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ORIGINAL PAPER

Bioactivity of sandalwood oil (*Santalum austrocaledonicum*) and its main components against the cotton aphid, *Aphis gossypii*

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Abstract Control efficacy of sandalwood oil (*Santalum austrocaledonicum* Vieill) and its main components, α- and β-santalol, which have not been tested before against *Aphis gossypii* Glover (Hemiptera:Aphididae), was investigated in the laboratory, greenhouse and field bioassays. The main constituents of the commercial sandalwood oil were found to be α-santalol (47.5 %), β-santalol (18.7 %), bergamotol (7.2 %) and lanceol (9.1 %) according to GC and GC-MS analyses. The sandalwood oil was fractionated into an α-santalol-rich fraction (RF), β-santalol RF, and a mixture of α- and β-santalols by silica gel column chromatography. The purity of α-santalol RF was 75.4 % (ratio of α-:β-santalol was 94:6) and that of β-santalol RF was 34.6 % (ratio of α-:β-santalol was 16:84). Laboratory bioassay showed that sandalwood oil, α- and β-santalol RFs had the same and significantly higher repellency and control efficacy against *A. gossypii* than the two control treatments and that a mixture of α- and β-santalols was toxic to the aphid. Santalol was comparable to imidacloprid (a neonicotinoid insecticide) in its efficacy against *A. gossypii* infesting Rose of Sharon, *Hibiscus syriacus* L., with 98.8 % mortality. The control efficacies of sandalwood oil (94.0 %), α-santalol RF (84.2 %), β-santalol RF (90.6 %) and a mixture of α- and β-santalols (88.7 %) against the *A. gossypii* infesting hot peppers were also comparable to each other in the greenhouse bioassay. Considering its control efficacy against *A. gossypii*, the application of sandalwood oil and its components may open the possibility of environmentally friendly management of *A. gossypii*.

Keywords *Aphis gossypii* · Aphid · Sandalwood oil · Essential oil · Santalol · *Hibiscus syriacus* · Hot peppers

Key message

- The components of sandalwood oil, α- and β-santalol-rich fractions, were provided.
- The repellency of sandalwood oil and its components for *Aphis gossypii* as well as the mortality induced to the aphid were evaluated.
- Sandalwood oil and its components showed good control efficacy.
- The application of sandalwood oil and its components may open the possibility of environmentally friendly management of *A. gossypii*.

Hyun Sik Roh and Junheon Kim contributed equally to this work.

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Introduction

Aphis gossypii Glover (Hemiptera:Aphididae) is a highly polyphagous insect pest that damages cultivated plants worldwide (Blackman and Eastop 2000). Although chemical insecticides such as neonicotinoids have outstanding potency and systemic action against piercing-sucking pests (Nauen et al. 1998; Tomizawa and Casida 2005), aphids possess an ability to evolve resistance against most chemical insecticides (Martin and Workman 1997; Amad et al. 2003; Nauen and Elbert 2003; Shi et al. 2010; Herron and Wilson 2011). Besides, repeated use of chemical insecticides can cause environmental and human health hazards. Therefore, the development and application of environmentally friendly technologies is needed to achieve sustainable management of the pest.

Plant essential oils are good sources for the development of insect pest control agents. They account for pesticidal and sublethal effects and are relatively nontoxic to mammals and environmentally nonpersistent owing to their volatility (Isman et al. 2011). Many essential oils and plant extracts have insecticidal and repellent activities against *A. gossypii* (Tunç and Şahinkaya 1998; Hori 1999; Kim et al. 2005; Soliman 2006; Mareggiani et al. 2008; Bagavan et al. 2009; Salari et al. 2010; Sharma and Vidyarthi 2010; Ebrahimi et al. 2013). Sandalwood oil extracted from *Santalum austrocaledonicum* Vieill (Santalaceae:Santalales) is widely used as a flavor component in food products and as an ingredient in perfumes, and it is known to be effective against chemically induced skin cancer (Burdock and Carabin 2008). The main components of sandalwood oil are α- and β-santalol (Shellie et al. 2004; Roh et al. 2011). The insecticidal and repellent activities of sandalwood oil against *Tetranychus urticae* Koch (Acari:Tetranychidae) (Roh et al. 2011, 2012), *Aedes albopictus* Skuse, *A. aegypti* L. and *Culex pipiens* L. (Zhu et al. 2008) were reported. In this study, we investigated the efficacy of sandalwood oil, α- and β-santalols, against the cotton aphid, *A. gossypii*, in the laboratory, greenhouse and field bioassays.

Materials and methods

Insects and plants

Aphis gossypii infesting Rose of Sharon, *Hibiscus syriacus* L. (Malvaceae:Malvales), and hot pepper, *Capsicum annuum* L. (Solanaceae:Solanales), at Gyeongsang National University (GNU) was used in the experiments. A number of *H. syriacus* shrubs grow as garden plants on the university campus. *Aphisgossypii* overwinters on *H. syriacus* and infests it heavily in the spring season. Pepper plants were grown in pots under greenhouse conditions. *Aphis gossypii* populations occurring naturally on *H. syriacus* were collected and inoculated onto the hot peppers to increase their density. To ensure successful colonization of the aphids, both sides of the greenhouse were opened to let the aphids outside move into the greenhouse freely.

Essential oils and chemicals

Sandalwood oil was obtained from La Drome Provencales (Die, France). Authentic santalol [a mixture of α-santalol (50 %) and β-santalol (22 %); MP Bio Inc., Thuringer, Germany], imidacloprid (8 % suspension concentration; Bayer CropScience, Seoul, Korea), ethanol (Burdik and Jackson, Muskegon, MI) and triton X-100 (Sigma-Aldrich, St. Louis, MO) were procured.

Isolation of α- and β-santalol

To isolate α- and β-santalol, the sandalwood oil was subjected to silica gel column chromatography (Wakogel-200; Wako Pure Chem., Osaka, Japan) using hexane and diethyl ether-hexane as eluents (Fig. 1) (Gritter et al. 1985).

Instrumental analysis

Chemical analysis of sandalwood oil and its fractionation was carried out using gas chromatography (GC) and a GC-mass spectrometer (MS). GC analysis was performed on a GC-17A (Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detector and a DB-5 MS column (30 m × 0.25 mm i.d. × 0.25 μm, J & W Scientific Co.). The oven temperature was programmed as follows: isothermal at 40 °C for 1 min, then rising to 6 °C/min–250 °C; this temperature was held for 4 min. Helium was used as the carrier gas at a rate of 1.5 ml/min. GC-MS analysis was performed on a GCMS-QP2010 plus (Shimadzu Co.) interfaced with a GC2010 (Shimadzu Co.) equipped with an HP-Innowax column (30 m × 0.25 mm i.d. × 0.25 μm, J & W Scientific Co.). The oven temperature program was the same as for GC analysis. The samples were adjusted to 5,000 ppm with hexane, and 1 μl of the sample was injected. Compounds were identified by comparing mass spectral data of essential oils with those of the mass spectral library (the Wiley Registry of Mass Spectra Data, 2000, Agilent) and comparing retention indices of compounds with those in the literature (Shellie et al. 2004).

Laboratory bioassay

Potted hot pepper seedlings (around 30 cm high) were procured from commercial nurseries and maintained in the laboratory. Only two top leaves were retained by removing the remaining leaves. These plants were moved to a

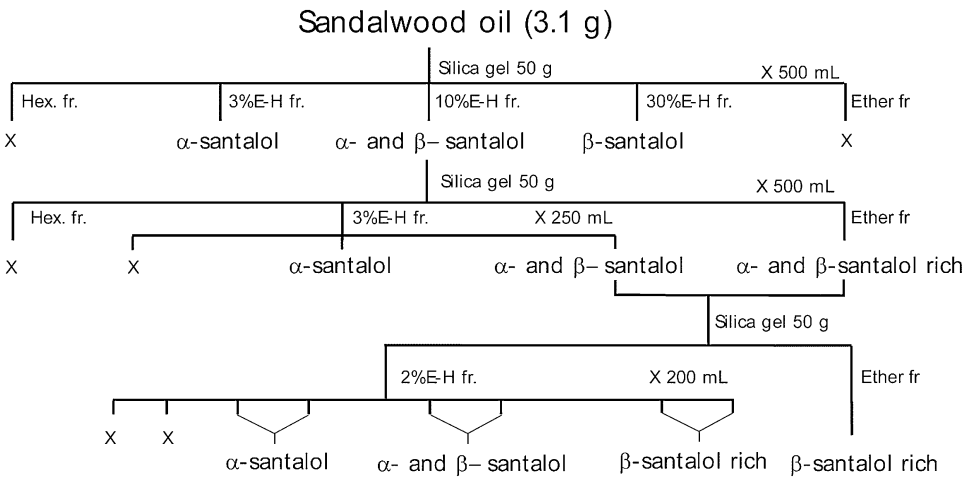

Fig. 1 Procedure for the isolation of α- and β-santalol. × indicates there was no α- or β-santalol. E and H indicate diethyl ether and hexane, respectively

greenhouse where mature hot pepper plants infested with a high density of *A. gossypii* were being cultivated. Two or three leaves from the mature plants were cut and put on the test seedlings for a day to allow the aphids to migrate to the leaves of the test seedlings. These seedlings were used in the laboratory bioassay. The laboratory bioassay was performed by modifying the method described by Roh et al. (2012). *Aphis gossypii*-infested seedlings were brought to the laboratory and painted with insect glue (Fujitangle, Fuji Pharmaceutical, Inc., Tokyo, Japan) at two-thirds height of the main stem from the soil surface to prevent escaping. A rectangular yellow sticky board (13 × 15 cm) was placed horizontally just above the soil surface of the pot surrounding the stem to cover the underside of the whole plant canopy.

Previously, a 0.1 % (v/v) concentration of sandalwood oil showed good acaricidal and repellent activity against *T. urticae* without showing phytotoxicity (Roh et al. 2011, 2012). Therefore, the applied concentration was determined to be 0.1 % (v/v) in this study to evaluate the control efficacy against *A. gossypii*. Each 400 µl of sandalwood oil, α-santalol-rich fraction (RF), β-santalol-rich fraction (RF) and mixture of α- and β-santalol was dissolved in 40 ml of ethanol. This solution was mixed with 360 ml distilled water and triton X-100 (20 µl) to make up a final concentration of 0.1 % (v/v). As a solvent control (Triton X treatment), a mixture of ethanol (40 ml), triton X-100 (20 µl) and distilled water (360 ml) was used. No material was treated in the blank control. Using a hand sprayer, treatment solutions (20 ml) were sprayed on both sides of *A. gossypii*-inoculated leaves. The treated seedlings were kept in a growth room maintained at 24 ± 2 °C, 40–80 % RH and 16 L:8 D conditions. The number of *A. gossypii* on the seedlings was recorded before spraying the solution. After 24 h, the numbers of *A. gossypii* on the seedlings and sticky boards were recorded. Four replicates were made in a randomized complete block design.

Field bioassay

A field bioassay was conducted at GNU (38°09′N, 128°06′E). *Aphis gossypii* infesting *H. syriacus* was tested with santalol (0.1 % v/v in ethanol–water), imidacloprid (0.1 % v/v in water), triton X-100 (0.005 % v/v in ethanol–water), and a blank control from 22–26 May 2009 (average temperature: 20.2 °C; no rain during the experiment). Imidacloprid (a necotinoid insecticide) is one of the most commonly used insecticides for controlling aphids in Korea. Santalol and triton X-100 solutions were prepared in the same manner as stated above for laboratory bioassay. Each stand of *H. syriacus* was 2 m apart in a row. A twig infested with *A. gossypii* within a stand was selected and 20 ml test solution applied using a hand sprayer. The number of live *A. gossypii* on the twig and leaves before and 24 h after spraying was recorded. The experiment was conducted in a randomized complete block design with five replications.

Greenhouse bioassay

The greenhouse bioassay was conducted in the greenhouse at GNU. *Aphis gossypii* infesting mature hot pepper plants were tested in the greenhouse with the same treatments as described above for the laboratory bioassay from 13–15 June 2012 (average temperature: 20.8 °C; no rain during the experiment). Solutions of sandalwood, α-santalol RF, β-santalol RF, a mixture of α- and β-santalol, triton X solution and a blank control were applied using a hand spray bottle. The hot pepper plants were planted in the greenhouse and infested with *A. gossypii*. Each plant was 0.3 m apart in a row. The number of live *A. gossypii* on the leaves before spraying the test solutions (20 ml) and 24 h after the treatment was recorded. Four replications were made in a randomized complete block design.

Table 1 Retention index and chemical composition of sandalwood oil and its fractions

Compounds	Retention index[a]		Composition (%)			
	Nonpolar (5 MS)	Polar (Innowax)	Sandalwood oil	α-Santalol rich raction	β-Santalol rich fraction	Mixture of α- and β-santalol
α-Santalol	2,355	1,679	47.5	75.4	6.6	29.0
Bergamotol	2,360	1,692	7.2	11.5	1.5	5.2
Epi-β-santalol	2,425	1,707	3.6	1.0	6.2	6.9
β-Santalol	2,439	1,720	18.7	4.7	34.6	38.0
Lanceol	2,500	1,763	9.1	0.8	23.9	10.8
Ratio of α- and β-santalol			72:28	94:6	16:84	43:57
			(7:3)	(9:1)	(2:8)	(4:6)

[a] Retention index (van Den Dool and Kratz 1963) on DB-5MS and HP-Innowax columns, according to *n*-alkanes (C15–C25)

Statistics

To evaluate the bioactivity of sandalwood oil and its components against *A. gossypii*, repellency, mortality and control efficacy were calculated by percentage using the following formula:

$$\%\,\text{Repellency} = \frac{\text{Total number of } A.gossypii \text{ on the sticky board after treatment}}{\text{Total number of } A.gossypii \text{ on the hot pepper plant before treatment}} \times 100$$

$$\%\,\text{Mortality} = \frac{\text{Total number of dead } A.gossypii \text{ on the hot pepper plant after treatment}}{\text{Total number of } A.gossypii \text{ on the hot pepper plant before treatment}} \times 100,$$

and control efficacy (%) = (SC−ST)/SC × 100, where SC and ST are the mean percent survival of control and treatments, respectively. Percent survival of control and treatments was calculated as follows: the total number of live *A. gossypii* on a hot pepper plant after treatment was divided by the total number of *A. gossypii* on the hot pepper plant before treatment. The corrected mortality was calculated using Abbott's formula (Abbott 1925). The values of repellency, mortality and control efficacy were subjected to arcsine transformation and analyzed by one-way analysis of variance (ANOVA) for a randomized complete block design. Treatment means were separated and compared by Tukey-Kramer HSD test using JMP, version 9.0.2 (SAS Institute Inc., Cary, NC). Mean (±SD) values of untransformed data were reported.

Results

Composition of sandalwood oil

The GC and GC–MS analyses revealed that the main constituents identified from sandalwood oil were α-santalol (47.5 %), β-santalol (18.7 %), bergamotol (7.2 %) and lanceol (9.1 %) (Table 1). The commercially available santalols showed the same profile as sandalwood oil (data not shown). Using SiO_2 column chromatography, α-santalol RF (1.35 g) and β-santalol RF (0.96 g) could be isolated with purities of 75.4 and 34.6 %, respectively. A mixture of α- and β-santalol (0.72 g) was obtained at a 4:6 ratio (Table 1). These isolated compounds were used for the bioassays.

Laboratory bioassay

Repellency, mortality and control efficacy of sandalwood oil and its components are summarized in Table 2. The repellencies of sandalwood oil, α- and β-santalol RFs were similar, although all of them were significantly superior to controls ($F_{5,15} = 42.90$, $p < 0.001$). A mixture of α- and β-santalol showed weaker repellency than sandalwood oil and β-santalol RF, but showed significantly higher repellency to *A. gossypii* than controls. Mortality effects of sandalwood oil and its components were in the range of 10.9–37.6 %. The mixture of α- and β-santalol showed significantly higher effects than other treatments ($F_{5,15} = 11.86$, $p < 0.001$). Although control efficacies of sandalwood oil, α- and β-santalol RFs, and the mixture were significantly higher than that of the controls, they were similar to each other ($F_{5,15} = 146.13$, $p < 0.001$). Triton X treatment caused an increase in the number of *A. gossypii*.

Table 2 Bioactivities of sandalwood oil and its components against *A. gossypii* infesting hot pepper in the laboratory bioassay (%, mean ± SD, $N = 4$)

Treatment[a]	Repellency[b,c]	Corrected mortality[b,c]	Control efficacy[b,c]
Sandalwood	77.1 ± 9.7a (0.89 ± 0.15)	10.9 ± 12.6bc (0.11 ± 0.13)	90.1 ± 3.5a (1.26 ± 0.30)
α-Santalol rich fraction	70.3 ± 10.1ab (0.79 ± 0.15)	17.1 ± 7.8b (0.17 ± 0.08)	88.2 ± 9.4a (1.11 ± 0.21)
β-Santalol rich fraction	77.1 ± 13.4a (0.90 ± 0.21)	12.0 ± 11.0bc (0.12 ± 0.11)	90.8 ± 10.6a (1.22 ± 0.30)
Mixture of α- and β-santalol	53.3 ± 12.1b (0.57 ± 0.14)	37.6 ± 11.5a (0.39 ± 0.13)	92.1 ± 10.9a (1.26 ± 0.30)
Triton X	18.4 ± 4.2c (0.19 ± 0.04)	−2.7 ± 3.4c (−0.03 ± 0.13)	−9.2 ± 15.6b (−0.09 ± 0.09)
Blank	21.8 ± 6.7c (0.22 ± 0.07)	0c (0)	0b (0)

[a] Applied concentrations of sandalwood oil and its components were 0.1 % (v/v) in triton X (0.005 % v/v), ethanol and water

[b] Means in a column followed by the same letter are not significantly different (Tukey–Kramer test, $\alpha = 0.05$)

[c] Arcsine-transformed values are given in parentheses

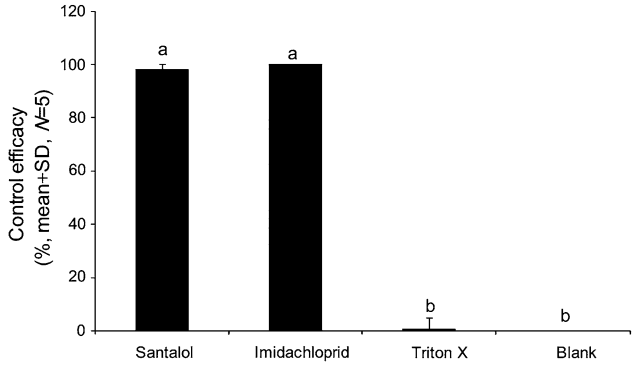

Fig. 2 Control efficacy against *Aphis gossypii* on *H. syriacus* in the field bioassay in 2009. The *different letters* on the *error bar* indicate significantly different means (Tukey–Kramer test, $\alpha = 0.05$)

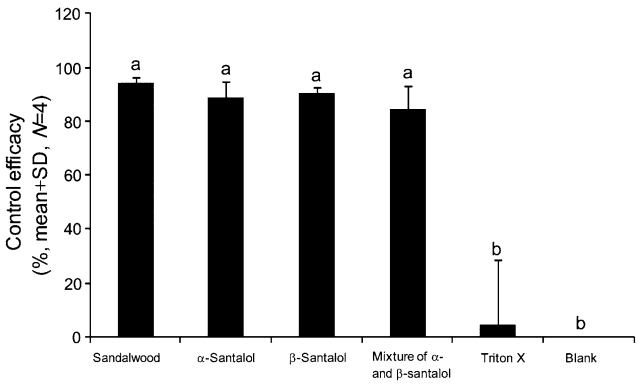

Fig. 3 Control efficacy of sandalwood oil and its components against *Aphis gossypii* on hot pepper plants in the green bioassay in 2012. The *different letters* on the *error bar* indicate significantly different means (Tukey–Kramer test, $\alpha = 0.05$)

Field bioassay

In the field bioassay performed on *H. syriacus* in 2009, the control efficacy of santalol was similar to that of imidacloprid, although the control efficacies of the two treatments were significantly higher than that of the controls ($F_{3,12} = 3{,}577.94$, $p < 0.001$; Fig. 2).

Greenhouse bioassay

In the greenhouse bioassay performed on hot pepper plants in 2012, the sandalwood oil solution (94.0 %) showed the highest control efficacy, followed by α-santalol RF (90.6 %), the mixture of α- and β-santalol (88.7 %), and β-santalol RF (84.2 %). However, control efficacies of sandalwood oil and its components were similar ($F_{5,15} = 88.47$, $p < 0.001$; Fig. 3).

Discussion

Our study clearly showed that sandalwood oil has a significant effect against *A. gossypii* in laboratory, greenhouse and field bioassays. This study is probably the first of its kind reporting the effect of sandalwood oil on *A. gossypii*. In the field bioassay, santalol, a major component of sandalwood oil, was as effective as imidacloprid, which is widely used in Korea for the control of several aphid species in many crops. In the laboratory bioassay, triton X and blank controls showed 18.4 and 21.8 % repellency. This relatively high repellency may be attributed to the insufficient settlement of *A. gossypii* on the two leaves of pot-reared hot pepper seedlings. The lower mortality of sandalwood and its components may be attributed to the low number of *A. gossypii* left on the treated leaves. When the mortality was considered as the number of *A. gossypii* left on the leaves, sandalwood, α- and β-santalol RFs, and their mixture led to significantly higher mortality than the two controls ($F_{5,15} = 29.4$, $p < 0.001$; Supplemental material Table S1).

In the field bioassay, control efficacy showed a similar trend as the laboratory bioassay. This higher control efficacy was mainly due to the insecticidal activity of sandalwood and its components, not the repellent activity. The density of *A. gossypii* in the field bioassay was 5–7 times

higher than that in the laboratory bioassay, and *A. gossypii* settled down sufficiently on the hosts. These different parameters may be the result of the different bioactive effects of sandalwood oil and its components in the laboratory and field bioassay.

Some active compounds in essential oils are known to be responsible for the insecticidal activity against *A. gossypii*. Anethole, carvacrol, p-cymene, γ-terpinene, α-pinene and β-caryophyllene have exhibited strong insecticidal activity against *A. gossypii* (Erler and Tunç 2005; Liu et al. 2010). For investigating responsible compounds in sandalwood oil for control efficacy, α- and β-santalol were isolated from sandalwood oil and tested. In our study, α-santalol, one of the main components of sandalwood oil, was responsible for the control efficacy. α-Santalol showed high repellency in the laboratory bioassay and high control efficacy in the field bioassay. Although β-santalol also showed higher repellency and control efficacy, due to the low purity of β-santalol isolated from sandalwood oil, its control efficacy could not be concluded from this study.

The structure-activity relationships of plant compounds against insect pests and nematodes have been well studied. Oxygenated monoterpenoids exhibited stronger activity than hydrocarbon monoterpenoids (Regnault-Roger and Hamraoui 1995). Seo et al. (2010) found that aliphatic alcohol, aldehyde and acid showed stronger nematicidal activity than the corresponding hydrocarbon. Interestingly, the identified components in sandalwood oil have allylic alcohol as a functional group. The repellent and mortality activity of sandalwood oil, santalol and α-santalol may be attributed to the structure of this component. β-Santalol and lanceol, which are the main components of β-santalol RF, possess an allylic alcohol structure, and this structure may contribute the good control efficacy against *A. gossypii*.

Conclusion

This is the first study demonstrating that sandalwood oil and its components, α- and β-santalol, have repellent and insecticidal activities against *A. gossypii*. Thus, considering its repellent and insecticidal activities against *A. gossypii*, the application of sandalwood oil and its components may open the possibility of environmentally friendly management of *A. gossypii*.

Author contributions

CGP conceived and designed the research. HSR and ESS conducted the experiments. JK, DWL and HYC analyzed the data. JK and CGP wrote the manuscript. All authors read and approved the manuscript.

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