Elemol	30.42	18.22	–	27.40
γ-Selinene	32.77	4.36	–	6.57
γ-Cadinene	32.92	0.68	–	0.50
β-Eudesmol	33.35	5.70	–	8.78
16-Kaurene	43.17	11.63	–	43.03

[a] Emission quantified by solid-phase microextraction and gas chromatography
[b] Not detected

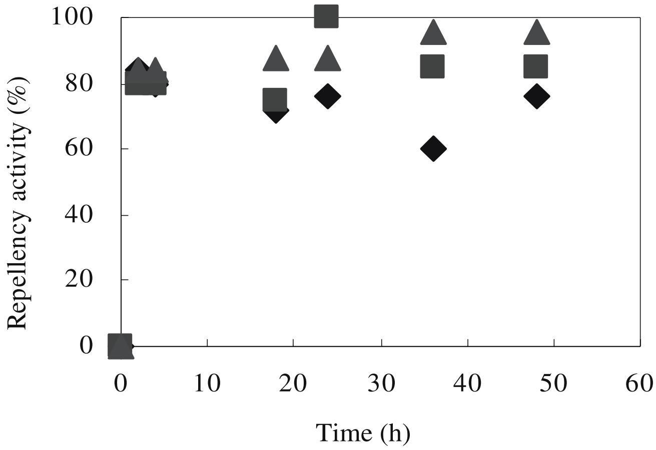

Fig. 3. Repellency activities of different concentrations of essential oil from *Cryptomeria japonica* against silverfish. *Triangles*, 0.03 mg/cm³; *squares*, 0.02 mg/cm³; *diamonds*, 0.01 mg/cm³

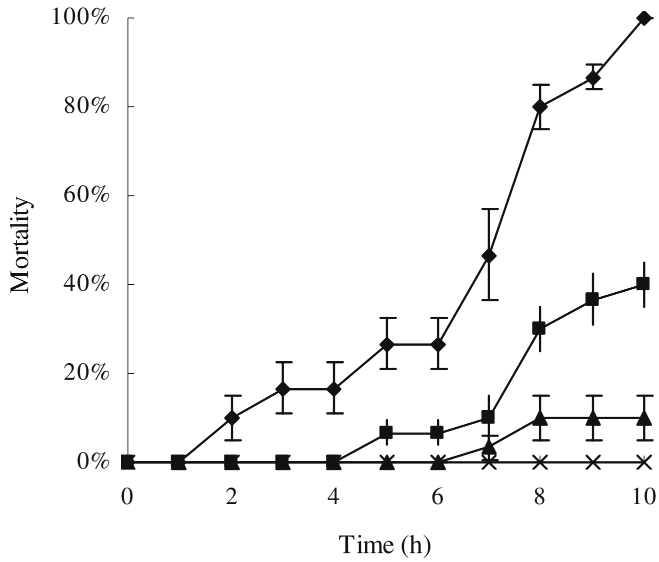

Fig. 4. Toxic effects of the essential oil from *Cryptomeria japonica* against silverfish. *Diamonds*, 0.16 mg/cm³; *squares*, 0.08 mg/cm³; *triangles*, 0.02 mg/cm³; *crosses*, control groups, 0.00 mg/cm³

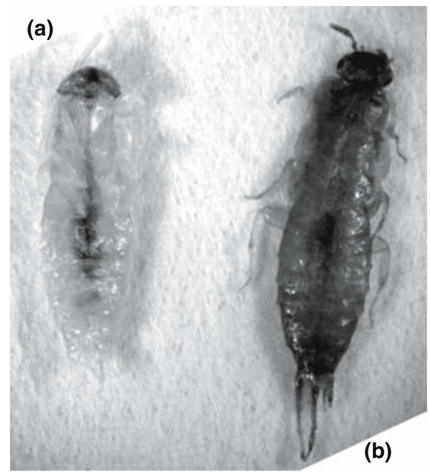

Fig. 5a,b. Dead silverfish in the toxic assay. **a** Silverfish that died without contacting the essential-oil-impregnated filter paper. **b** Silverfish that died on the essential-oil-impregnated filter paper

age constituents of essential oil in the leaves of *C. japonica* are similar to those reported by Lee and Lin.[11] However, our results are different from those observed by Cheng et al.[4] 16-Kaurene (40.62%) was also the dominant compound in Cheng's study, but the second most abundant compound was valencene (19.91%). This discrepancy may be due to the different seasons in which samples were collected and the different age of the trees.

In this study, we used a two-choice bioassay to evaluate the degree to which the essential oil from the leaf of *C. japonica* repelled silverfish. During the experiment, no dead silverfish were found in either the high dosage (0.03 mg/cm³) or low dosage (0.01 mg/cm³) test chambers. Figure 3 shows the degree to which the essential oil repelled silverfish over 48 h. It is very clear that in both high or low dosages the essential oil caused more than 80% of the silverfish to move to the chamber containing no essential oil (chamber A). Even after a long exposure time (48 h), the high dosage (0.03 mg/cm³) of essential oil still caused 90% repellency. The low dosage (0.01 mg/cm³) caused 76% repellency after 48 h. As shown in Fig. 2b and Table 1, the main volatile compounds from the essential oil to be evaporated from the impregnated paper in the test chamber were 3-carene, *p*-cymene, limonene, *β*-myrcene, *γ*-terpinene, *α*-terpinene, and 4-terpineol. All of these emissions were monoterpenoids. The residue compounds on the filter papers were elemol, *β*-eudesmol, and 16-kaurene (Fig. 2c and Table 1). The compounds remaining in the filter paper were sesquiterpenoids and diterpenoids.

Figure 4 demonstrates the toxic effects of the essential oil of *C. japonica* on silverfish. In the control group (0.00 mg/cm³), silverfish survived with active motion during the test period. When the different amounts of essential oil were added to the test dishes, a dose-dependant insecticidal effect was observed in the test chambers. All the test silverfish were killed at the highest dosage (0.16 mg/cm³) after 10 h of exposure. The percentage mortalities at other concentrations of essential oil were 40.0% (dosage 0.08 mg/cm³) and 10.0% (dosage 0.02 mg/cm³). The LD_{50} (lethal dose for 50% of the population) for silverfish death was 0.087 mg/cm³. After the assay, we found that dead silverfish had two distinct appearances in the test dishes (Fig. 5). Figure 5a shows a silverfish collected in a test dish that died without contacting the filter paper. Figure 5b shows a silverfish that died on the filter paper. It was noticed that the silverfish that died on the filter paper had a dark brown color; the body of the silverfish that died without contacting with filter paper was more transparent. Thus, it is speculated that there may be two mechanisms by which the essential oil kills the silverfish. In one case, it is speculated that silverfish absorbed volatile compounds, such as 3-carene, *p*-cymene, limonene, which then induced a toxic effect; in the other case the silverfish may have directly absorbed nonvolatile compounds, such as 16-kaurene, *β*-eudesmol, elemol, and others, and then been poisoned by these compounds. Further toxicological mechanisms need to be investigated.

Based on the results obtained in this pilot research, we conclude that there may be great potential for the development of the essential oil from the leaves of *C. japonica* for use as a reagent to prevent the damage caused by silverfish. This product would be an environmentally friendly chemical and safe for application.

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