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Efficacy of various synthetic pyrethroid-impregnated encasement materials against house dust mite under laboratory conditions

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Abstract. The acaricidal activity of synthetic pyrethroid and benzyl benzoate against *Dermatophagoides pteronyssinus* was examined in the laboratory, using a specially designed test set up. On the basis of median lethal dose (LD_{50}) values, the compound found to be most toxic to *D. pteronyssinus* was benzyl benzoate (LD_{50} = 50 mg/m^2), followed by permethrin (LD_{50} = 76.7 mg/m^2), deltamethrin (LD_{50} = 146.7 mg/m^2), esbioallenthrin (LD_{50} = 186.6 mg/m^2) and lamdacyhalothrin (LD_{50} = 756.6 mg/m^2). Very low toxicity was observed with bifenthrin (LD_{50} = 5157.8 mg/m^2). A laboratory control trial was also carried out to compare the acaricidal activity (residual effect) of four pyrethroids impregnated on woven and non-woven encasement materials against house dust mites during a 4-month period. Of the pyrethroids used in this study, esbioallenthrin demonstrated the highest acaricidal activity, and of the pyrethroid impregnated materials, the non-woven encasement material was more effective than the woven encasement material.

Introduction

House dust mites (HDM) are a well-known cause of asthma and other respiratory allergies (Quek et al. 1994; Eraso et al. 1998). It has been demonstrated that exposure to high levels of HDM is associated with severe asthma (Zock et al. 1994; Custovic et al. 1996). The most common species of HDM are *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Dt). They are mainly found in bedding, carpet and upholstered furniture. Several strategies have been used in an attempt to control house dust mite, including killing mites by physical or chemical methods. Chemicals with acaricidal activity have been used to treat upholstered furniture, carpets and bedding with the aim to reduce HDM allergen exposure (Platts-Mills et al. 1997).

Pyrethroids are a class of chemicals that have proven to be very effective in controlling arthropods of medical and veterinary importance. They have a very low toxicity in humans and other mammals and its structural analogues are related to six biologically active compounds, natural pyrethrins (pyrethrum), extracted from chrysanthemum flower heads (WHO 1988; Zerba 1988). Currently, in Thailand bedding materials impregnated with a natural pyrethrin are commercially available (Pikasso®) and the product is claimed to have mite protection properties.

Bed encasement with a mite-proof cover is one way of controlling HDM antigen exposure (Owen et al. 1990). Various types of material have been used for HDM barriers, including tight-woven and non-woven fabrics. Both woven and non-woven encasement materials have been highly recommended to patients with dust mite allergy. It was previously suggested that a woven cover with pore size of 2–10 μm could prevent the passage of house dust mites (Vaughan et al. 1999). Woven encasement is made of tightly-woven and well-organized micro-fibers, whereas non-woven fabrics are usually made of spun-bonded polypropylene or polyethylene fibers (Mahakittikun et al. 2003). The combination of chemical and encasement control methods, i.e. acaricide-impregnation on mite-proof encasement materials, may be an alternative HDM control method.

The evaluation of the effectiveness of anti-mite agents has to start under laboratory conditions, but no standard set-up was available. Several techniques and devices have been especially developed for *in vitro* study, such as the Robinson chamber, or a micro-well plate cover with black filter paper and a glass slide, or a test chamber made from a 24-well tissue culture plate (Mitchell 1985; Kalpakhlioglu et al. 1996; Raynaud et al. 2000). In this study we developed an alternative inexpensive and effective laboratory technique for *in vitro* study of the efficacy of acaricides against HDM. The primary objective of this study was to test pyrethroid-impregnated encasing materials as an alternative effective control method against house dust mites.

Materials and methods

Acaricidal activity of six compounds and test set-up effectiveness

Dermatophagoides pteronyssinus mites were maintained in a culture medium made of rat chaw (CPF, Thailand) and liver extract (Diffco®) at 25 °C and 75% relative air humidity (RH). Emulsifiable concentrate formulations of permethrin, deltamethrin, lamdacyhalothrin, esbioallenthrin and bifenthrin (Wellcome Ltd.) were used during the study. Benzyl benzoate (Wellcome Ltd.), an acaricide widely used as anti-HDM agent (Platts-Mills et al. 1997), was used as a toxic reference compound.

To determine the initial testing concentrations of the acaricides, tests were conducted by continuously exposing groups of 20 adult mites to a series of

concentrations of each compound. The mortality rate of the mites was assessed after 24 h, following previous testing of acaricidal activity in HDM (Kalpaklioglu et al. 1996). The lowest concentration of each acaricide that caused 100% mortality was chosen for further acaricidal activity study: groups of 20 adult mites were continuously exposed to 500 mg/m^2 permethrin, 900 mg/m^2 deltamethrin, 5000 mg/m^2 lamdacyhalothrin, 1250 mg/m^2 esbioallenthrin, 40000 mg/m^2 bifenthrin, or 312.5 mg/m^2 benzyl benzoate. The mortality rate of the mites was assessed at 30 min, 1, 3, 24 and 48 h.

In order to determine the median lethal dose (LD_{50}) and the 95% lethal dose (LD_{95}) of each synthetic pyrethroid, a series of six two-fold dilutions of each acaricide (permethrin at 1000, 500, 250, 125, 62.5 and 31.25 mg/m^2; deltamethrin at 900, 450, 225, 112.5, 56.2 and 28.1 mg/m^2; lamdacyhalothrin at 5000, 2500, 1250, 625, 312.5 and 156.2 mg/m^2; esbioallenthrin at 2415, 1207.5, 603.8, 301.9, 150.9 and 75.5 mg/m^2; bifenthrin at 40000, 20000, 10000, 5000, 2500 and 1250 mg/m^2 and benzyl benzoate at 312.4, 156.2, 78.1, 39.5, 19.8 and 9.9 mg/m^2) and an untreated control were prepared using acetone as diluent.

The experiment was performed using a specially designed test set up which consisted of a glass-slide unit (5 × 5 cm), a disposable rubber ring (Ø 2 cm), a plastic unit (5 × 5 cm) with a hole (Ø 2 cm) in the middle, and four clips. A piece of filter paper was cut to 2.5 × 2.5 cm. One hundred microliters of a diluted test compound was applied to the filter paper and the rubber ring and both were left to dry for 24 h before use in the experiment. After drying the filter paper was placed on a glass slide unit and the ring was placed on the paper. Twenty adult *D. pteronyssinus* were picked randomly from a laboratory culture and placed in the middle of the ring using a fine needle made of human hair under a stereomicroscope. The rubber ring was subsequently covered with another piece of acaricide-impregnated filter paper and a plastic unit put on top. The four edges of the set-up were held together with clips. In the control set-up only acetone was used.

Mortality was assessed under a stereomicroscope after 24 h exposure. The test set-ups were placed at room temperature and 75% RH. A mite was considered dead if it showed morphological change (brown and shrunken) or if it did not move even on prodding with a blunt needle. For each concentration of each chemical three replicates were performed and the average mortality was calculated. LD_{50} and LD_{95} (in mg/m^2) of each acaricide were obtained from log-dose probit lines, drawn by plotting the percentage killed against the various concentrations (Finney 1989). The relative toxicity index was calculated by comparing the LD_{50} values of each acaricide against the LD_{50} of benzyl benzoate, the reference compound.

Residual effects of pyrethroid impregnated-encasement materials against HDM

A bioassay was performed to determine the residual effect of each pyrethroid when impregnated on woven and non-woven encasement materials, made of

tightly woven fabric (2–10 μm pore size) and of spun-bonded polypropylene fibers, respectively. Bifenthrin was not chosen for further study due to its extremely high LD_{50} value. Pieces of 2.5 × 2.5 cm of each of the materials were impregnated with an acaricide at the lowest concentration that caused 100% mortality of the HDM: 500 mg/m^2 permethrin, 900 mg/m^2 deltamethrin, 5000 mg/m^2 lamdacyhalothrin and 1250 mg/m^2 esbioallenthrin. These impregnated encasement materials were kept in the dark at room temperature and were used in a bioassay against *D. pteronyssinus* once a month over a four-month period. The bioassay was performed using the test set-up as previously described, except that the filter paper was replaced by the chemically treated woven and non-woven encasement materials. Twenty adult *D. pteronyssinus* were used in each assay. The mortality rate was recorded after 24 h and three replicates were done with each acaricide. Every month, new mites were used in the experiment.

Statistical analysis

Probit analysis was used to calculate LD_{50} and LD_{95} for the acaricides. One-way ANOVA and Bonferroni's multiple comparison test were employed to compare the acaricidal activity among the pyrethroid-impregnated woven and non-woven encasement materials. All analyses were performed using SPSS/PC Version 10.0.

Results

No mites in the new set-up escaped during the test process and no dead mites were found in the control. Survival curves in Figure 1 indicate that permethrin had slowest acaricidal activity, with 35.4% of mites still alive after 3 h of continuous exposure, followed by deltamethrin, with 18% of mites still alive after 3 h. No mites were alive after 3 h of exposure to benzyl benzoate or any of the other pyrethroids. Figure 1 also shows that all mites were killed within 24 h. There was no evidence of any mite tolerance after 24 h of constant exposure *in vitro*.

The LD_{50} values for the acaricidal pyrethroids confirm that the house dust mites were susceptible to all compounds studied, most to permethrin and least to bifenthrin (Table 1). Comparison of the relative toxicity indices, based on LD_{50} values, showed that the toxic reference benzyl benzoate was 1.5, 2.9, 3.7, 14.9 and 102 times more toxic than permethrin, deltamethrin, esbioallenthrin, lamdacyhalothrin and bifenthrin, respectively.

The residual mortality of house dust mites by four synthetic pyrethroids impregnated on woven and non-woven encasement materials is displayed in

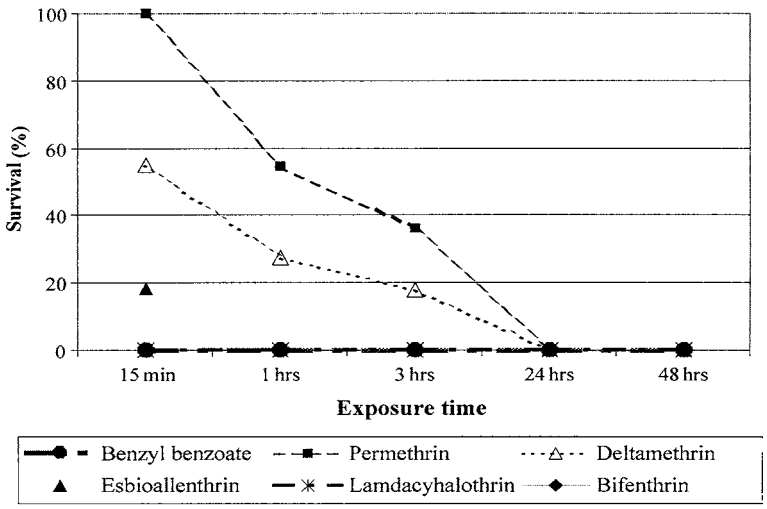

Figure 1. Survival curves of *Dermatophagoides pteronyssinus* after exposure to acaricides at different time intervals

Table 1. Relative efficacy of five synthetic pyrethroids and benzyl benzoate on filter papers against *Dermatophagoides pteronyssinus*, expressed in median and 95%-lethal doses (LD_{50} and LD_{95}; mg/m^2). In brackets: 95% confidence intervals.

Acaricides	LD_{50}	LD_{95}
Benzyl benzoate	50 (32.2–78.8)	306.6 (220–553.3)
Permethrin	76.7 (46.7–131.1)	471.1 (278.9–856.7)
Deltamethrin	146.7 (30–301.1)	892.2 (508.9–1694.4)
Esbioallenthrin	186.6 (115.6–301.1)	1140 (690–2040)
Lamdacyhalothrin	756.6 (462.2–1240)	4604.4 (2720–8501)
Bifenthrin	5157.8 (3853.3–6864.4)	31368.9 (22107.8–159129.7)

Figure 2. The efficacy of the four pyrethroids was different in the woven (1-way ANOVA, $F = 168$, df $= 3, 956$, $p < 0.0001$) as well as the non-woven material (1-way ANOVA, $F = 430$, df $= 3, 956$, $p < 0.0001$). Among the four pyrethroids studied, esbioallenthrin showed the highest residual effect in both woven and non-woven materials, followed by lamdacyhalothrin, deltamethrin and permethrin (Bonferroni's Multiple Comparison Test; $p < 0.001$ for all comparisons).

The mortality rate of *D. pteronyssinus* at monthly intervals over a four-month period appeared higher on the non-woven than on the woven encasement material for all four pyrethroids (independent t-tests, df $= 38$, $p < 0.0001$; permethrin: $t = 6.96$, delamethrin: $t = 20.94$, esbioalenthrin: $t = 20.71$, lamdacyhalothrin: $t = 9.78$).

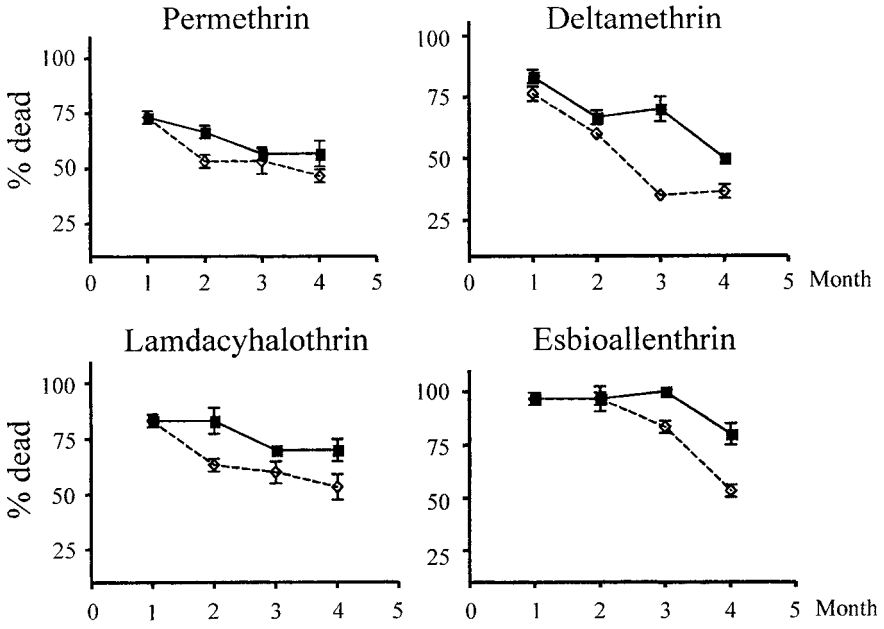

Figure 2. Comparison of the mortality rate (mean % dead ± SE) of *Dermatophagopides pteronyssinus* when exposed to synthetic pyrethroid-impregnated woven (open symbols, dashed lines) and non-woven (closed symbols, drawn lines) encasement materials during a four-month period.

Discussion

This study compared the acaricidal effectiveness of a variety of pyrethroids with that of benzyl benzoate, under laboratory conditions with a specially designed set-up. The new set-up appeared effective since no mite escaped during the experiment and no mite died in the control group. In addition, it is cheap and simple and may thus be used for *in vitro* studies on contact effect, persistence, and residual effects of chemicals or natural herb products with acaricidal activity against HDM, or for the *in vitro* study of other mites of medical and agricultural importance.

The relative toxicity assays infer that benzyl benzoate is superior to the pyrethroids tested, based on the LD_{50} values. Compared to benzyl benzoate, however, pyrethroids are distinctly less toxic to humans and other mammals (WHO 1988). The safety benefit has led to the widespread acceptance and utilization of pyrethroids to control insects of medical importance (Lindsay 1989). For example, permethrin is stressed to be a photo-stable insecticide that is very effective against a large variety of insects and mites with low mammalian toxicity and virtually no allergic side effect. It is approved for use on/

around livestock and pets, and for scabies therapy in infants, little children and patients with seizures and neurological complications (Haustein 1991).

Overall, the pyrethroid-treated encasement materials caused significantly higher mite mortality than the untreated ones. Furthermore pyrethroid-impregnated non-woven encasement material was significantly more effective than the woven type. Perhaps the compressed fiber structure of non-woven encasement material allows penetration and habitation of HDM into the fiber, providing greater contact with the acaricide. In contrast, woven encasement material has a tight and well-organized texture and therefore penetration or habitation of HDM was nonexistent on the surface (Mahakittikun et al. 2003).

One limitation of the impregnation of pyrethroid on encasement materials is that it cannot provide continuous protection since all acaricides have a half-life. For example, in the study of Wu et al. (1991), the half-life of deltamethrin impregnated on cotton and nylon bednets was 65.6 and 55.4 days, respectively, and the half-life of permethrin was 35.0 and 27.4 days. Thus, a deltamethrin-impregnated bednet (10 mg/m^2) is effective for 6–7 months. The protection efficacy of these pyrethroids is reduced when these materials are washed (Graham et al. 2002). Thus, a disposable pyrethroid-impregnated non-woven type encasing is more promising for control of HDM.

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