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THE EFFICACY OF BENZYL BENZOATE SPRAYS IN KILLING THE STORAGE MITE *TYROPHAGUS PUTRESCENTIAE* (ACARI: ACARIDAE)

Anu T.K. Harju[1], Sirpa M.A. Pennanen[1], Jyrki Liesivuori[1, 2]

[1]Kuopio Regional Institute of Occupational Health, Laboratory of Environmental Microbiology, Kuopio, Finland
[2]University of Kuopio, Department of Pharmacology and Toxicology, Kuopio, Finland

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Abstract: This study tested the effectiveness of benzyl benzoate (BB) in killing *Tyrophagus putrescentiae* (Schrank) mites when using the method utilized by regular consumers. The efficacy of the BB sprays was determined in laboratory experiments and semi-field experiments with mattress and mattress pad pieces. The mites were counted and their living status determined at different time points microscopically. In the laboratory experiment, the sprays containing either 0.5%/0.9% BB with 70% ethanol or 0.1% BB with absolute ethanol were highly efficient, resulting in over 90% mite mortality within 20–30 minutes. In the semi-field experiment, mimicking the home application, the sprays were applied to pieces of a mattress and a mattress pad, and allowed to affect the area for 30 minutes before thorough vacuuming. The recovery of mites was usually less than 10%. The sprays containing BB were effective in killing the mites in the laboratory, but success was less prominent in the semi-field tests. This method could be used in testing other compounds for their efficacy in killing mites.

Address for correspondence: Dr. Sirpa Pennanen, Kuopio Regional Institute of Occupational Health, Laboratory of Environmental Microbiology, P.O. Box 93, FIN-70701 Kuopio, Finland. E-mail: sirpa.pennanen@ttl.fi

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INTRODUCTION

Several studies have shown that exposure to dust mites increases the risk of allergic reactions [23-25]. To avoid the onset of symptoms, whether caused by HDM or SM, reducing exposure to the allergen seems to work best [25], though both mites and their faeces need to be removed. Thorough vacuuming of floors, carpets and beds is often advised, but since mite density is not necessarily correlated with the amount of dust, it is doubtful whether such cleaning can drastically affect the number of mites [1, 11, 34]. Other suggested methods include reducing indoor humidity, airing objects in the cold [9], exposure to direct sun light or dry heat [10], using mattress and pillow casings [35], or using an acaricide to kill the mites [5, 7, 21, 23, 38]. However, none of these methods result in permanently eradicating mites, and their efficacy is often questionable [12, 27, 28, 34].

In dwellings, the most common mites are the house dust mites (HDM) *Dermatophagoides pteronyssinus* (Trouessart) and *D. farinae* (Hughes) [1, 23, 31]. In addition to house dust mites, storage mites (SM) such as *Tyrophagus putrescentiae* (Schrank) can sometimes be found in dwellings [23, 29] and may occasionally be numerous [1, 14, 18]. Especially in rural settings, storage mites may comprise a large portion of the mites in beds [14]. Exposure to storage mites may cause symptoms similar to those for house dust mites [23].

In the home, usual HDM and SM habitats include beds, bedding, stuffed furniture, rugs, soft toys and clothing [1,

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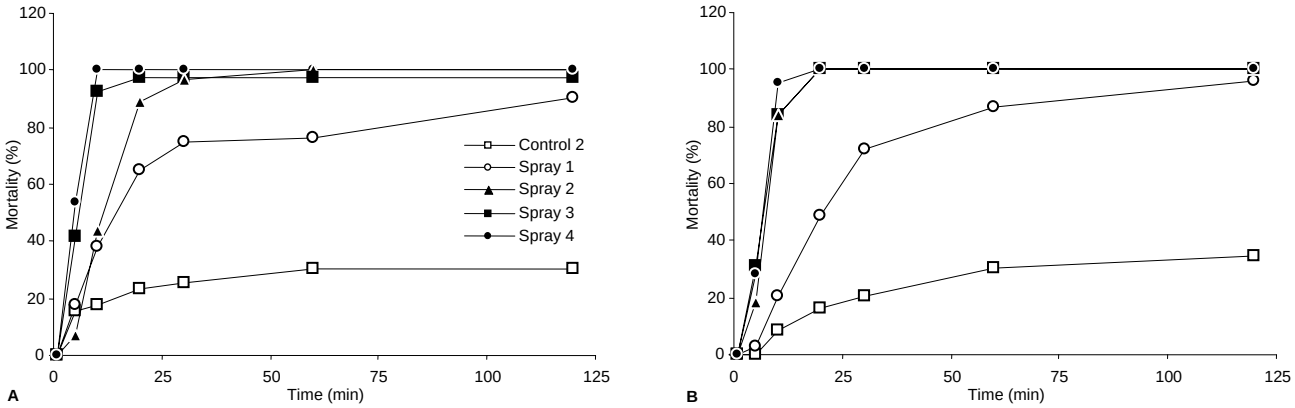

Figure 1. Mortality of *Tyrophagus putrescentiae* mites in petri-dish experiments. A. Treatment with 1-second spraying. Treatments as in Table 1. Each point represents a mean of 3 determinations. B. Treatment with 2-second spraying. Treatments as in Table 1. Each point represents a mean of three determinations.

6, 8, 11, 30, 33]. Beds especially and bedrooms provide important habitats since the microclimate in the bed is often highly favourable to mites [8]. Furthermore, dampness in the home can result in high Der p 1 content of dust [22, 32] or high mite numbers [15, 37].

Using a spry for reducing the number of mites in the environment is a relatively new phenomenon in Finland. Before using or recommending an acaricide spray for this purpose, it is important to know if the spray also works when used by the consumer. The aim of this study was to ascertain the efficacy of 4 different benzyl benzoate (BB) sprays in killing *Tyrophagus putrescentiae* mites. The efficacy of the BB sprays was determined in laboratory experiments, while semi-field experiments with mattress and mattress pad pieces simulated the conditions at home and the actions taken by users. The experiment was carried out just as the users would, by spraying and cleaning-up, in order to see the actual impact of BB and vacuuming on mites. *Tyrophagus* mites were chosen for the experiment because they are relatively common in both rural and urban environment. Furthermore, it seems that *T. putrescentiae* is a more important sensitiser than HDM in Finland.

MATERIALS AND METHODS

Mites. The *Tyrophagus putrescentiae* (Schrank) mites used for experiments were cultured at the Kuopio Regional Institute of Occupational Health, Finland. The mites were grown in small glass jars, kept at room temperature, and fed with dry yeast - wheat germ mixture (1:1 w/w). The small jars were kept in larger jars containing about 50 ml of saturated NaCl. The large jars were covered with an aluminium-foil lid resulting in 75% relative humidity within the jars. When the population density was sufficiently high and the food was almost depleted, the mites were moved from the jar to the lid where they were collected. A mite was considered to be alive if it moved around or moved its legs or other appendages while stationary, and it was considered dead when no movement was apparent.

Sprays. Six different sprays were tested (Tab. 1). The active ingredient in the sprays was benzyl benzoate, which is insoluble in water and has an aromatic odour [29]. It can irritate skin and eyes, and its vapours at high temperatures can cause irritation to the respiratory tract. The solvent in the sprays was either 70% or absolute ethanol. Citric acid was used as a conservation component. The sprays were received as ready mixtures and we did not have any say about their ingredients or the mixing ratio.

The available user's instructions indicated that a mattress should be sprayed for 30–60 seconds and allowed to affect the area for 30 minutes before thorough vacuuming. This information was used to calculate the treatment times both for the laboratory and mattress experiments.

Experimental design: laboratory experiments. The first set of tests was carried out to test the 4 experimental sprays, while 70% ethanol (70% EtOH) was used as a control. Since mites disappeared from the 70% EtOH dishes and died in large numbers, a second set of tests was conducted in which de-ionised water (H_2O) was compared with 70% ethanol in order to determine whether mite mortality had been induced by ethanol alone. All tests were done in 3.3-litre (12.5 × 12.5 × 21 cm) glass jars. One glass petri dish (Ø 6 cm) was placed in each of the jars. Each solution was sprayed into its own jar from rim level, with treatment time being either about 1–2

Table 1. The sprays used in the experiments.

Treatment	Composition
Control 1	de-ionised water
Control 2	70% ethanol
Spray 1	0.1% benzyl benzoate, 70% ethanol, 1% citric acid
Spray 2	0.5% benzyl benzoate, 70% ethanol, 1% citric acid
Spray 3	0.9% benzyl benzoate, 70% ethanol, 1% citric acid
Spray 4	0.1% benzyl benzoate, absolute ethanol, 1% citric acid

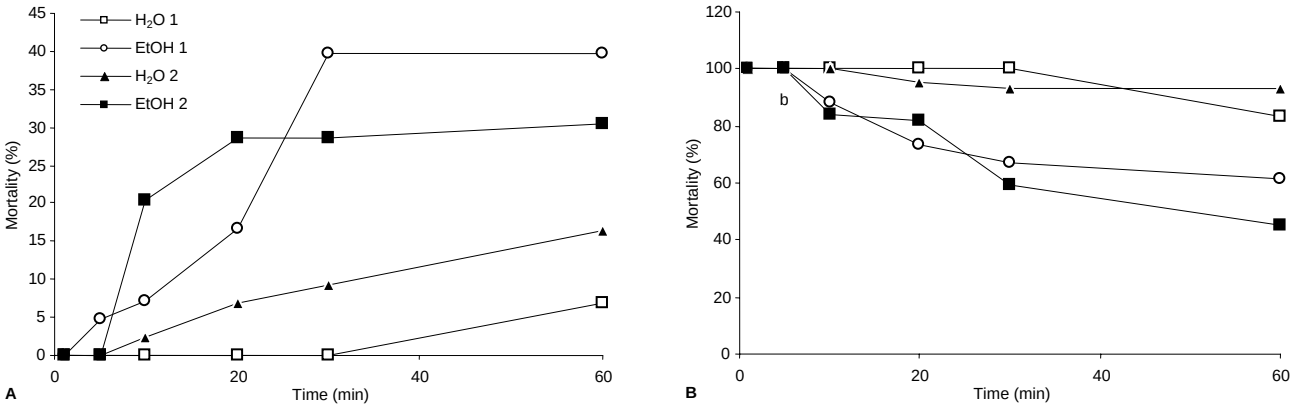

Figure 2. A. Mortality of *Tyrophagus putrescentiae* mites in water and 70% ethanol treatments. H_2O 1 = one spray of de-ionised water, H_2O 2 = two sprays of de-ionised water, EtOH 1 = one spray of 70% ethanol, and EtOH 2 = two sprays of 70% ethanol. Each point represents a mean of 3 determinations. B. Disappearance of mites from the petri-dishes. Treatments as in A. Each point represents a mean of 3 determinations.

seconds, corresponding approximately to a 60-second or 120-second spraying of the bed, respectively. Within 20 seconds after the spraying, about 15–20 mites (15 ± 8.5 and 21.6 ± 14.8 for the first and second sets, respectively) were placed onto the petri dish (= time 0) and an aluminium-foil lid was placed on top. The mites were counted after 1 minute of exposure (= original number of mites), and further checks on mite numbers and their living status were performed at 5, 10, 20, 30 60 and 120 minutes, with a stereomicroscope (Wild M3Z, Heerbrugg, Switzerland, 20× magnification). All treatments were carried out in triplicate.

Experimental design: mattress tests. In these tests we wanted to simulate the actual use of the sprays. A mattress with springs (80 × 200 × 20 cm) and its pad (80 × 200 × 7 cm) were both cut into twelve 30 × 39 cm pieces that were placed into plastic boxes (31.5 × 39.9 × 23 cm). For each pad and mattress piece, 67.9 (± 13.1) and 122.8 (± 14.0) mites were transferred to a petri dish, which was then placed overnight upside down on top of the mattress pieces for the mites to move onto (and into) them. Each piece was then treated with 1 of the 6 different solutions: 2 controls (H_2O, 70% EtOH) and the four sprays containing BB (Tab. 1). Each piece was sprayed for about 3 seconds from a distance of 15–20 cm above the surface. The spray was allowed to take effect for 30 minutes before the pieces were thoroughly vacuumed (Miele electronic S2511 air clean, 1400 W) and the mites were collected onto a filter (glass fibre filter, MN 640w, Ø 9 cm). Both sides of the mattress pad pieces were treated and vacuumed while only the upper side of the mattress pieces were treated and vacuumed. The vacuuming time was 2.5–3.8 minutes per square meter, which exceeds the 2 minutes per square meter recommended for HDM sampling [22, 23]. The treatments were performed twice with both mattress and pad pieces. The filters were checked for mites with a stereomicroscope (Wild M3Z, Heerbrugg, Switzerland, 20 × magnification).

Statistical analyses. Since the data was not normally distributed, we used the non-parametric Friedman test to evalute the differences between treatments. The p-value of 0.05 was set as a limit for statistically significant difference. The correlation between mites placed on the mattress or pad pieces and the number of mites recovered were tested with Pearsons correlation test.

RESULTS

Laboratory tests. All 4 BB sprays were effective in killing *Tyrophagus*-mites. The first deaths were recorded at 5 minutes, and within 30 minutes of exposure each of these sprays had resulted in mite mortality rates of at least 75% (Fig. 1a and b). Treatment time did not have any noticeable effect on mite mortality. Sprays 2, 3 and 4 (Tab. 1) were more effective than spray 1, causing at least 95% mortality within 30 minutes with both treatment times, and were clearly more effective than 70% ethanol alone ($p = 0.022$ - $p= 0.049$ and $p = 0.017$ - $p = 0.046$ for the first and second sprayings respectively). After 2 hours of exposure, no differences could be found in mite mortality among the BB sprays, while the mortality caused by 70% EtOH was still significantly lower. However, mites on ethanol dishes had a higher incidence of disappearance than those on the spray dishes. Only 3 mites from the spray treatments disappeared, representing 0.8% of the mites placed on the 24 dishes, while almost half (43.4%) of the mites put on the 6 ethanol dishes disappeared. When escaping was taken into account and mite mortality calculated, only with the mites remaining at the end of the exposure (2 hours), were the ethanol treatments found to have killed about 60% of the mites.

Because of the high ethanol mortality in the first set of tests, we performed a second set of tests with de-ionised water (H_2O) and 70% ethanol. H_2O caused significantly ($p = 0.014$ - 0.046) lower mortality among the mites than ethanol (11.7% and 35%, respectively, Fig. 2a) and less mites disappeared from the H_2O dishes than from the ethanol dishes (11.8% and 53.2%, respectively, Fig. 2b). Treatment time (1 second *vs.* 2 seconds) did not affect either the escaping or the mortality of the *Tyrophagus*-mites. When disappearance was taken into account, the

Table 2. Number of mites recovered from mattress-pad pieces after spraying. Treatments as in Table 1.

Treatment	Initial number of mites	Number of mites recovered	Recovery rate (%)
Control 1	70 ± 17.0	2 ± 2.8	2.4 ± 3.5
Control 2	65 ± 21.2	3.5 ± 0.7	5.5 ± 0.7
Spray 1	66 ± 11.3	6 ± 0	9.2 ± 1.6
Spray 2	64.5 ± 27.6	10.5 ± 9.2	21.3 ± 23.4
Spray 3	67 ± 7.1	4 ± 0	6 ± 0.6
Spray 4	75 ± 7.1	4.5 ± 4.9	5.7 ± 6.1

Table 3. Number of mites recovered from the mattress pieces after spraying. Treatments as in Table 1.

Treatment	Initial number of mites	Number of mites recovered	Recovery rate (%)
Control 1	111.5 ± 6.4	0	0
Control 2	125.5 ± 31.8	2.5 ± 0.7	2 ± 0.1
Spray 1	122.5 ± 2.1	4 ± 2.8	3.3 ± 2.4
Spray 2	104 ± 7.1	4.5 ± 2.1	4.3 ± 1.8
Spray 3	110 ± 9.9	3 ± 1.4	2.7 ± 1.0
Spray 4	103 ± 8.5	6 ± 4.2	5.7 ± 3.7

difference between water and ethanol treatment in mite mortality became greater (14.3% and 65.6% of mites dead, respectively).

Mattress tests. Only a few mites were found from the mattress and pad pieces after treatment and vacuuming. The recovery rate (number of mites caught/initial number of mites) was 2–21% (Tab. 2) and 0 to 6% (Tab. 3) with pad and mattress pieces, respectively. The number of recovered mites did not seem to depend on the number of mites placed on the mattress or pad pieces ($r = 0.3592$, $p = 0.278$ and $r = 0.0274$, $p = 0.933$ for pad and mattress pieces, respectively). Moreover, the treatment used did not appear to affect the observed mortality since all mites, except one, were dead when found. There were, however, some differences among the treatments in recovery. In the mattress pad tests, the lowest recovery was found in water treatments and the highest recovery with spray 2. Recovery was lower in the mattress tests, and the main difference among the treatments was the absence of recovered mites in the water treatment.

DISCUSSION

In regions with dry winters, such as most of Finland, the HDM numbers remain low even in the summer and autumn [30], the usual peak seasons for mite populations. Hence, it has been suggested that mites are a minor problem in Finland [26]. In their study, Raunio *et al.* [26] found no Der p 1, allergen in any of the rugs tested. Similarly, only a few of the bed/sofa samples contained Der p 1 and even then in extremely low concentrations (unpublished data). However, Hanhela [14] showed that both SM and HDM are frequently found in Finnish beds in rural areas. Furthermore, dampness in houses - a growing problem in Finland - is often associated with increased risk of mite exposure [22], especially of storage mites [37]. In Finland, using an acaricide to control house dust or storage mites in dwellings has been a recent development. Our experimental design was chosen because people trying to control mites do not have elaborate methods for measuring the exact amount of spray used, and it is important to know that the method used will work. Our results show that all the BB sprays used in this study were effective in killing *T. putrescentiae* in the laboratory, but a similar effect was not confirmed for the semi-field experiments.

In the laboratory tests, sprays 2, 3 and 4 were the most effective, causing at least 90% mortality in 20–30 minutes. The higher efficacy of sprays 2 and 3 compared to spray 1 results from the increasing concentration of benzyl benzoate. The efficacy of spray 4, on the other hand, can be attributed to both benzyl benzoate and the increased ethanol content, suggesting an interaction between the two. Benzyl benzoate is typically used as an insecticide to treat mite and lice infestations. It has been found to be effective as an acaricide [5, 7, 17], although its efficacy in reducing the allergen load has been questioned [5, 28, 36, 38]. Using tannic acid simultaneously with benzyl benzoate has produced better results [38], but the effects do not seem to be long lasting. In contrast, Bischoff *et al.* [7] found that using benzyl benzoate as an additive when washing garments at low temperatures results in a large reduction in mite numbers.

In addition to the BB sprays, both H_2O and 70% EtOH caused mortality among mites, though water less so than ethanol. When the sprays or controls were used in the jars, a slight sheet of moisture remained on the dishes during the experiment. Since ethanol is more volatile than water, the water layer stayed in place longer. The comparison between 70% ethanol and water treatments showed that the mortality seen was not, however, due to drowning. Indeed, van Bronswijk and Sinha [9] have been able to demonstrate that it takes more than 2 days for *Dermatophagoides* mites to drown in soapy water. The individuals that died during the water treatments might have been in a poorer condition to begin with, or *Tyrophagus* sp. might be more prone to drowning than *Dermatophagoides*.

Similarly, although ethanol in high concentrations and large quantities is poisonous to mites [13], the most pronounced effect of ethanol seen here was dispersal. The 70% ethanol seemed to act as a repellent for the mites, since almost half of the mites in each repetition disappeared from the dishes. The fate of the disappeared mites was unclear. Conversely, relatively few mites disappeared from the water and the spray dishes. This would suggest that benzyl benzoate may have affected the mites quickly, and thus killed them before they could leave the dishes.

On the other hand, no treatment effect was seen in the mattress experiments other than a low number or lack of mites captured from water-treated mattress samples. The recovery of mites was very low in most cases, which agrees well with other studies [4]. According to Carswell *et al.* [11], vacuuming may be more efficient in removing dust than mites. This suggests that the mites burrowed deeply into the mattress pieces and were able to stay there - either dead or alive - during the vacuuming. Clearly, survival of any of the mites inside the mattress could subsequently result in a new build up of the population. *T. putrescentiae* populations are known to increases very rapidly in favourable conditions, since a female can produce 300–500 eggs during the oviposition period [3, 20]. This can lead to over a 100-fold increase in population per generation [3]. This vastly exceeds the reproductive potential of *Dermatophagoides farinae*, 30–100 eggs per oviposition period [2] or *D. pteronyssinus*, with an average of 50 eggs [37].

The results of this study show that benzyl benzoate is effective in killing mites under laboratory conditions. It also shows that BB sprays are probably not as effective when used at home to deal with mites and their allergens. Thorough vacuuming is strongly recommended to remove the dead mites and their allergens. However, even that might not be sufficient, and other methods are needed. Since our purpose was to simulate the conditions and practises performed in the homes, we suggest that the model established here could be useful in testing other compounds for their efficacy in killing mites.

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