Acaricidal Abilities and Chemical Composition of *Forsythia suspense* Fruit Oil against Storage and Pyroglyphid Mites

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Abstract This research is aimed at evaluating the potential abilities of the natural acaricide of *F. suspense* oil against *Tyrophagus putrescentiae* and *Dermatophagoides* spp. Based on the LD₅₀ values, in contact bioassay, *F. suspense* oil (8.19, 3.28, and 4.35 µg/cm²) showed acaricidal effects against *T. putrescentiae*, *D. farinae*, and *D. pteronyssinus*, respectively. Fumigant toxicities of *F. suspense* oil showed similar patterns as those observed with contact toxicities. GC/MS analysis showed the major components of *F. suspense* oil to be β-pinene (45.88%), myrtenol (13.86%), (+)-α-pinene (13.09%), (–)-trans-pinocarveol (7.34%), sabinene (6.64%) and pinocarvone (4.13%). These findings indicate that *F. suspense* oil has potential as a natural acaricide.

Keywords acaricidal effect · *Dermatophagoides farinae* · *Dermatophagoides pteronyssinus* · *Forsythia suspense* · *Tyrophagus putrescentiae*

Storage and pyroglyphid mites are major allergens worldwide causing bronchial asthma, rhinitis and atopic dermatitis (Kim et al., 2003; Lee, 2004; Saad et al., 2006; Song et al., 2011). Pyroglyphid mites found in indoor apartments belong to the genus *Dermatophagoides* (Lim et al., 2008). In particular, *D. farinae* and *D. pteronyssinus* are the major factors causing sudden infant death syndrome (Rim and Jee, 2006). The typical storage mite,

Tyrophagus putrescentiae, lives in preserved eggs, cheese and grain (Gulati and Mathur, 1995). Moreover, storage mites have been recognized as an etiological factor in allergic diseases affecting food workers and farmers (Song et al., 2011). Controlling mite populations has been done mainly through the use of pirimiphos-methyl and benzene hexachloride (Wu et al., 2012). Although effective, these resulted in resistance and mammal toxicity (Kang et al., 2006). In more recent studies, natural substances of plant oils are reported to be good alternatives (Pasay et al., 2010).

Forsythia suspensa (Thunb.), a member of the Oleaceae family, is widely distributed in Asia (China, Korea and Japan) and many European nations. (Qu et al., 2008). It contains isolaricresinol, succinic acid, erythritol and wogonin-7-O-glucoside (Liu et al., 2003). Furthermore, it is reported to have biological effects including antibacterial (Bae et al., 2005; Qu et al., 2008), antioxidant (Qu et al., 2008; Wang et al., 2008) and antihypertensive (Nishibe et al., 1982). However, the acaricidal activities of *F. suspense* fruits against *T. putrescentiae*, *D. farinae* and *D. pteronyssinus* have not been reported. In this study, we aimed to look into the potent ability of *F. suspensa* oil against storage and pyroglyphid mites.

F. suspense fruits were ground into powder and extracted by the simultaneous steam distillation-extraction method (Boutekedjiret et al., 2003). The analysis of *F. suspense* fruit oil was performed using GC-Mass (6890; Agilent Technologies) (5973; Agilent) with a DB-5 (0.25 mm) fused capillary column (30 m×0.25 mm i.d.). The GC injector temperature was 210°C. The initial temperature of the column was 50°C and it was programmed to rise to 201°C at a ramp rate of 1.9°C/min and stay at this temperature for 16 min. The ion source temperature was 231°C and the helium gas flow was set at 0.81 mL/min. The GC effluent entered the mass spectra electron ionization (70 eV) chamber. The mass analyzer was set to scan from 49 to 602 amu for 2 s. Volatile constituents were identified by comparison to retention indices, retention times and mass spectra in the Wiley Registry of Mass Spectral Data.

Storage and pyroglyphid mites were sustained on yeast (1:1 by

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weight) and fry forage no. 1 (Korea Special Feed Meal Co. Ltd., Korea) in a completely dark area. They were reared in a cage (15×12×6 cm) containing the diet and kept at 74% relative humidity and 25°C. Acaricidal effect against three storage and pyroglyphid mites was evaluated using both fumigant and contact bioassays. Fumigant bioassay was modified and performed following the method described by Yang et al. (2014). Different quantities (40–1 µg/cm³) of *F. suspense* oil were applied to paper disks (0.8 cm diameter and 0.1 cm thick). Each treated disk was dried in a fume hood for 14 min and then placed in the top of a microtube (2 mL). Twenty mites were inoculated into each microtube, which was then sealed using the cap containing the treated disk. The acaricidal effects of *F. suspense* oil were measured using contact bioassay modified as indicated by Yang et al. (2014). *F. suspense* oil concentrations (20–1 µg/cm²) were liquefied in pure methanol (50 µL). *F. suspense* oil was injected into the bottom of a petri dish (35 mm i.d.×10 mm L.) and excess solvent was dried for 13 min. Twenty mites were inoculated into a petri dish and the lid was closed. The systems were maintained at 75% relative humidity and 25±1°C in the dark for 24 h. The LD₅₀ values were estimated and transformed to arcsine square root values for analysis of variance (SAS, 2004). The effect of each treatment was determined to be significantly different at *p* < 0.05.

The acaricidal activities of essential oil (yield 0.36%) from *F. suspense* fruits against three storage and pyroglyphid mites are shown in Table 1. To determine acaricidal effect, the potent activity of *F. suspense* oil was estimated using fumigant and contact bioassays against *Dermatophagoides* spp. and *T. putrescentiae*. Compared to the LD₅₀ values of benzyl benzoate, in fumigant bioassay, the acaricidal effects of *F. suspense* oil (10.63, 6.80, 9.21 µg/cm³) was more potent than those of benzyl benzoate (11.13, 12.18, 11.75 µg/cm³) against *T. putrescentiae*, *D. farinae*, and *D. pteronyssinus*. Contact bioassay results showed *F. suspense* oil (8.19, 3.28, 4.35 µg/cm²) to be more active than benzyl benzoate (8.31, 8.48, 7.72 µg/cm²) against *T. putrescentiae*, *D. farinae*, and *D. pteronyssinus*. *F. suspense* oil has a possibility of acting as a

natural acaricide against three storage and pyroglyphid mites. Our findings show that contact toxicities of *F. suspense* oil were more potent against *T. putrescentiae*, *D. farinae*, and *D. pteronyssinus* than fumigant toxicities. In other words, these results showed that the mode of acaricidal action of *F. suspense* oil was suggested by insect's skin. Our study is the first to report the acaricidal effects of *F. suspense* oil against three acaridae and pyroglyphid mites.

Volatile compounds of *F. suspense* oil were recognized by GC-MS and matched the retention indices and mass spectra of compounds in the literature (Wenlu and Benlian, 2008). As shown in Table 2 and Fig. 1, the composition (%) of the volatile constituents of *F. suspense* oil was found to be: β-pinene (45.88%), myrtenol (13.86%), (+)-α-pinene (13.09%), (–)-trans-pinocarveol (7.34%), sabinene (6.64%), pinocarvone (4.13%), (–)-terpinen-4-ol (2.69%), dipentene (1.02%), (S)-cis-verbenol (0.74%), γ-terpinene (0.60%), (1S)-(–)-verbenone (0.55%), cymene (0.53%), myrcene (0.45%), α-terpineol (0.44%), α-phellandrene (0.42%), camphene (0.40%), 2,5-cyclooctadien-1-ol (0.36%), α-terpinene (0.31%), eucalyptol (0.30%) and campholenic aldehyde (0.27%). The volatile constituents were grouped as alcohols (2,5-cyclooctadien-1-ol, eucalyptol, myrtenol, (–)-trans-pinocarveol, (–)-terpinen-4-ol, α-terpineol and (S)-cis-verbenol), aldehydes (campholenic aldehyde), monoterpene hydrocarbons (camphene, o-cymene, dipentene, myrcene, (+)-α-pinene, β-pinene, α-phellandrene, sabinene, α-terpinene and γ-terpinene) and monoterpene ketones (pinocarvone and (1S)-(–)-verbenone). Compared with the previous study (Wenlu and Benlian, 2008), the major compounds of *F. suspense* oil were geraniol, linoleic acid, myristic acid, octacosane, β-pinene, squalene, and 1-terpinen-4-ol. Some constituents derived from plants are affected by geographic location, harvest time, extraction procedures and extracted parts of herbs (Yang and Lee, 2013).

Current results indicate that *F. suspense* oil could be useful in the development of plant acaricide against three acaridae and pyroglyphid mites. Further study should be conducted on human health safety of *F. suspense* oil to increase acaricidal potency and stability.

Table 1 Acaricidal activities of *F. suspense* oil and a synthetic acaricide against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*^a

Samples	Bioassay	Mite species	LD ₅₀	95% CL	RT ^b
<i>F. suspense</i> oil	Fumigant (µg/cm ³)	<i>D. farinae</i>	6.80	6.21-7.39	1.79
		<i>D. pteronyssinus</i>	9.21	8.60-9.82	1.28
		<i>T. putrescentiae</i>	10.63	13.41-13.85	0.82
	Contact (µg/cm ²)	<i>D. farinae</i>	3.28	2.68-3.88	2.59
		<i>D. pteronyssinus</i>	4.35	3.84-4.86	1.77
		<i>T. putrescentiae</i>	8.19	8.64-9.94	0.89
Benzyl benzoate	Fumigant (µg/cm ³)	<i>D. farinae</i>	12.18	11.15-13.21	1.00
		<i>D. pteronyssinus</i>	11.75	11.06-12.44	1.00
		<i>T. putrescentiae</i>	11.13	10.45-11.81	1.00
	Contact (µg/cm ²)	<i>D. farinae</i>	8.48	7.99-8.97	1.00
		<i>D. pteronyssinus</i>	7.72	7.02-8.42	1.00
		<i>T. putrescentiae</i>	8.31	7.78-8.84	1.00

^aExposed for 24 h.

^bRT, Relative toxicity=LD₅₀ value of benzyl benzoate/LD₅₀ value of each compound.

Fig. 1 Analysis of various constituents from *F. suspensa* oil by GCMS.

Table 2 Analysis of various constituents from *F. suspensa* oil identified by GCMS

Peak number	Retention time (min)	Compound	Relative area (%)
1	4.818	α -Phellandrene	0.42
2	4.973	(+)- α -Pinene	13.09
3	5.256	Camphene	0.40
4	5.692	Sabinene	6.64
5	5.803	β -Pinene	45.88
6	5.959	Myrcene	0.45
7	6.483	α -Terpinene	0.31
8	6.626	o-Cymene	0.53
9	6.715	Dipentene	1.02
10	6.774	Eucalyptol (1,8-cineole)	0.30
11	7.268	γ -Terpinene	0.60
12	8.514	Campholenic aldehyde	0.27
13	8.795	(-)- <i>trans</i> -Pinocarveol	7.34
14	8.857	(S)- <i>cis</i> -Verbenol	0.74
15	9.040	2,5-Cyclooctadien-1-ol	0.36
16	9.201	Pinocarvone	4.13
17	9.437	(-)-Terpinen-4-ol	2.69
18	9.650	α -Terpineol	0.44
19	9.781	Myrtenol	13.86
20	9.991	(1S)-(-)-Verbenone	0.55

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