Effectiveness of essential oils of medicinal plants at reducing the amounts of allergen produced by the European house dust mite, *Dermatophagoides pteronyssinus* (Trouessart)

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ABSTRACT

Essential oils obtained from three medicinal plant species, namely, cinnamon (*Cinnamomum bejolghota* [Buch.-Ham.] Sweet), citronella grass (*Cymbopogon nardus* Rendle), and clove (*Syzygium aromaticum* [L.] Merr. & L. M. Perry), were sprayed on allergenic materials (house dust plus spent mite medium produced by the European house dust mite, *Dermatophagoides pteronyssinus* [Trouessart]) to evaluate the effects of the oils on allergen levels. A 1% solution of each essential oil in 95% ethanol was sprayed on the allergenic materials (which contained about 50 µg of allergen per gram of material), and 95% ethanol, water, and untreated allergenic materials were used as controls. The sprayed materials were allowed to stand for 48 h, and then allergen levels were analyzed by means of an enzyme-linked immunosorbent assay method. The results showed that the essential oil from *C. bejolghota* reduced the amount of allergen by 99.4%, and the essential oils from *C. nardus* and *S. aromaticum* reduced the amounts by 77.1% and 74.0%, respectively. In contrast, the 95% ethanol control reduced the amount of allergen by only 9.8%.

Key words: ELISA, spent mite medium, direct spray

INTRODUCTION

The European house dust mite (HDM), *Dermatophagoides pteronyssinus* (Trouessart), is one of the most important HDM species and is a common cause of allergies worldwide (Boese 1985; Hughes 1976). It is the most abundant HDM species found in Thailand (69.94%), followed by the tropical HDM *Blomia tropicalis* (Bronswijk) (23.46%) and the American HDM *Dermatophagoides farinae* Hughes (5.35%) (Insung et al. 2010). The adult *D. pteronyssinus*,...
which is globular in shape and creamy white, has a striated cuticle and measures approximately 420 µm in length and 320 µm in width (Suggars 1987). The body parts and fecal excreta of these mites are initially 10-50 nm in diameter but break down into smaller fragments that become airborne when dust is disturbed. Mite allergens cause symptoms of an allergic reaction include sneezing, nasal stuffiness, runny nose, itchy or watery eyes, and asthma.

There are many mite control methods, such as washing bedding in hot water, encasing mattresses and pillows in allergen-impermeable covers or tightly woven-material covers, maintaining room relative humidity below 50%, removing carpets, and vacuuming regularly (Jeong et al. 2006; Miller et al. 2007; Vyszenski-Moher et al. 2002). Abidin and Ming (2012) also demonstrated that a commercial air ionizer kills HDMs and can be used to reduce natural mite populations on exposed surfaces. In addition, various chemicals such as benzyl benzoate, DEET, and dibutyl phthalate have been used to control HDM populations (Suhaili and Ho 2008; Uehara et al. 2006).

Research into plant-derived acaricides is now being intensified as it becomes evident that they have enormous potential in this regard. The biological activity of plant extracts against mites has been reported in the literature, and there have been many articles dealing with mites in stored products, including mushroom mites and HDMs (Chang et al. 2001; Insung 1995; Insung and Boczek 1995; Kim et al. 2003a, b; Macchioni et al. 2002; Miyazaki et al. 1989). Clove essential oil, which contains eugenol and its derivatives, was reported as having high effectiveness against *D. pteronyssinus* (Kim et al. 2003a). Insung and Pumnuan (2009) and Veeraphant et al. (2011) demonstrated that this mite species can be effectively controlled by many plant essential oils, including essential oils from medicinal plants such as cinnamon (*Cinnamomum bejolghota* [Buch.-Ham.]), citronella grass (*Cymbopogon nardus* Rendle), and clove (*Syzygium aromaticum* [L.] Merr. & L. M. Perry).

In this study, we evaluated the effects of these three plant essential oils on the amounts of allergen produced by *D. pteronyssinus* after allergenic materials were sprayed with the essential oils.

**MATERIALS AND METHODS**

1. **Mite stock culture**
   *D. pteronyssinus* were maintained in mite bottles kept in a mite chamber at 25 ± 1°C and 86 ± 1% relative humidity. Saturated KCl was used to control humidity. The mites were fed a mixture made from rice (6 g), rat food (4 g), wheat germ (4 g), and yeast (1 g) (Insung and Boczek 1995).

2. **Extraction of plant essential oils**
   The essential oils were extracted from the medicinal plants by water distillation (Table 1). Specifically, 1 kg of plant parts was boiled in 2 L of water for 6 h. The obtained essential oils were stored in a refrigerator at 10°C.

3. **Treatment of allergenic materials with essential oils, and allergen extraction**
   House dust was mixed 1:1 with spent mite medium (SMM, mite cultures including food
remains, feces, exuviae, and dead mites), which contains large amounts of allergen; this mixture contains about 50 µg of allergen per gram of mixture. Five-gram portions of the mixture were put in petri dishes (10 cm diameter), and each dish was sprayed with 3 mL of a 1% solution of an essential oil diluted in 95% ethanol (three dishes were sprayed with each diluted essential oil). Control dishes were sprayed with 95% ethanol or with distilled water. All treatments and controls including the third one as untreated house dust were air dried for 48 h. Then 0.1 g of material from each of the control groups and the treated mixtures was put in a microtube, 2 mL of phosphate-buffered saline (pH 7) was added, and the microtubes were placed in a rotator at 4°C. After 24 h, the contents of the microtubes were homogenized at 2,500 rpm for 15 min. Finally, 1 mL of the supernatant was removed and kept at 4°C for further study.

4. Enzyme-linked immunosorbent assay of the Der p1 allergen

The amount of Der p1 in the supernatant was measured by an enzyme-linked immunosorbent assay method using mouse monoclonal antibodies (Indoor Biotechnologies). Three replicates were performed for each experiment.

The reduction in the amount of Der p1 was calculated by the following formula:

Reduction rate (％) = \( \frac{\text{Amount of allergen in control} - \text{Amount of allergen in treatment dish}}{\text{Amount of allergen in control}} \times 100\% \)

where “control” refers to untreated house dust + spent mite medium.

RESULTS AND DISCUSSION

Essential oils from the three medicinal plants investigated in this study have been reported as being highly toxic to *D. pteronyssinus*; specifically, 1% solutions of the essential oils could completely control the mites by both contact and fumigation methods (Insung and Pumnuan 2009; Veeraphant et al. 2011). The current study was conducted to prove that these essential oils would also show antiallergenic properties if house dust allergens were sprayed with the essential oil. Allergen amounts in the treatment groups were compared with amounts in the control groups. Three replicates of each experiment showed similar results.

Spraying with essential oil from cinnamon reduced the amount of Der p1, one of the most important allergenic proteins in *D. pteronyssinus* (Platts-Mills and Chapman 1987), >99.4±0.0%. This reduction was significantly higher than that observed for the other essential oils. Citronella grass oil, water, and clove oil reduced the amounts of Der p1 by 77.1±18.6%, 75.8±2.5%, and 74.0±12.4%, respectively (there were no significant differences between these
percentages. The 95% ethanol control had little effect on the amount of allergen (9.8 ± 13.1% reduction, Table 2). Mite cultures provide very much allergen even if extracts diluted to 0.000001% still produce skin reactions in persons sensitive to house dust (Voorhorst et al. 1967). Vyszenski-Moher et al. (2002) reported that cold and hot water alone (McDonald and Tovey 1992) or containing detergents can reduce the amount of house dust allergen, as was observed in this study (water resulted in a 75.8% reduction). The allergenic reduction caused by contact with the plant essential oils was extremely high, particularly for cinnamon oil, which contains 82.05% eugenol, along with 3.80% trans-caryophyllene, 1.85% trans-cinnamyl acetate, and other components. Clove oil contains 97.1% eugenol, 1.69% trans-caryophyllene, and 0.53% alpha-humulene (Pumnuan and Insung 2012); and citronella grass oil contains 18-20% geraniol, 9-11% limonene, 7-11% methylisoeugenol, 6-8% citronellol, and 5-11% citronellal (Chang 2007). The chemical composition of cinnamon oil is similar to that of clove oil, whereas the chemical compositions of citronella grass oil and cinnamon oil are quite different. These three plant essential oils markedly reduced the amount of allergen. Many other kinds of plant essential oils have been shown to reduce the amounts of dust mite allergen, as was first reported by Wangapai et al. (2011). These obtained results indicate that it would be promising to use plant essential oils to control HDMs. The oils not only kill HDMs but also reduce the amounts of allergens produced by the mites. The reduction observed for the water control may have been due to dissolution of the allergen in water when house dust was sprayed previously. Higher reductions were observed when house dust was treated with various essential oils and compared with untreated house dust. The higher activity of the three oils may have been due to the ability of the oil to dissolve the allergen, as for the water control, or to some other mode of action. Hence, investigation of their dissolvability or modes of action is still needed.

**CONCLUSION**

The effects of spraying 1% solutions of essential oils obtained from three medicinal plant species, namely, cinnamon (*C. bejolghota*), citronella grass (*C. nardus*), and clove (*S. aromaticum*), on allergic materials (house dust plus spent mite medium produced by the HDM *D. pteronyssinus*), were analyzed by an enzyme-linked immunosorbent assay method. The results showed that the essential oil from *C. bejolghota* was extremely effective in reducing allergen

| Experiment                                      | Reduction (%) |
|------------------------------------------------|---------------|
| Control 1, untreated house dust + spent mite medium | 0.0 ± 10.7 c  |
| Control 2, treatment with water                  | 75.8 ± 2.5 b  |
| Control 3, treatment with 95% ethanol            | 9.8 ± 13.1 c  |
| Treatment with clove oil                         | 74.0 ± 12.4 b |
| Treatment with citronella grass oil              | 77.1 ± 18.6 b |
| Treatment with cinnamon oil                      | >99.4 ± 0.0 a |

%CV 20.5

1 Data are mean ± SD (n = 3). Means followed by the same lowercase letter were not significantly different (P < 0.01) according to Duncan’s multiple range test.
levels (99.4%), followed by the essential oils from *C. nardus* and *S. aromaticum* (77.1 and 74.0% reductions, respectively). Water treatment also showed high allergen-reduction activity (75.8%), whereas the control (95% ethanol) had little effect (9.8% reduction). The obtained results indicate that the use of plant essential oils is a promising method to control HDMs.

REFERENCES

Abidin, S. Z. and H. T. Ming (2012) Effect of a commercial air ionizer on dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae) in the laboratory. Asian Pacific Journal of Tropical Biomedicine. 2: 156–158.

Boese, J. L. (1985) Mites. In Principles of Food Analysis for Filth, Decomposition, and Foreign Matter. (ed., Gorham, J. R.), pp. 63–82, FDA Technical Bulletin No. 1, U. S. Department of Health and Human Services. Public Health Service, Food and Drug Administration, Washington.

Chang, S. T., Chen, P. F., Wang, S. Y. and H. H. Wu. 2001. Antimite activity of essential oils and their constituents from *Taiwania cryptomerioides*. Journal of Medical Entomology. 38: 455–457.

Chang, Y. S. (2007) 8 Map species from Malaysia for ICS. Forest Research Institute Malaysia, Workshop on NFP, 28–29 May 2007, Nanchang, PR China.

Hughes, A. M. (1976) The Mites of Stored Food and Houses. 2nd edition, Ministry of Agriculture, Fisheries and Food, Technical Bulletin 9. London.

Insung, A. (1995) Influence of some active substances of plant extracts on the mold mite, *Tyrophagus putrescentiae* (Schrank). In: Proceedings of Symposium on “Advances of Acarology in Poland” Siedce, September 26–27, 1995. (eds., Boczek, J. and S. Ignatowicz), pp. 234–241, Polish Academy of Science, Warsaw.

Insung, A. and J. Boczek (1995) Effect of some extracts of medicinal and spicy plants on Acarid mites. In: Proceedings of Symposium on “Advances of Acarology in Poland” Siedce, September 26–27, 1995. (eds., Boczek, J. and S. Ignatowicz), pp. 211–223, Polish Academy of Science, Warsaw.

Insung, A. and J. Pumnuan (2009) Acaricidal activity of essential oils of medicinal plants against the house dust mite, *Dermatophagoides pteronyssinus* (Trouessart). KKU Science Journal. 37: 183–191. (in Thai with English abstract)

Insung, A., Pumnuan, J. and P. Konvipasrung (2010) Species diversity of house dust mite in central Thailand. Entomology and Zoology Gazette. 28: 31–39. (in Thai with English abstract)

Jeong, K. Y., Lee, I. Y., Lee, J., Ree, H. I., Hong, C. S. and T. S. Yong (2006) Effectiveness of reduction for control of house dust mites and cockroaches in Seoul. Korea. Korean Journal of Parasitology. 2006. 44: 73–79.

Kim, E. H., H. K. Kim, and Y. J. Ahn (2003a) Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). Journal of Agricultural and Food Chemistry. 51: 885–889.

Kim, E. H., H. K. Kim, D. H. Choi, and Y. J. Ahn (2003b) Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). Applied Entomological Zoology. 38: 261–266.

Macchioni, F., P. L. Cioni,, G. Flamini, I. Morelli, S. Perrucci, A. Franceschi, G. Macchioni and L. Ceccarini (2002) Acaricidal activity of pine essential oils and their main components against *Tyrophagus putrescentiae*, a stored food mite. Journal of Agricultural and Food Chemistry. 50: 4586–4588.

McDonald, L. G. and E. Tovey (1992) The role of water temperature and laundry procedures in reducing house dust mite populations and allergen content of bedding. Journal of Allergy and Clinical Immunology. 90: 599–608.

Miller, J. D., L. Naccara, S. Satinover and T. A. E. Platts-Mills (2007). Nonwoven in contrast to woven mattress encasing accumulate mite and cat allergen. Journal of Allergy and Clinical Immunology. 120: 977–979.

Miyazaki, Y., Yatagai, M. and M. Takaoka. 1989. Effect of essential oils on the activity of house dust mite. Japanese Journal of Biometeorology. 26: 105–108.

Platts-Mills, T. A. E. and M. D. Chapman (1987) Dust mites: immunology, allergic disease and environmental control. Journal of Allergy and Clinical Immunology. 80: 755–75.

Pumnuan, J. and A. Insung (2012) Effectiveness of essential oils from clove and cinnamon in controlling stored product...
mite, *Suidasia pontifica* Oudemans. KKU Science Journal. 40: 1205–1213. (in Thai with English abstract)

Suggars, A. L. (1987) House dust mites: a review. J. Entomol. Sci. Suppl. 1: 3–15.

Suhaili, Z. A. and T. M. Ho. 2008. Residual activity of benzyl benzoate against *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). The Southeast Asian Journal of Tropical Medicine and Public Health. 39: 507–510.

Uehara, S., M. R. Franzolin, S. Chiesa, D. Moreira, W. Gambale, and C. R. Paula (2006) Effectiveness of house dust mite acaricide tri-n-butyl tin maleate on carpets, fabrics and mattress foam: a standardization of methodology. Revista do Instituto de Medicina Tropical de São Paulo. 48: 171–174.

Veeraphant, C., Mahakittikun, V. and N. Soonthorncharoennon (2011) Acaricidal effects of Thai herbal essential oils against *Dermatophagoides pteronyssinus*. Mahidol University Journal of Pharmaceutical Science. 38: 1–12.

Voorhorst R., F. T. M., Spieksma, H. Varekamp, M. J. Leupen and A. W. Lyklema (1967) The house-dust mite, *Dermatophagoides pteronyssinus* and the allergens it produces. Identity with the house-dust allergen. The Journal of Allergy. 39: 325–339.

Vyszenski-Moher, D. L., L. G. Arlian and J. S. Neal (2002) Effects of laundry detergents on *Dermatophagoides farinae, Dermatophagoides pteronyssinus* and *Euroglyphus maynei*, Annals of Allergy, Asthma and Immunology. 88: 578–583.

Wangapai, T., Insung, A., Pumnuan, J. and A. Chandrapaty (2011) Effectiveness of essential oils from wild plants on reduction of house dust mite, *Dermatophagoides pteronyssinus* (Trouessart). Agricultural Science Journal. 42(Suppl.): 323–326. (in Thai with English abstract)