

RESEARCH NOTE

RESIDUAL ACTIVITY OF BENZYL BENZOATE AGAINST *DERMATOPHAGOIDES PTERONYSSINUS* (ACARI: PYROGLYPHIDAE)

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Abstract. The residual effect of a commercial spray containing 2.0% benzyl benzoate against *Dermatophagoides pteronyssinus* was evaluated in the laboratory. Filter papers treated with benzyl benzoate were dried for 2 hours and placed in Petri dishes. Adult mites were then added and mortality was assessed following 24 hour contact and at succeeding 4 weeks intervals up to week 28 post-treatment using the same treated filter papers. There was 100% mortality at 24 hours post-treatment, declining progressively to 71% by week 28. Probit analysis estimated a median lethal time (LT₅₀) of 59 weeks.

INTRODUCTION

Dermatophagoides pteronyssinus (Trouessant) is one of the most common and abundant house dust mite species in tropical regions worldwide. Alive and dead, they represent a major source of indoor allergens to humans (Wharton, 1976; Fain *et al*, 1990; Arlian and Platts-Mills, 2001). The development of more effective methods for reducing mite infestations and prevention of dust mite allergies in homes has been an area of research for decades. Two major strategies have been commonly advocated: (i) allergen avoidance or inactivation, and (ii) reduction or elimination of mites (Colloff *et al*, 1992).

Multiple strategies have been recommended to reduce mites and associated allergen levels in homes such as reducing in-

door relative humidity to below 50%, coupled with regular washing or cleaning of carpets, mattresses, stuffed furniture, and use of dust-proof encasement on mattresses and pillows (Arlian and Platts-Mills, 2001; Vyszenski-Moher *et al*, 2002). Several chemicals have been recommended for use in homes to kill dust mites, including lindane, diethyl-m-toluamide, permethrin, pirimiphos-methyl, sumithrin and tannic acid (Heller-Haupt and Busvine, 1974; Lau-Schadendorf *et al*, 1991; Dietemann *et al*, 1993; Vyszenski-Moher *et al*, 2002).

Other studies have demonstrated benzyl benzoate is effective at denaturing dust mite allergen (Chang *et al*, 1996; Van Der Heide *et al*, 1997; Vanlaar *et al*, 2000) and can actually killing mites and reduce their populations (Hayden *et al*, 1992; Rebmann *et al*, 1996). In Malaysia, a commercial spray containing liquid benzyl benzoate is available. The objective of this laboratory study was to investigate the immediate and residual effectiveness of this commercial spray in killing *D. pteronyssinus* mites.

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MATERIALS AND METHODS

Mites

Dermatophagoides pteronyssinus mites were obtained from colonies established in 1960 in the Acarology Unit, Institute for Medical Research (IMR), Malaysia. The colonies were reared in small glass bottles and routinely fed ground rat chow. All bottles were kept in a desiccation jar containing a solution of sodium chloride to maintain ideal relative humidity (RH) near 75%. The colonies were maintained at an average temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

Chemical

A commercial spray (Demitze™, Approved Allergy Fighters Sdn Bhd, Malaysia) containing 2.0% benzyl benzoate and 5% anionic surfactants was used.

Bioassay procedure

A filter paper bioassay method was used based on modification of Braun *et al* (1987). A recently produced bottle of Demitze™ was obtained from the manufacturer. The benzyl benzoate solution was applied at a rate 0.5 ml/cm^2 to each piece of Whatman no. 1 filter paper (9.8 cm^2). A similar number of control papers were treated with 10 ml of distilled water only. After drying under a fume hood for two hours, each treated filter paper was placed in the 14 cm diameter plastic Petri dish. A thin film of Vaseline was applied on the inside surface of the Petri dish bordering the edges of the filter paper to prevent escape of mites. Thirty adult *D. pteronyssinus* mixed male and female were placed on each filter paper. A total of 10 replicates were tested for both treatment and control papers per time interval.

Treatment and control dishes were held in two separate glass chambers at 25°C and 75% RH. Mortality was assessed after 24 hours contact exposure. Mites were carefully examined using a dissecting microscope and

those that did not move when prodded were recorded as dead. Following the bioassay, all mites were removed and the test filter papers stored inside storage box without lid at normal room conditions. The papers were tested at 24 hours post-treatment and again using the same procedures at 4 week intervals up to week 28.

Statistical analysis

Percent mortality was recorded for each time interval. The median lethal time (LT_{50}) was determined by probit analysis (Finney, 1971) using SPSS ver 13.0 (SPSS, Chicago, IL).

RESULTS

The residual effect of 2.0% benzyl benzoate treated papers against *D. pteronyssinus* at 24 hours and 4, 8, 12, 16, 20, 24 and 28 weeks post-treatment is presented in Fig 1. One hundred percent mortality was recorded at 24 hours post-treatment. At week 4, 83% mortality was recorded, followed by a gradual decline in % mortalities with each succeeding 1 month interval. At week 28, overall mortality was 71%. Control mortality was 0% throughout the study. Probit analysis estimated a median lethal time (LT_{50}) of 59.0 weeks with $y = 0.97 - 4.36x$ (y = mortality, x = week post treatment).

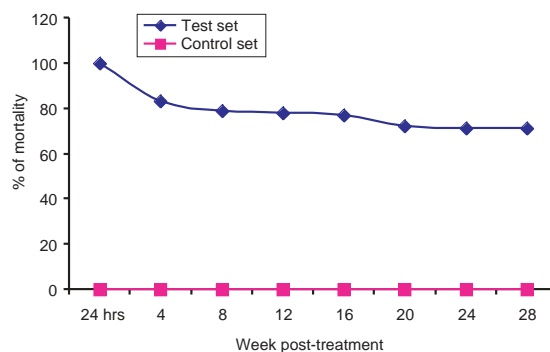


Fig 1—Mortality of *D. pteronyssinus* mites on filter paper treated with 2.0% benzyl benzoate.

Table 1
The residual effect of 2.0% benzyl benzoate solution dried on filter paper against *D. pteronyssinus*.

Acaricide	LT ₅₀ (week)	Regression equation	95% Fiducial confidence limits		Chi-square
			LCL	UCL	
2.0% benzyl benzoate	59.0	Y = 0.97-4.36X	45.5	93.9	1.753 ^a (df=5)

^ap = 0.88 not significant at p < 0.05 level.

Probit analysis estimated a median lethal time (LT₅₀) of 59.0 weeks with $y = 0.97 - 4.36x$ ($\chi^2 = 1.753$, $df=5$, $p = 0.882$) (Table 1).

DISCUSSION

Low percentage concentrations of benzyl benzoate have been used successfully as miticides against scabies (*Sarcoptes* species) and preventing contact with disease-carrying (Scrub typhus) *Leptotrombidium* mites; however, it has proven far less effective as an insecticide for controlling head and body lice (*Pediculus* species and *Phthirus pubis*). This study demonstrated that Demitze™ is very effective against the common house dust mite, *D. pteronyssinus* when applied as a 2% spray and allowed to dry. Hayden *et al* (1992) has reported similar findings. As seen in our study, Rebmann *et al* (1996) and Kalpaklioglu *et al* (1996) also found % mortality decreased over time. Following 24-hour contact with the treated filter papers, residual activity decreased from 100% at first test (2 hours post-treatment) to 71% mean mortality at 28 weeks post-treatment. The manufacturer recommends that Demitze™ be used on mattresses with a reapplication every 3-4 months. Our results indicate that under laboratory conditions, the spray can theoretically kill 50% of a mite population up to 59 weeks. Such a prolonged residual activity may not be possible under more normal conditions due to various

factors. The bioassay technique described herein is an easy-to-use procedure to quantitatively assess residual effectiveness of contact acaricidal agents. It is not known if or how much benzyl benzoate from the treated surface is able to permeate into materials such as a mattress. Chemical penetration and the effectiveness of the spray on reduction of other common species of dust mites and their associated allergens require further study.

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