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Acaricidal Properties of Star Anise (*Illicium verum* Hook.f.) Essential Oil against House Dust Mite [*Dermatophagoides pteronyssinus* (Trouessart)]

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Acaricidal properties of essential oil from star anise (*Illicium verum* Hook.f.) were evaluated by using fumigation, residual contact and fibers coating method against house dust mite (*Dermatophagoides pteronyssinus* (Trouessart)) and compared with standard trans-anethole and essential oils from clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry). The fumigation method was made within 25 L knockdown chamber. The various concentrations of essential oils at 0 (95% ethanol), 0.15, 0.30, 0.45, 0.60, 0.75, 0.90, 1.05 and 1.2 µl/L air were used. The fumigation period was 1 h and mortality of mite was observed at 24 h after fumigation. The residual contact method was done in a glass tube 0.4 cm in diameter and 3 cm long and covered with filter paper on both ends. The various essential oil concentrations at 0 (95% ethanol), 0.02, 0.04, 0.06 and 0.08 µl/cm^2 were applied and mortalities of mite were observed at 12 and 24 h after treatment. The various essential oil concentrations at 1 and 2% in 95% ethanol with MU and PG as additive agents were used to coat the fiber. The tested synthetic fibers were soaked in all treatments for 30 min, dried in a hot air oven at 50 ℃ for 2 h. The coated fibers were separately kept in cloth bags at 25±2^{o}C. The effectiveness of those coated fibers were evaluated by direct contact method and observed on the beginning day and every week as well as the mortality of mite was observed at 24 h after treatment. The result showed that all fumigation experiments showed low effectiveness to control house dust mite, when clove essential oil had a high residual contact property, showed 100% mortality of exposure period of 12 h at 0.02 µl/cm^2 and showed the LC_{50} and LC_{90} at 0.006 and 0.014 µl/cm^2, respectively. In addition, this essential oil was also highly toxic against the house dust mite, by using fibers coating method. It could control the mite effectively as long as 49 days. The coated fibers with star anise essential oils and standard trans-anethole could kill the mite quite well within 2 weeks but lower than 30% mortality within 28 and 35 days, respectively. Whereas, the essential oil of clove was highly effective to control house dust mite, it could kill the mite more than 30% within 56 days.

Keywords: fumigation, direct contact, coated fibers, house dust mite

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Introduction

The house dust mite *Dermatophagoides pteronyssinus* Trouessart, (Phylum Arthopoda) which is the dominant species in the household and considered to be the most important mite causing allergic diseases such as asthma, allergic rhinitis, allergic conjunctivitis and atopic eczema affected of people worldwide (Eggleston and Bush, 2001). Controlling to house dust mite can be done by removing carpets, replacing upholstered furniture, washing clothing and bedding in hot water (McDonald *et al*., 1992; Tovey *et al*., 1994). Nowadays, allergic diseases become a serious human health problem and these problems have need for the development of new strategies for control dust mites.

In addition, chemical methods to control house dust mites in the chemical acaricidal and physical methods have been applied to eliminate allergens from carpets, upholstery, and bedding (Woodfolk *et al*., 1994), category such as benzyl benzoate and pyrethroids (Kalpaklioglu *et al*., 1996). However, not recommended these agents use can cause acaricidal resistance, posing risks to the environment and human health.

The essential oil is an alternative control method for house dust mites because of their insecticidal and acaricidal properties. Chang *et al*. (2001) reported that the essential oil of Taiwania cryptomerioides Hayata at concentration of 12.6 μg/cm^2 caused 67.0 and 36.7% mortalities of the house dust mites, *D. pteronyssinus* (Trouessart) and *D. farinae* Hughes, respectively. Kim *et al*. (2003) found that eugenol and its derivatives, acetyleugenol, isoeugenol and methyleugenol showed much lower LD_{50} values of 0.67, 1.55, 3.71 and 5.41 μg/cm2, respectively, in controlling *D. pteronyssinus* by contact. Clove and cinnamon extracts at concentration of 125 μg/cm^2 was effective with more than 88.7% mortality of mite. Beside, dichloromethane extracts of clove and cinnamon showed the highest toxicity against *L. perniciosus* with LD_{50} values of 34.97 and 35.57 μg/cm^2, respectively. (Pumnuan *et al*., 2008)

The purpose of this study was to investigate the acaricidal properties of essential oil from star anise (*I. verum* Hook.f.) against house dust mite (*D. pteronyssinus* (Trouessart)) by using fumigation, residual contact and fibers coating method.

Materials and methods

House dust mites culture

House dust mite, *D. pteronyssinus* was cultured in the laboratory of Department of Plantproduction Technology, Faculty Agricultural Technology,

(King Mongkut's Institute of Technology Ladkrabang), Bangkok, Thailand. They were cultured in mite bottles kept in mite chamber at temperature (25.0±1.0℃ and 86±1% relative humidity) and were feed a diet (powdered rat feed, wheat grain, and yeast, at the ratio 4:4:1 by weight) (Insung and Boczek, 1995).

Essential oil preparation

The essential oils from star anise (*I. verum* Hook.f.), and clove (*S. aromaticum* (L.) Merr. & L.M. Perry) were procurement from Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand. The chemical standard trans-anethole, the main component found in star anise (more than 80%) was also prepared for further experiment.

Experimental treatment

Fumigation test

Ten to fifteen adult mites were put into the mite cage likes acrylic sheet (3x5x0.45 cm) perforated into frustum of cone. The essential oils at various concentrations of 0 (95% ethanol), 0.15, 0.30, 0.45, 0.60, 0.75, 0.90, 1.05 and 1.2 µl/L air against the mite were evaluated by fumigation method performed in 25 L knockdown chamber with 1 h fumigation time. The mortality of mite was observed at 24 h after treatment. The experiment with 5 replications was applied.

Contact test

The various essential oil concentrations of 0 (95% ethanol), 0.02, 0.04, 0.06, 0.08 and 0.10 µl/cm^2 were applied in a glass tube 0.4 cm in diameter and 3 cm long and covered with filter paper on both ends. The mortalities of mite were observed at 12 and 24 h after treatment. The experiment was 5 replications.

Fiber Coating test

The essential oils at the concentrations of 1 and 2% in 95% ethanol in which Musk, MU and Propylene Glycol, PG (by 1:1:1) as additive agents were used to coat the fiber and compared with standard trans-anethole (1 and 2%). The tested synthetic fibers were soaked in all treatments for 30 min, dried in a hot air oven at 50℃ for 2 h. The coated fibers were separately kept in cloth bags at 25±2oC. The effectiveness of those coated fibers were evaluated by direct contact method and observed on the beginning day and every week. Coated fibers 0.5 g were placed in the mite cage. Ten adult mites were put into

the mite cage and mortality of mite was observed at 24 h after treatment. Experiments was made under 5 replications.

Statistical analysis

Mites were considered dead if their appendages did not move when probed with a small hair brush. Abbott's (1987) formula was used to calculate the actual death rates. The experiment was designed in five completely randomized replicates (CRD). The data obtained was statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). The LC_{50} was calculated by the probit method.

Results and Discussion

The result from fumigations experiment showed that clove essential oil presented the highest toxicity to the house dust mite at only 0.6 µl/L air caused 100% mortality of mite. For the standard strans-anethole had high fumigations property showed 61.6% mortality at the concentration of 1.2 µl/L air. Remarkably, the essential oil from star anise showed low effectiveness to control house dust mite, which was much different when compared the essential oil from clove. That was similar to the report of Insung and Pumnuan (2009) (Figure 1).

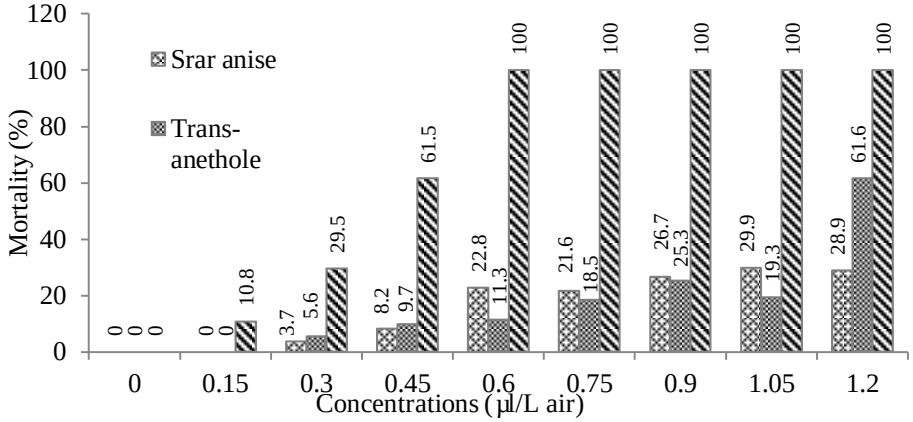

Figure 1. Mortality percentage of house dust mites, *Dermatophagoides pteronyssinus* after fumigation with plant essential oils at various concentrations.

The various essential oil of clove (*S. aromaticum* (L.) Merr. and L.M. Perry) had a high residual contact property, showed 100% mortality of

exposure period of 12 h at 0.02 µl/cm^2 and showed the LC_{50} and LC_{90} at 0.006 and 0.014 µl/cm^2, respectively. Followed by standard trans-anethole could kill completely mite at of 0.3 µl/cm^2 that showed the LC_{50} and LC_{90} at 0.032 and 0.047 µl/cm^2, respectively. Whereas, essential oils of star anise gave 100% mortality of exposure period of 24 h at 0.06 µl/cm^2 that showed the LC_{50} and LC_{90} at 0.032 and 0.050 µl/cm^2, respectively. In addition, the essential oil of clove was highly effective when it caused 100% mite mortality of exposure period of 24 h at 0.01 µl/cm^2 and showed the LC_{50} and LC_{90} at 0.006 and 0.013 µl/cm^2, respectively (Table 1).

For the fibers coating method, the coated fibers with only MU and PG as additive agents could kill mite 75.4% within 0 day. In addition, the coated fibers with clove essential oil at the concentration of 2% was highly effective to control house dust mite, it could kill the mite 31.4% within 56 days that showed the LRT_{50} and LRT_{90} at 46.90 and 26.00 days, respectively. For the coated fibers with star anise essential oil and standard trans-anethole could kill the mite lower than 25% within 28 days with, the LRT_{50} at 18.94-24.92 days and LRT_{90} at 5.21-10.81 days, respectively. Whereas, the fibers coated with clove essential oil and standard trans-anethole at concentration of 2% were killed completely the mite within 21 days that showed the LRT_{50} and LRT_{90} at 10.84-11.68 and 0.97-0.70 days (Table 2).

Coating of synthetic fibers with clove oil was a promising agent with a potential use in house dust mite laden mattresses (Mahakittikun *et al.*, 2014). Cinnamon and clove were the most phytotoxic causing electrolyte leakage resulting in cell death, and that eugenol was determined to be the major component of cinnamon oil (84% v/v), as it was for clove oil (Tworkoski, 2002). Clove and cinnamon oils were successful in killing *D. pteronyssinus* with 100% mortality at concentration of 1.2 µg/cm^3 and had LD_{50} at 0.092 and 0.232 µg/cm^3, respectively (Insung and Pumnuan, 2008). Disappointment, when the essential oil of star anise in corporated with petroleum oil was reported as most toxic, it caused 68.8% mortality of brown planthopper (BPH), respectively (Chantawee *et al*., 2012).

Conclusion

Acaricidal properties of essential oils were evaluated by using fumigation, residual contact and fibers coating method against house dust mite (*D. pteronyssinus* (Trouessart)). The result showed that the fumigation toxicity of star anise was rather low performance but as residual contact, it had high. For the fibers coating method, the coated fibers with star anise essential oil could kill the mite as 85.1% at 7 days.

Table 1. Mortality percentage of *D. pteronyssinus* after treated with essential oils at various concentrations at 12 and 24 h by Residual contact method

Essential oils	Mortality (%)[1] Concentrations (µl/cm²)									LC_{50}	LC_{90}	Slope	S.E.
	0.0	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08				
12 h													
Star anise	0.0±0.0^{a}	0.0±0.0^{b}	2.9±6.4^{c}	8.7±8.7^{c}	37.6±9.9^{c}	87.3±14.2^{a}	90.3±13.6^{a}	94.2±8.9^{a}	98.2±4.1^{a}	0.044	0.060	77.861	4.533
Trans-anethole	0.0±0.0^{a}	2.2±5.0^{b}	28.9±6.5^{b}	21.3±7.4^{b}	82.7±14.8^{b}	94.1±8.4^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	0.032	0.047	83.114	5.177
Clove	0.0±0.0^{a}	94.0±8.9^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	0.006	0.014	180.845	18.696
%CV	-	19.16	12.02	15.13	14.02	10.16	8.15	5.21	2.36				
24 h													
Star anise	2.2±5.0^{a}	2.2±5.0^{c}	29.5±13.6^{b}	36.9±11.4^{c}	57.3±12.0^{b}	94.0±8.9^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	0.032	0.050	72.091	4.282
Trans-anethole	2.2±5.0^{a}	13.1±10.4^{b}	33.4±3.5^{b}	49.3±7.9^{b}	90.7±6.0^{a}	98.2±4.1^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	0.026	0.042	79.265	4.973
Clove	2.2±5.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	100.0±0.0^{a}	0.006	0.013	169.320	17.823
%CV	223.16	17.24	14.90	12.90	9.35	5.83	-	-	-				

[1/] Means ± SD in column followed by the same common letter were not significantly different (P<0.05) according to DMR.

Table 2. Mortality percentages of *D. pteronyssinus* caused by fibers coated with essential oils and kept in cloth bag.

Essential oils formulation	Concentrations (%)	% Mortality (Means)[1]									LRT_{50} (days)	LRT_{90} (days)
		Days										
		0	7	14	21	28	35	42	49	56		
Control (Acl+PG+MU[2])	0	75.4±16.1	-	-	-	-	-	-	-	-	-	-
Star anise+PG+MU	1	92.3±12.4	40.0±9.3	7.3±10.1	-	-	-	-	-	-	5.77	-
	2	100.0±0.0	85.1±11.2	63.3±17.0	35.5±7.1	27.1±11.0	-	-	-	-	18.94	5.21
Trans-anethole+PG+MU	1	97.8±5.0	59.1±13.0	39.4±24.9	17.3±10.9	-	-	-	-	-	11.68	0.70
	2	100.0±0.0	98.0±4.5	80.0±23.2	57.9±7.8	37.6±8.6	23.1±10.8	-	-	-	24.92	10.81
Clove+PG+MU	1	100.0±0.0	55.6±11.0	28.6±8.4	18.6±7.4	-	-	-	-	-	10.84	0.97
	2	100.0±0.0	100.0±0.0	100.0±0.0	97.8±4.5	84.6±9.6	62.2±12.5	60.0±16.9	53.0±18.0	31.4±9.2	46.90	26.00

[1/]Means ± SD in column followed by the same common letter were not significantly different (P<0.05) according to DMR, [2/]PG, MU: additive agents.

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