Research paper

Antimite Activity of the Essential Oil from *Cunninghamia lanceolata* Heartwood

Jui-Chung Shieh,[1,3] Huai-Hui Wu[2]

【Summary】

The essential oil in the heartwood of *Cunninghamia lanceolata* was extracted and separated with a water steaming distillation method followed by column chromatography. Three major components, i.e., α-cedrene, α-terpineol, and α-cedrol, were obtained through identification by gas chromatography (GC) and gas chromatograph-mass spectrophotometry (GC-MS). They were then used to test their antimite activities against *Dermatophagoides pteronyssinuss*, *D. farinae*, and *Blomia tropicatus*. Results showed that α-terpineol was generally the best at killing the 3 mites, and α-cedrol was most effective in killing *D. pteronyssinuss*. It was somewhat less effective against *D. farinae*, while α-cedrene was only promising for killing *B. tropicatus*. The essential oil of *C. lanceolata* heartwood is effective in suppressing *D. pteronyssinuss* and *B. tropicatus*.

Key words: essential oil, chemical component, dust mite, corrected mortality (%), heartwood.
Shieh JC, Wu HH. 2007. Antimite activity of the essential oil from *Cunninghamia lanceolata* heartwood. Taiwan J For Sci 22(1):47-56.

研究報告

杉木精油抗蟎活性之研究

謝瑞忠[1,3] 吳懷慧[2]

摘 要

試驗用杉木心材，以水蒸氣蒸餾法抽取精油，精油中成分用column chromatography分離，並純化獲得3種成分，成分鑑定配合GC, GC-MS，證實為α-cedrene, α-terpineol及α-cedorl，分別將分離出3種成分，對歐洲室塵蟎(DP)，美洲室塵蟎(DF)及熱帶無爪塵蟎(BT)進行抗蟎活性，結果發現α-terpineol對3種塵蟎抗蟎活性最好，α-cedrol對歐洲室塵蟎效果最好，美洲室塵蟎次之，α-cedrene僅對熱帶無爪蟎有效。杉木心材精油對歐洲室塵蟎及熱帶無爪塵蟎抗蟎效果顯著。

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Received September 2006, Accepted November 2006. 2006年9月送審 2006年11月通過。

關鍵詞：精油、化學成分、塵蟎、校正致死率、心材。
謝瑞忠、吳懷慧。2007。杉木精油抗蟎活性之研究。台灣林業科學22(1):47-56。

INTRODUCTION

Taiwan is located in the tropics and subtropics, an environment with high temperatures and high humidities that is favorable for the survival and propagation of dust mites. Therefore, dust mites can be found almost everywhere here. Human health problems such as asthma are known to be correlated with dust mites, and life may be threatened in serious cases, with children being most often affected.

Organic phosphate chemicals are commonly used to control and prevent the propagation of dust mites. However, long-term use of such chemicals may jeopardize human health. So, many studies in recent years have tried to extract secondary metabolites such as essential oils from wood for the control of dust mites, and significant results were observed in many cases. Essential oils have been proven to be harmless to humans and are capable of easing asthma syndrome. Furuno et al. (1994) reported that the essential oils extracted from 6 species of Lauraceae trees were able to inhibit the activity of dust mites, and the essential oil extracted from leaves of shirodano (*Neolitsea sericea*) was able to inhibit dust mites such as *Dermatophagoides pteronyssinuss* (DP) and *D. farinae* (DF), because the essential oil contains isosericenine which was found to have the greatest antimite activity in the experiments. Morita et al. (1994) also reported that the essential oil from Japanese cedar extracted with n-hexane was able to inhibit the activity of DP, and the key compound was identified to be cryptomerione. Miyazaki (1996) reported that the essential oil extracted from wood of hiba (*Thujopsis dolaberata* var. *hondal*) was able to inhibit the activity of DP.

Yatagai and Ding (1996) reported that the essential oils extracted with n-hexane from the wood and leaves of *Pinus massoniana* were able to inhibit the activity of DP. Ho et al. (1998) used n-hexane to extract the wood of 6 tree species, and found that the extract from *Cunninghamia lanceolata* inhibited the activity of DF. Chen et al. (2002) observed that the cinnamic aldehyde type of essential oil extracted from leaves of *Cinnamomum osmophloeum* was able to inhibit mite activity. Lin et al. (2003) also reported the same results with cedrol from the heartwood of *C. lanceolata* which inhibited the activity of DF.

Wu (1996, 1997) revealed that 16 species of dust mites including DP and DF were commonly observed in environments in Taiwan, and *D. pteronyssinuss* topped the others, followed by DF. Therefore DP, DF and *B. tropicatus* (BT) were used throughout the experiments in this study. Essential oil from *C. lanceolata* was evaluated for its antimite activity, and also used to identify the key active ingredients/compounds. The final purpose was to try to use these key components in clinical experiments.

MATERIALS AND METHODS

Materials

Fifteen year-old logs of *C. lanceolata* used in this study were obtained from the Lienhuachih Research Center of the Taiwan Forest Research Institute, in central Taiwan. The heartwood and sapwood of *C. lanceolata* were indvidually sawn into planks, then cut into shavings, and finally ground to a pow-

der or sawdust. The moisture contents of the sawdust were determined in an oven at 105℃ for several hours until the weight remained unchanged.

Methods

Extraction of essential oils

The essential oil of *C. lanceolata* sawdust was obtained by steam distillation for 6 h. The components in the essential oil were separated by column chromatography with a fraction collector (Model SF-160), manufactured by Advantec Toyo Kaisha (Tokyo, Japan). The eluant was a mixed solution of n-hexane and ethyl ether in varying proportions.

Analyses and identification of the components in the essential oil

Melting points were determined on a Fisher-Johns melting-point apparatus. Thin-layer chromatography (TLC) was conducted on precoated silica-gel G plates (E. Merck, Darmstadt, Germany, 20×20 cm, layer 0.25 mm thick); the plates were developed with n-hexane-ethyl ether (2/1, v/v) and n-hexane-acetone (7/3, v/v). The spots were detected by spraying the plates with 10% aqueous H_2SO_4 followed by heating.

Gas chromatography (GC) was performed using a Hewlett-Packard 5890A instrument equipped with a flame ionization detector (FID) and a 30 m×0.25 mm HP-5MS fused silica capillary column. The oven temperature was programmed between 70 (1 min isothermal) and 220℃ at an increasing rate of 3℃ min^{-1}.

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Hewlett-Packard 5985 mass spectrometer combined with a Hewlett-Packard 5890A GC equipped with the same fused silica capillary column. The mass spectra were obtained at an ionization voltage of 70 eV. Identification of individual components was established by comparison of their mass spectra and retention indices with those of authentic samples (Yukawa et al. 1979, Noever et al. 1987).

Anti-mite activity test

1. Rearing of dust mites

Fifty pairs each of the DF (Fig. 1), DP (Fig. 2) and BT (Fig. 3) mites after mating were reared in Petri dishes (5.5 cm in diameter and 2 cm in height) containing yeast and wheat bran (1:1, w/w). The dishes were covered with a transparent polyethylene (PE) sheet, kept in a transparent PE box containing a saturated sodium chloride solution, and placed in an incubator at 25~28℃ and 75% relative humidity. Three replications were used for each mite experiment.

2. Antimite activity experiments

The test set was composed of 3 layers of transparent acrylic plates. The top and bottom layers were 9.0 (L)×4.6 (W)×0.2 (H) cm

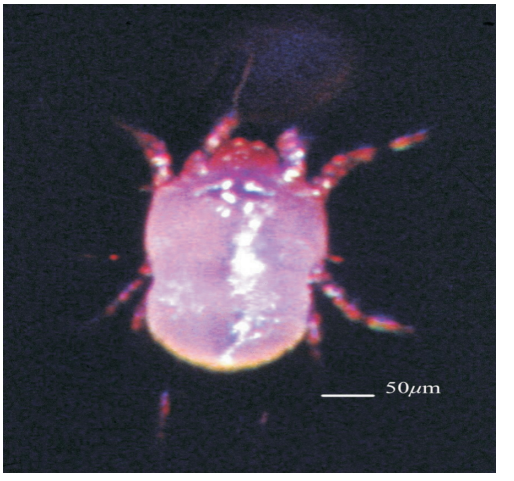

Fig. 1. Dust mite (*Dermatophagoides farinae*, ♀).

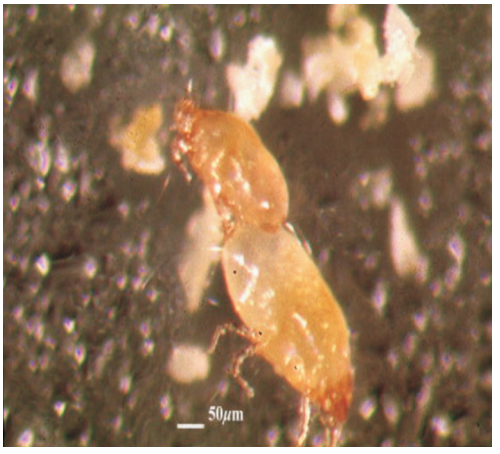

Fig. 2. Mating of dust mites (*Dermatophagoides pteronyssinuss*).

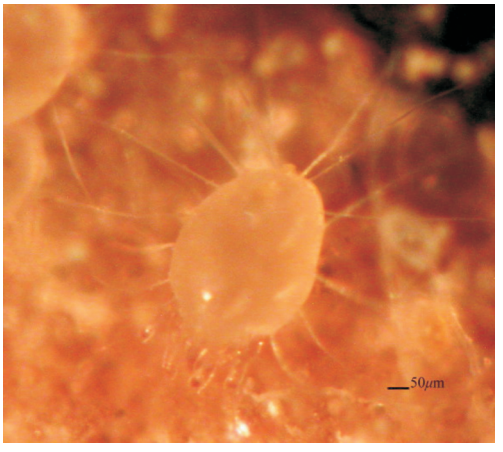

Fig. 3. Dust mite (*Blomia tropicatus*, ♀).

and the middle layer was 4.6 (L)×4.6 (W)×0.8 (H) cm with a circular hole (3 cm in diameter) in the center. A piece of filter paper was placed on the bottom of the hole (3.5 mm in diameter). Ethanol (95%) was used to dilute the stock essential oil of *C. lanceolata* 500, 1000, and 2000 times, producing concentrations of 0.5, 0.25, and 0.125 ppm, respectively. The filter paper under the hole of the middle layer was drenched with 300 ul of the different-concentration solutions, and 10~20 adult female mites were kept in the hole. The 3 acrylic layers were bound for observation. Four replicates were used in this experiment and filter paper containing only ethanol (95 %) served as the control. Mortality were observed under a dissecting microscope 24 and 48 h after incubation in the incubator described above. The percentage corrected mortality was calculated by Abbott's formula according to Rosenheim and Hoy (1989):

Corrected mortality (%) = [Treatment mortality − Control mortality / (100 − control mortality)]×100.

RESULTS AND DISCUSSION

Yield, analysis, and identification of *C. lanceolata* heartwood essential oil

The yield of essential oil in the heartwood of *C. lanceolata* ranged between 1.3 and 2.3% v/w (dry wood), while a trace amount of essential oil was found in sapwood. Thirty grams of the essential oil from *C. lanceolata* heartwood was put in a silica gel column and then mixed solvents of n-hexane: ether at 100:0, 90:10, 80:20, 70:40, 60:80, 50:50, and 40:60 (v/v) were respectively used as eluants to separate the components of the essential oil (Table 1).

After concentrating under reduced pressure, 3.8 g of condensed material was obtained from the fraction 1 solution eluted with n-hexane: ether (100:0 v/v). Fraction 1 condensed material was further purified by silica gel preparative thin-layer chromatography (TLC) using n-hexane:ethyl ether (4:1 v/v) as the developer, and 3.5 g of liquid material was finally obtained. The major component, making up 98% of the 3.5 g of liquid material by GC integrator, had a retention time (RT) of 0.16 min in the GC analysis. The mass spectrum is shown in Fig. 4. The analysis showed that m/z 204 was the parent peak $[M]^+$ with a molecular weight of 204, and the compound

Table 1. Separation of essential oil from the heartwood of *Cunninghamia lanceolata* by column chromatography

Extraction solvents n-hexane: ether (v/v)	Solvent volume (ml)	Fractions	Dry weight[1)] of extracts (g)
100:00	500	1	3.8
90:10	800	2	2.9
80:20	800		
70:40	800	3	22.0
60:80	800		
50:50	1000		
40:60	1000	4	0.5

[1)] Essential oil (30 g) of *C. lanceolata* was separated by silica-gel column chromatography [100×6 cm, silica gel (160 g)].

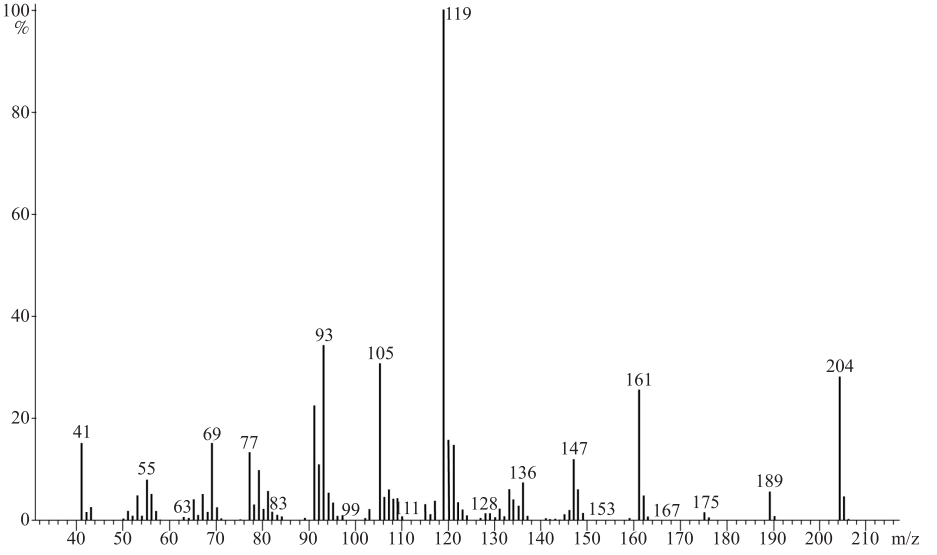

Fig. 4. Mass spectrum of α-cedrene isolated from the essential oil of *Cunninghamia lanceolata* heartwood.

was possibly $C_{15}H_{24}$. The m/z 189 was a fragment M-CH_3, m/z 161 was fragment M-C_3H_5, and m/z 119 was the peak of fragment C_9H_{11}. Results of the ms analyses were the same as in the reports by Yukawa and Ito (1979) and Noever et al. (1987) in their mass spectrum of α-cedrene. Therefore, we are certain that the major component of the mass spectrum in Fig. 4. is α-cedrene, the molecular formula and molecular weight of which are $C_{15}H_{24}$ and 204, respectively.

Fraction 2 was obtained from 800 ml of the eluant composed of n-hexane: ether (90:10, v/v), 2.9 g of condensed material was collected, and 2.6 g of further purified liquid material was obtained after silica gel preparative TLC. Ninety six percent of the major component was located at a retention

time of 11.5 min on the GC chromatogram. Its mass spectrum is shown in Fig. 5. Because the molecular ion peak of an alcohol is often very small, the fragment of $[M\text{-}H_2O]^+$ was easily identified. Therefore, the m/z 136 was the fragment $[M\text{-}H_2O]^+$, its molecular weight was 154, and its formula was established as $C_{10}H_{18}O$. The base peak m/z 59 had similar characteristis to those of α-terpineol in the monocylic terpene. The m/z 59 was fragment $[C_3H_7O]^+$. The m/z 121 was $[M\text{-}H_2O\text{-}CH_3]^+$, and m/z 93 was fragment $[M\text{-}H_2O\text{-}C_3H_7]^+$. This mass spectrum was identified as α-terpineol according to Yukawa et al. (1979), the molecular formula and molecular weight of which are $C_{10}H_{18}O$ and 154, respectively.

Fraction 3 was obtained from 3400 ml of the eluant composed of a mixture of 800 ml each of n-hexane: ether at 80:20 (v/v), 70:40 (v/v), and 60:80 (v/v) and 1000 ml at 50:50 (v/v) (Table 1). Twenty-two grams of crude crystal material was obtained after silica gel column chromatography, and 21.95 g of further purified crystal was obtained by silica gel preparative TLC; almost 100% of the major component was obtained at an RT of 26.9 min in the GC analysis. The 21.95 g of the further purified crystal was repurified with spectrum-grade methanol for recrystalization, and 21.8 g of pure crystal was obtained. The crystal was analyzed on a 2-dimensional TLC plate with n-hexane: ethyl ether (4:1 v/v) and n-hexane: acetone (7:2 v/v) as the developer, and the Rf values of the pure crystal on the TLC were 0.25 and 0.53, respectively. There was only a single spot on the silica gel glass plate. Therefore, the component was a pure single compound. The melting point was between 85 and 86℃, and its RT was 26.98 min in the GC analysis. The mass spectrum of the crystal is shown in Fig. 6. The molecular ion peak $[M]^+$ was at 222. Therefore, it is a compound of sesquiterpene with the formula $C_{15}H_{26}O$. The base peak m/z 95 was fragment $[C_7H_{11}]^+$, while the other main peak at m/z 150 was fragment $[M\text{-}H_2O\text{-}C_4H_6]^+$, m/z 135 was fragment $[C_{10}H_{15}]^+$, m/z 207 was fragment $[M\text{-}CH_3]^+$, and m/z 107 was fragment

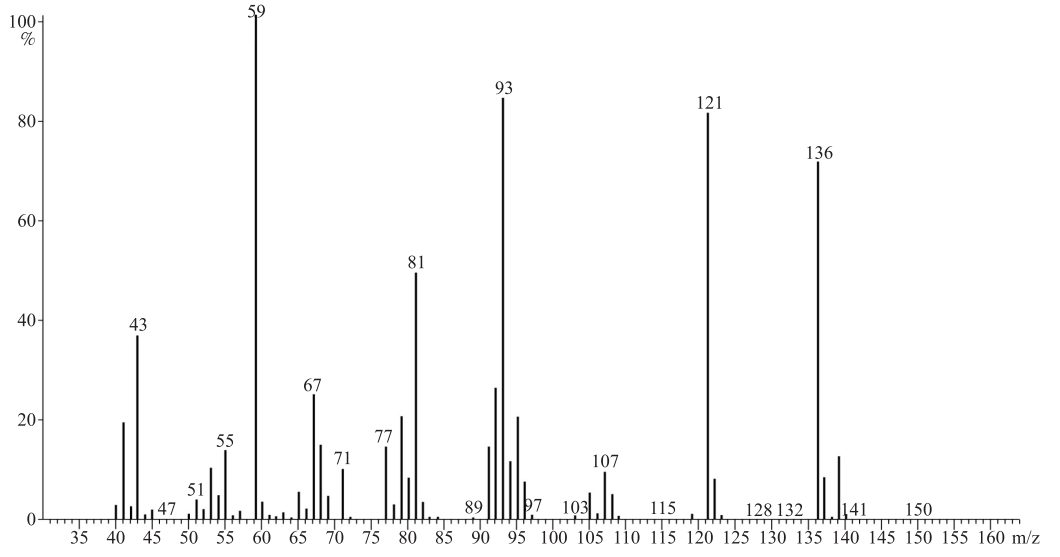

Fig. 5. Mass spectrum of α-terpineol isolated from the essential oil of *Cunninghamia lanceolata* heartwood.

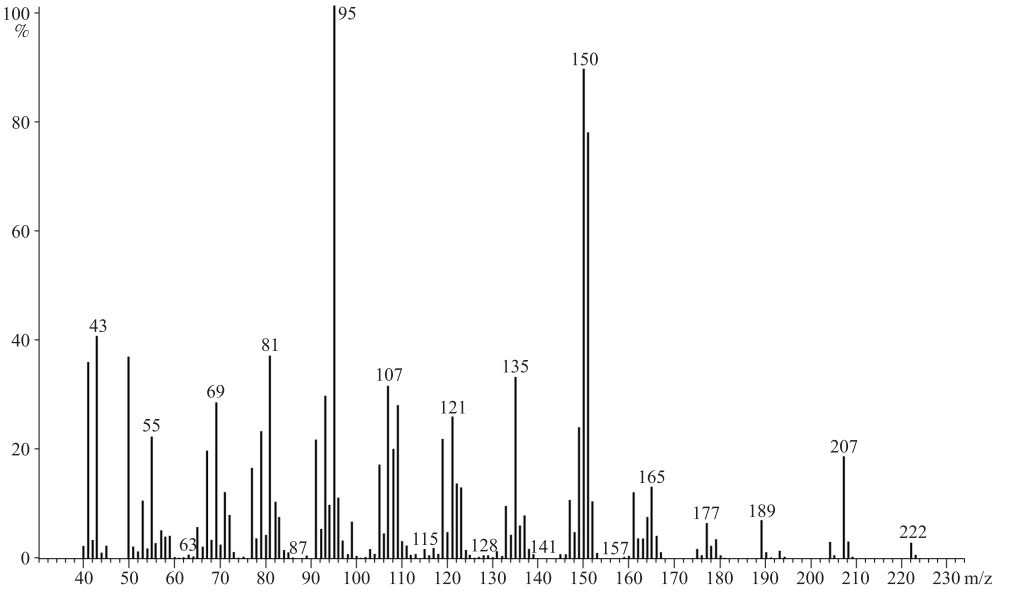

Fig. 6. Mass spectrum of α-cedrol separated from the essential oil of *Cunninghmia lanceolata* heartwood.

$[C_8H_{11}]^+$. This crystal component was identified as α-cedrol according to Yukawan and Ito (1979) and Noever et al. (1987), and its molecular formula and molecular weight are $C_{15}H_{26}O$ and 222, respectively.

As shown in Table 1, only 0.5 g of dry material was obtained from fraction 4 eluted with n-hexane: ether (40:60 v/v). Following the TLC analysis, the dry material contained at least 1/3 of the crystal α-cedrol, therefore, no further purification was conducted.

Anti-mite activity test

The 3 major components i.e., α-cedrene, α-terpined, and α-cedrol, separated from the essential oil of *C. lanceolata* heartwood were diluted with 95% ethanol to obtain concentrations of 0.5, 0.25 and 0.125 ppm for each compound and to test their anti-mite activities against DP, DF, and BT. Results was shown in Table 2.

1. Twenty-four hours after incubation, the corrected mortalities of DP for *C. lanceolata* essential oil, α-terpineol, and α-cedrol at the concentrations of 0.5 and 0.25 ppm all reached 100% as shown in Figs. 7 and 9. When the concentration was reduced to 0.125 ppm, 100% of the corrected mortality was only observed for *C. lanceolata* essential oil and α-cedrol, but for α-terpineol, it was reduced to 88.3% as shown in Fig. 11. In the case of α-cedrene, the corrected mortality of DP at concentration of 0.5, 0.25 and 0.125 ppm were 66.7, 66.7, and 6.7%, respectively. It was the least effective mitecide among the 4 samples tested. Results observed at 48 h after incubation revealed that the corrected mortalities of DP for *C. lanceolata* essential oil, α-cedrol, and α-terpineol at the 3 test concentrations all reached 100% as shown in Fig. 10. However, for α-cedrene, at the concentrations of 0.125, 0.25, and 0.5 ppm, the corrected mortalities were 56.7, 75.0, and 93.3%, respectively, as shown in Figs. 8, 10, and 12. α-cedrene was the least effective among the 4 samples tested.

Table 2. Corrected mortalities (%) of the 3 tested dust mites to the 4 *Cunninghamia lanceolata* essential oils/components at different times and concentrations

Essential oils	DF[1)]						DP[1)]						BT[1)]					
	24 h			48 h			24 h			48 h			24 h			48 h		
	0.5 ppm	0.25 ppm	0.125 ppm	0.5 ppm	0.25 ppm	0.125 ppm	0.5 ppm	0.25 ppm	0.125 ppm	0.5 ppm	0.25 ppm	0.125 ppm	0.5 ppm	0.25 ppm	0.125 ppm	0.5 ppm	0.25 ppm	0.125 ppm
C. lanceolata oil	46.7[2)]	8.3	3.3	53.3	18.3	8.3	100[2)]	100	100	100	100	100	100[2)]	100	100	100	100	100
α-cedrene	0	0	0	20	11.7	8.3	66.7	66.7	6.7	93.3	75	56.7	100	85	78.3	100	93.2	86.4
α-terpineol	100	100	100	100	100	100	100	100	88.3	100	100	100	100	100	88.3	100	100	100
α-cedrol	100	6.7	5	100	11.7	6.7	100	100	100	100	100	100	80	63.3	30	83	74.6	38.9

[1)] DP: *Dermatophagoides pteronyssinuss*; DF: *Dermatophagoides farinae*; BT: *Blomia tropicatus*.

[2)] Corrected mortality (%).

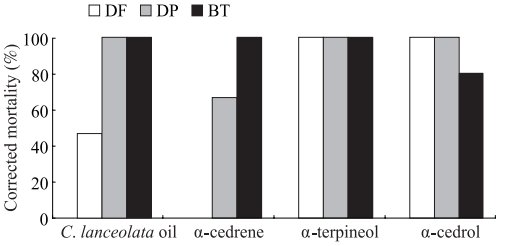

Fig. 7. Corrected mortalities (%) of the antimite activity of the major components from *Cunninghamia lanceolata* essential oil at the concentration of 0.5 ppm, 24 h after incubation.
DP, *Dermatophagoides pteronyssinuss*; DF, *D. farinae*; BT, *Blomia tropicatus*.

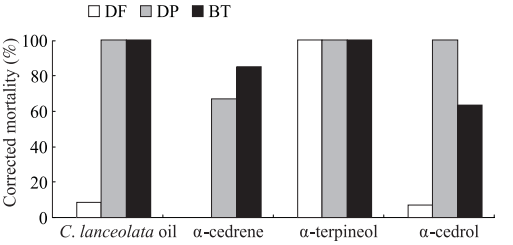

Fig. 9. Corrected mortalities (%) of the antimite activity of the major components from *Cunninghamia lanceolata* essential oil at concentration of 0.25 ppm, 24 h after incubation.
DP, *Dermatophagoides pteronyssinuss*; DF, *D. farinae*; BT, *Blomia tropicatus*.

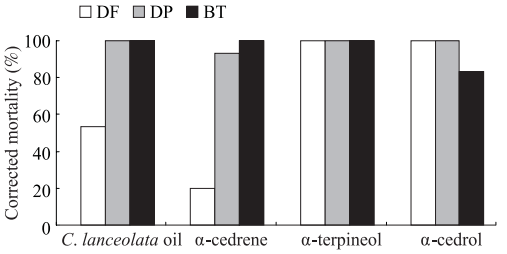

Fig. 8. Corrected mortalities (%) of the antimite activity of the major components from *Cunninghamia lanceolata* essential oil at the concentration of 0.5 ppm 48 h after incubation.
DP, *Dermatophagoides pteronyssinuss*; DF, *D. farinae*; BT, *Blomia tropicatus*.

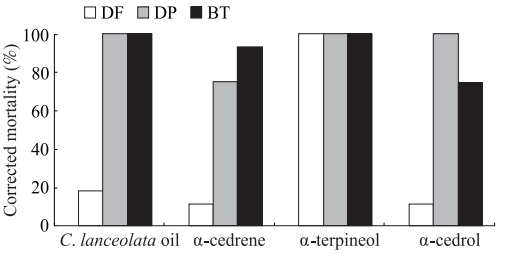

Fig. 10. Corrected mortalities (%) of the antimite activity of the major components from *Cunninghamia lanceolata* essential oil at concentration of 0.25 ppm, 48 h after incubation.
DP, *Dermatophagoides pteronyssinuss*; DF, *D. farinae*; BT, *Blomia tropicatus*.

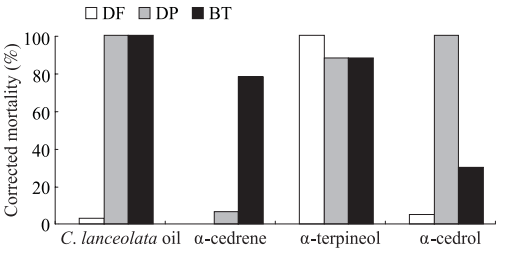

Fig. 11. Corrected mortalities (%) of the antimite activity of the major components from *Cunninghamia lanceolata* essential oil at concentration of 0.125 ppm 24 h after incubation.
DP, *Dermatophagoides pteronyssinuss*; DF, *D. farinae*; BT, *Blomia tropicatus*.

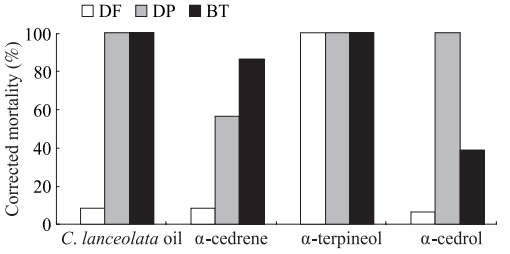

Fig. 12. Corrected mortalities (%) of the antimite activity of the major components from *Cunninghamia lanceolata* essential oil at concentration of 0.125 ppm, 48 h after incubation.
DP, *Dermatophagoides pteronyssinuss*; DF, *D. farinae*; BT, *Blomia tropicatus*.

2. In the anti-mite activity tests on DF 24 h after incubation, 100% corrected mortality was observed with α-terpineol at all the 3 concentrations in Figs. 7, 9, and 11. For α-cedrol, the corrected mortalities at 0.5, 0.25, and 0.125 ppm were 100, 6.7, and 5.0%, respectively. These 2 compounds were better than α-cedrene or *C. lanceolata* essential oil. The same results were also reported by Lin et al. (2003). However, only α-cedrol was used in the anti-mite activities against DF, DP, and BT, while α-terpineol and α-cedrene were not tested. As shown in Table 2, the corrected mortalities of DF in the α-terpineol test, 48 h after incubation, at the 3 tested concentrations were all 100% as shown in Figs. 8, 10, and 12. In the case of cedrol, 100% corrected mortality was only observed at 0.5 ppm. The performances at 0.125 and 0.25 ppm were not very good; still α-cedrol was better than *C. lanceolata* essential oil and α-cedrene. Overall, for the anti-mite activity, α-terpineol was the most effective, followed by cedrol, and *C. lanceolata* oil, and α-cedrene was the least effective.

3. The corrected mortalities of BT in α-terpineol 24 h after incubation, at 0.125, 0.25 and 0.5 ppm, were 88.3, 100, and 100% at the 3 concentration respectively. In the case of α-cedrene, the corrected mortalities at 0.125, 0.25, and 0.5 ppm were 78.3, 85.0, and 100%, respectively. The performance of α-cedrol was the poorest, and the corrected mortalities were all less than 80%. The same results were also obtained 48 h after incubation. With α-terpineol and *C. lanceolata* oil, 100% corrected mortalities were observed at all 3 different concentrations. The corrected mortalities of α-cedrene at 0.125, 0.25, and 0.5 ppm were 86.4, 93.2, and 100%, respectively. Among these, α-cedrol was also the poorest.

CONCLUSIONS

The heartwood of *C. lanceolata* contains 1.3~2.3% (v/w) essential oil. The essential oil of *C. lanceolata* can be separated and purified into α-cedrene, α-terpineol, and α -cedrol by silica gel column chromatography. α-Terpineol and α-cedrol were found to have the best antimite activities against test to

D. pteronyssinuss. α-terpineol was the best against *D. farinae*, followed by α-cedrol. For *B. tropicatus*, α-terpineol was the best, followed by α-cedrene. The essential oil of *C. lanceolata* heartwood was very effective against *D. pteronyssinuss* and *B. tropicatus*.

ACKNOWLEDGEMENTS

The authors thank Dr. Yeh Chung-Chuan, Deputy Director of the Tainan District Agricultural Research and Extension Station, for his valuable technical assistance.

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