

Comparative efficacy of house dust mite extermination products

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Summary

The acaricidal efficacy of nine marketed products, i.e. Acardust, Acarosan (foam and powder), Actelic 50, Artilin 3A (spirit and water base), liquid nitrogen, Paragerm AK, and Tymasil, and of intensive vacuum-cleaning have been compared on four different test surfaces: mattress, tufted carpet, gypsum board and rough wooden board, all covered with artificial house dust. They were inoculated with the house dust mite, *Dermatophagoides pteronyssinus* or the house-dust fungus *Aspergillus repens* for evaluation of the fungistatic claims of some products. The acaricidal activity of Tymasil did not surpass that of vacuuming; its fungistatic effect was not apparent. The other products showed complete to almost complete eradication on at least one of the substrates tested. Taking into account the results of acaricidal efficacy as well as the data on safety and practicality acquired earlier, Acarosan powder was considered first choice for carpet treatment. Acarosan and liquid nitrogen, were found to be effective in the treatment of mattress, pillow, upholstered furniture and heavy curtains. On wooden surfaces Acarosan was found to be both effective and safe, while Acardust, Actelic 50, Artilin 3A (both fungistatic as well as acaricidal), liquid nitrogen and Paragerm also passed the efficiency test.

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Introduction

House dust mite allergy is a world-wide problem [1]. Preventive measures are widely advocated, including the avoidance of allergen exposure by reducing the mite content of dwellings. Some recent studies have been published in this journal [2–5], as well as elsewhere [6–11].

One of the problems encountered in practical mite reduction studies is the omnipresence of the mites, which inhabit a great variety of niches inside dwellings, i.e. textile floor coverings, badly sealed wooden floors, non-washable heavy curtains, padded furniture, mattresses, pillows, blankets, quilts and eiderdowns, as well as other home textiles [12].

In order to decrease the allergen content of homes produced by house dust mites (Pyroglyphidae), a number of steps have to be taken. These consist of establishing an inventory of the mite-situation in the individual home and of formulating hygienic limits to support each step. Actual management of the allergen content starts after careful examination both of the patient and of his home. Management comprises of three phases: prevention of infestation, extermination of harmful mites, and removal of allergenic products [13]. In a preliminary report, safety, efficacy and practicality of different domestic acaricides available on the market were compared [6]. In the present experiment, the acaricidal efficacy of products was tested on *Dermatophagoides pteronyssinus* in standardized semi-natural conditions and compared with the results of vacuum-cleaning alone, as a normal household cleaning measure, or with those results in the case of no treatment. In a second experiment, the fungistatic activity of some products was tested against a common dust-inhabiting fungus (*Aspergillus repens*).

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Table 1. Description of the test surfaces

Test surface	Origin of material	Preparation of substrate units (10 × 10 cm)
Mattress	Kenton Polyether, Utrecht; composite of shiny acetate/viscose mattress tick, 0.4 cm thick polyurethane foam	Pieces were cut, doubled and sewn at rims and + form to obtain 10 × 10 cm 'mattresses' with shiny cotton at the outer side
Tufted carpets	Nederlandse Tapijt Unie, Utrecht, The Netherlands; polyamide poles on polyester foam base	No finishing work needed; pieces were cut
Rough wooden board	Do-it-yourself store; 0.6 cm thick unfinished spruce deal	Pieces were sawn and roughened with sand paper number 200
Gypsum board	Norgips b.v., Delfzijl, The Netherlands; 0.8 cm thick with protective paper on the side	Protective paper removed; roughened with sand paper number 200

Materials and methods

Acaricidal tests were performed on four different substrates covered (or soiled) with artificial house dust, and inoculated with mites or fungi.

Substrates

For the purpose of considering the great diversity of possibly inhabitable niches within a dwelling, four different test surfaces were selected: mattress, tufted carpet, roughened wooden board, and gypsum board (Table 1).

Pieces of 10 × 10 cm were cut of each material. Since all surfaces are normally more or less soiled under home conditions, the test substrates were covered with a modified artificial test dust [14,15]. Its base consisted of 5 g Catenex oil N945 (Deutsche Shell AG, Hamburg, Germany), 33 g kaoline O (Erbslöh, Düsseldorf, Germany), 25 g Quartz flour W 12 (Quarzwerte, Frechen, Germany), 25 g dried and pulverized peat (Euroflor AG, München, Germany), 10 g calcium-carbonate, 0.5 g soot FW 2 (Degussa GB Pigmente, Frankfurt, Germany), 0.5 g dried and pulverized cigarette ash and 1 g iron trioxide FKK/K (Simon & Werner, Schwalbach/Ts, Germany) [14]. The mixture was combined with dried yeast powder and beard shavings (dander as well as hair stubs) in the ratio 4:1:3 (w/w). One gram of artificial test dust was brushed into each 10 × 10 cm square piece of mattress and carpet with batches of 0.5 g being used for gypsum or wood samples so as to comply with maximum dust levels under home conditions.

Treatments

Nine acaricidal products were tested: Acardust, Acarosan (foam and powder), Actelic 50, Artilin 3A (on spirit and on water base), liquid nitrogen, Paragerm AK (as a spray

not in a nebulizer) and Tymasil (Table 2), all provided by courtesy of the manufacturers or bought in local stores. The formulations were used in concentrations recommended by the manufacturer. Intensive vacuuming (25 sec/10 × 10 cm substrate = 42 min/m²; 13 cm wide nozzle; 50 l air/min) was chosen by way of control.

Test organisms

Dermatophagoides pteronyssinus (Trouessart 1897), taken from cultures reared on a mixture of dried yeast and human dander media (1:1 w/w) at 25°C and 75% RH and *Aspergillus repens* (Corda) Saccardo, taken from cultures reared on malt agar slants in tubes (water-activity 0.99) were used. For inoculation purposes, a spore suspension was prepared by washing the fungal slant with distilled water containing 0.02% Tween-80. The spore concentration of the suspension was assessed at 3.5 million spores/ml using a blood-cell count chamber (Schmeck, Hofheim/Ts, Germany) and 0.1 ml of this suspension was inoculated on the experimental substrates.

Experimental set-up

Two different experiments were carried out, one with mites and one with fungi. All acaricidal formulations were used according to the directions furnished by the manufacturer. They were applied on all four test surfaces. Artilin being used only on wood and gypsum (Table 2). In the case of Artilin, all surfaces of the wood and gypsum plates were painted 2 days prior to soiling the surface, whereas for the other acaricides only the upper 'soiled' surfaces of the materials were treated. Control vacuuming took place in the mite experiment, but not in the fungal experiment. The following three different blank series were decided on: (i) no mites or fungi, and without any

Table 2. Product description [source: company information, 2, 3, 25] and use in both mite and mould experiment

Name	Description	Experimental use
Acardust	Applipharm, Marseille, France; aerosol spray 500 ml; for mattress, upholstered furniture, carpet 5 g/m ² advised; acaricidal substances esbiol 0.4%, piperonyl butoxide 3%; 4–6 treatments/year advised	54 ± 10 mg/10 × 10 cm; one application only
Acarosan foam	Werner & Mertz GmbH, Mainz, Germany; dry foam; 275.8 g; for upholstered furniture, mattresses, pillows and heavy curtains (50–100 g/m ² advised); acaricidal substance benzyl benzoate 3%; two treatments/year advised with vacuuming 14 days after treatment	550 ± 10 mg/10 × 10 cm; one application only
Acarosan powder	Werner & Mertz GmbH, Mainz, Germany; three bags cleaning powder 750 g each; for carpet; 50–100 g/m ² advised; acaricidal substance benzyl benzoate 5%; 1 or 2 treatments per year advised with subsequent vacuuming	630 ± 10 mg/10 × 10 cm; one application only
Actelic 50	ICI Ltd, Plant Protection Division, Kent, United Kingdom; 5% pirimiphos methyl—solution used as spray; for carpet and upholstered furniture; 40 ml/m ² advised; 8–12 treatments/year advised	70 ± 10 mg/10 × 10 cm; one application only
Artilin 3A	Artilin, Colayrac-St-Cirq, France; can of 5 kg, four types of paints; for walls and ceilings; 80–170 g/m ² advised; acaricidal substance benzyl benzoate; fungicidal substance imidazole; frequency of treatment not stated; anti-mite and anti-fungal effect claimed	10 × 10 cm; one application 48 hr before dust soiling
Liquid nitrogen	British Oxygen Company Ltd, United Kingdom or Union Carbide Ltd (Europe and United States); for mattress and carpet; 5 l/single bed, 7 l/per double bed, 5–10 l/m ² carpet advised; one treatment/year or in case of reappearance of symptoms; only anti-mite effect claimed	250 ± 20 ml/10 × 10 cm; one application only
Paragerm AK	Promedica, Levallois-Perret, France; aerosol spray 325 ml; for mattress (both sides), pillows, bed covers, padded furniture and heavy curtains; 1 ml/m ³ advised; acaricidal substances benzoic acid, terpineol and salolthymol (in total 24%); fungicidal substance chlorophenol 0.2%; 2–3 treatments/year advised; anti-mite and anti-fungal effect, as well as bactericidal effect claimed	39 ± 9 mg/10 × 10 cm; 2 applications, 2-week intervals
Tymasil	Gist-Brocades B.V., Brussels, Belgium; spray 192 g; for mattress and pillows; 0.5 g/m ² advised; active substance 2.17% natamycin, 0.2% benzylalkonium (reducing mites through extermination of necessary fungi); treatment: 6 times at 2-week intervals, followed by 1 treatment/month indefinitely; all treatments to be followed by vacuuming; anti-mite effect through fungistatic properties	87 ± 11 mg/10 × 10 cm; 3 applications at 2-week intervals

treatment; (ii) no mites but with vacuuming (mite experiment only); (iii) no fungi but 0.1 ml distilled water (fungal experiment only); (iv) with mites or fungi, but without any treatment. Treatments and blanks were executed in duplicate, and both fungal and mite experiments were repeated after one week.

Each soiled substrate was placed on the bottom of a glass petri-dish (diameter 15.0 cm). To prevent the mites from escaping, the rims were covered with 'Glu Lhomme Lefort' (Gilot Sep, Sartrouville, France), and a woven fabric (poplin, 65% polyester, 35% cotton, batiste). The dishes were stacked per series of treatment in polyethy-

Table 3. Acaricidal efficacy and possible fungistatic effect on mattress surfaces. Means and ranges of mite numbers assessed by the heat escape method. Means and ranges of 1×1 cm areas colonized by fungi. Significance at 1% confidence level. Treatments arranged in order of acaricidal efficacy. Products belonging to same group (as denoted with same letter a or b) do not differ significantly among each other

Treatment	Mite growth		Fungal growth		Fungistatic effect
	Geometric mean	Range	Arithmetic mean	Range	
None	264 a	111-600	96	75-100	
Liquid nitrogen + vacuuming	0 b	0-0	37	23-46	+
Acarosan powder + vacuuming	1 b	0-2	93	89-99	-
Paragerm + vacuuming	47 a	3-268	74	40-86	-
Actelic 50 + vacuuming	53 a	31-78	94	81-100	-
Acardust + vacuuming	79 a	59-86	82	70-92	-
Acarosan foam + vacuuming	86 a	57-166	92	88-95	-
Tymasil + vacuuming	150 a	125-171	95	86-100	-
Vacuuming only	153 a	91-197	Not done		Not done

+, yes

-, no

lene bags (ARE, Polypax b.v., Hilversum, The Netherlands; $1.2 \text{ g/m}^2/24 \text{ hr}$ water vapour exchange at 20°C and 85% relative humidity). The relative humidity at room temperature (22°C) was kept at 75% by using 200 ml of aqueous saturated NH_4SO_4 solution in a 250 ml beaker. The soiled substrates were pre-incubated for 8 days prior to inoculation with 50 mites (mite experiment) or with 0.1 ml spore suspension (fungal experiment). Incubation continued for 48 hr after inoculation (mite experiment) or 7 weeks (fungal experiment), followed by experimental treatment, except for the Artilin paints which had been applied 48 hr before dust soiling started. Paragerm and Tymasil treatment was repeated according to the stipulated directions for use (Table 2). In the case of the mite experiment, vacuuming (Wolf Sapphire 8793, Wolf Electric Tools Ltd, London, England) was performed 4 weeks after primary treatment or non-treatment.

Eight weeks after primary treatment the trials were terminated. In the mite experiment, living mites on the plates were detected by a heat-escape method according to Bischoff and co-workers [16] with the following modifications: the substrates were placed on heating plates (Salvis HP, Salvis AG, Luzern, Switzerland; Kurz HP-3p, Kurz Instruments APS, Denmark) with a temperature of 70°C for 1 hr (mattress, carpet) or 1.5 hr (gypsum, wood), while covered with adhesive foil and a 2 mm thick plate of plywood for pressing. In the case of the fungal experiment a glass plate with a $1 \times 1 \text{ cm}$ matrix was arranged about 5 mm above the $10 \times 10 \text{ cm}$ substrates

for counting the number of $1 \times 1 \text{ cm}$ squares with and without fungal growth. The results were expressed in number of positive squares (maximal possible range 0-100 squares).

Statistics

The results have been presented as geometric (mites) or arithmetic (fungi) means and ranges of number of mites or number of squares with fungal growth. For tests of significance, mite counts were subjected to $\log_{10}(1+x)$ transformation because of their wide range, which appeared almost proportional to the mean and the presence of 0-values [17,18].

In the mite experiment the significance of the difference for the respective treatments was tested on the same substrate to evaluate comparative efficacy. In the fungal experiment, only the difference between treatment and no-treatment was taken into account as only the presence or absence of the claimed fungistatic effect was under study here. A *t*-test based on range for independent samples was used at a 1% confidence level [18].

Results

In all cases the blanks without mite inoculation (with or without vacuum-cleaning) remained free of mites. In the artificial house dust used, the inoculated fungus *A. repens* remained the prevailing fungus on the treated surfaces.

Table 4. Acaricidal efficacy and possible fungistatic effect on carpet surface. Means and ranges of mite numbers recovered using heat escape method and mould colonized 1 × 1 cm squares. Significance at 1% confidence level. Treatments arranged in order of acaricidal efficacy. Products belonging to same group (as denoted with same letter a or b) do not differ significantly

Treatment	Mite growth		Fungal growth		Fungistatic effect
	Geometric mean	Range	Arithmetic mean	Range	
None	456 a	297–778	93	81–100	
Acarosan powder + vacuuming	0 b	0–0	90	68–100	–
Liquid nitrogen + vacuuming	0 b	0–0	46	30–55	+
Acarosan foam + vacuuming	3 b	1–17	75	60–99	–
Acardust + vacuuming	4 b	0–64	68	79–92	+
Actelic 50 + vacuuming	17 b	2–63	98	47–72	+
Paragerm + vacuuming	7 a/b	0–132	63	47–72	+
Vacuuming only	105 a	29–330	Not done		Not done
Tymasil + vacuuming	241 a	51–836	100	98–100	–

+, yes

–, no

Table 5. Acaricidal efficacy and possible fungistatic effect on gypsum surface. Means and ranges of mite numbers recovered using heat escape method and mould colonized 1 × 1 cm squares. Significance at 1% confidence level. Treatments arranged in order of their presumed acaricidal efficacy which could not be tested statistically due to lack of development of mites on this surface

Treatment	Mite growth		Fungal growth		Fungistatic effect
	Geometric mean	Range	Arithmetic mean	Range	
None	7	0–137	93	75–100	
Acarosan powder + vacuuming	0	0–0	84	60–100	–
Acarosan foam + vacuuming	0	0–0	83	63–100	–
Artilin aqueous + vacuuming	0	0–0	1	0–1	+
Artilin spirit + vacuuming	0	0–0	2	0–4	+
Liquid nitrogen + vacuuming	0	0–0	22	6–28	+
Actelic 50 + vacuuming	0	0–0	98	93–100	–
Paragerm + vacuuming	2	0–6	68	48–83	–
Acardust + vacuuming	2	0–24	79	52–100	–
Vacuuming only	3	0–10	Not done		Not done
Tymasil + vacuuming	8	3–16	92	80–100	–

+, yes

–, no

Table 6. Acaricidal efficacy and possible fungistatic effect on wooden surface. Means and ranges of mite numbers recovered using heat escape method and mould colonized 1 × 1 cm squares. Significance at 1% confidence level. Treatments arranged in order of their acaricidal efficacy. Products belonging to same group (as denoted with same letter a, b or c) do not differ significantly

Treatment	Mite growth		Fungal growth		Fungistatic effect
	Geometric mean	Range	Arithmetic mean	Range	
None	412 a	217–852	94	72–100	
Acarosan powder + vacuuming	0 c	0–0	84	59–99	–
Artilin aqueous + vacuuming	0 c	0–0	12	0–28	+
Artilin spirit + vacuuming	0 c	0–0	3	0–8	+
Actelic 50 + vacuuming	0 c	0–0	99	95–100	–
Acardust + vacuuming	1 c	0–1	94	84–100	–
Liquid nitrogen + vacuuming	1 c	0–1	44	26–58	+
Acarosan foam + vacuuming	2 b	0–24	66	54–82	–
Paragerm + vacuuming	11 b	5–18	51	22–99	–
Tymasil + vacuuming	90 a	62–167	85	76–100	–
Vacuuming only	168 a	50–282	Not done		Not done

+, yes

–, no

In the mite experiment, vacuuming did not differ from the case of no-treatment on any of the surfaces investigated.

As the treatment efficacy appeared to be related to the substrate, subsequent acarological and fungal results have been presented by surface.

Mattress (Table 3)

Significant mite reductions were found in the case of liquid nitrogen and Acarosan powder treatment, with a complete to nearly complete extermination of the mites, fungistatic activity only being apparent in case of liquid nitrogen.

Carpet (Table 4)

All treatments except Tymasil and vacuuming alone, had a significant mite reducing effect. Significant fungistatic effects were shown in the case of liquid nitrogen (not claimed by the manufacturer), Acardust (not claimed) and Paragerm (as claimed), but not for Tymasil (although claimed by the manufacturer).

Gypsum board (Table 5)

This substrate did not appear to constitute a suitable niche for *D. pteronyssinus*. Mite numbers remained low

(geometric mean 7) even without any treatment, and no statistical differences were present for mites. The two Artilin treatments showed a fungistatic effect (as claimed), as did liquid nitrogen. The fungistatic claims of Paragerm or Tymasil could not be proven.

Wooden board (Table 6)

On the roughened wooden board, a number of treatments (Acarosan powder, Artilin aqueous or white spirit, Actelic 50, Acardust and liquid nitrogen) showed complete or virtually complete eradication of the mites. Acarosan foam and Paragerm reduced the mite level significantly better than vacuuming alone or no treatment. Some fungicidal activity appeared in case of the two Artilin formulations and liquid nitrogen.

Discussion

The adopted testing method can probably be considered as the most severe ever used for establishing house-dust mite reducing capacity *in vitro*. The strong soiling of the substrates provides mites as well as fungi with protection against adverse influences, such as acaricidal formulations.

All manufacturers of the commercial products tested, recommend intensive vacuum-cleaning before treatment

of objects. So as to get an undisturbed high population development of mites the soiled materials were not cleaned prior to the application of the products. All products, except Artilin, were therefore tested on the heavily soiled substrates. For practical reasons in case of Artilin treatment, all surfaces of the materials (wood and gypsum) had to be painted in advance. According to the directions for use, the Paragerm and Tymasil treatments were repeated after 2 weeks (Paragerm) or after 2 and 4 weeks (Tymasil). In the case of Paragerm mention might be made here of the room mist device (not tested here) as a very handy alternative.

The efficacy level accepted under our experimental conditions probably corresponds to the 'worst' home conditions, where, for optimal clinical results, treatments should keep mite numbers and mite allergen below hygienic limits at all times. In a number of homes, an effective treatment with Acarosan, Paragerm, Actelic 50 or Tymasil was reported earlier [13,19–21].

Mattresses, pillows and upholstered furniture are still the niches hardest to remove mites from. Initial dramatic decrease of mite load by mechanical or chemical means is commonly followed by rapid recolonization [22–25]. In this experiment, the good results achieved with Acarosan or liquid nitrogen indicate that both products are useful under dwelling conditions, though Acarosan is the safer (Table 3). In the case of Acarosan foam as applied on the mattress, our results were in contrast to those reported in a double-blind sanitation trial with atopic rhinitis patients [26]. Therefore two times and four times higher concentrations were additionally tested in a second experiment. A complete to nearly complete extermination of the mites was seen, indicating that the amount of Acarosan needed in practice should be increased. For safety reasons (e.g. hazard of skin burning during application) liquid nitrogen should only be used by a trained operator and should therefore, at present be regarded as impracticable for other than experimental applications [27]. Despite its good results, Acarosan powder is not applicable to mattress under dwelling conditions, since it does not adhere to all mattress surfaces (on top, under, on the sides).

Acarosan powder would appear to be the first choice for exterminating house dust mites from carpets (Table 4). It is highly effective and toxicologically speaking well tested and safe. Moreover, it cleans the carpet while removing mite allergen and it does not damage the usual types of carpets [25,28–31]. Its high effectiveness is based on the physical nature of the powder mixing readily with the house dust and on its not being repugnant to the mites. In fact, the high water content of the powder grains may even attract mites that subsequently devour the benzyl benzoate adsorbed by zeolite.

The mineral gypsum surface, used in homes especially for inner walls, was found not to support mite growth. One reason for this could be that it was still too dry after 1 week pre-incubation. A weighing experiment showed that after 7 days about 95% of the total increase in weight of the gypsum at room temperature and 75% relative humidity was present, indicating that indeed the niche might be somewhat dry. However, in case of the wooden substrate only 80% of the total increase in weight was observed after a same period and on this substrate the mites thrived. Investigations in dwellings showed that house dust mites were able to colonize mineral surfaces with and without visible mould growth [32].

Rough wooden surfaces proved to constitute a suitable niche for house dust mite growth. At the moment, this niche frequently fails to be mentioned in the allergen inventory of individual homes. The two Acarosan formulations could be useful for the treatment of wood though such use of the products is not advocated by the manufacturer, probably because of the (unclear) possibility of damage to paint or varnish.

As stated above, some of the products tested claimed a fungistatic effect. This claim could be substantiated in case of the Artilin paints and Paragerm (carpet only), but not for Tymasil. The fungistatic properties of Acardust as shown on carpet only, could be related to the use of an unknown preservative in the formulation.

Without any acaricidal product involved, vacuum-cleaning alone did not reduce mite numbers significantly, as demonstrated earlier [22,33–35].

The good results found with Acarosan treatments support earlier findings of a complete or nearly complete eradication of mites in carpet, upholstery and mattresses after application of the products [20,25,28–30]. A (nearly) complete reduction of mites also occurred after treatment with liquid nitrogen, which was also demonstrated in dwellings [3].

Differences between our test results and other published data for the Paragerm-, Acardust-, Pirimiphos-methyl- and Tymasil-formulations might partly be explained by the heavily soiling of the plates, the lack of vacuum-cleaning prior to application of the products or the short-term observation period adopted for the present experimental conditions. Paragerm treatment resulted in an 80–100% mite mortality rate on mattresses [7,19,36,37]. More than 80% mortality occurred after Acardust application [8] and the use of pirimiphos-methyl (Actelic 50) lead to a complete eradication of house dust mites [29]. In accordance with the findings of other authors [38,39] the 65–96% mite mortality rate for Tymasil reported earlier could not be confirmed [10,11,21]. Recently it was demonstrated that after treatment with twice the commercial concentration of Tymasil

a long-term control of house-dust mites could be guaranteed [40].

To enhance clinical relevance of the proposed studies on efficacy in dwellings these should be aimed at achieving allergen levels below the (provisional) sensitization limit of 2 µg *Der p1* allergen or 0.6 mg guanine per gram house dust [26,41].

In addition, chemical extermination products should be compared with physical or mechanical preventive and extermination measures, such as cleaning procedures other than vacuuming and diminishing the humidity level in the home. In this way the theoretical management concept proposed recently [13] would become applicable and could be made readily available to a wide circle of patients as well as to not yet sensitized atopics.

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