Chemical control testing on foodstuff mites

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Abstract

Astigmata mites are of great importance both economically and medically. They cause allergies and dermatitis, and damage foodstuffs in storage.

In conservation environments the most important species are *Acarus siro, Tyrophagus putrescentiae, Glycyphagus domesticus*. In houses, the most important species is *Dermatophagoides pteronyssinus*.

Chemical disinfestation is a problem in storage areas and for this reason the activity of new and traditional formulations has been evaluated. These have a low level of mammalian toxicity and are therefore indicated for use in public environments. Three pyrethroids, 16 essential oils and 9 new synthesis molecules were used.

The results indicated that the pyrethroids had a good overall level of efficacy, and have potential for use in controlling domestic mites. The essential oils and the new molecules showed little acaricide activity.

Introduction

The Astigmata mites are very important both medically and commercially. Many species are responsible for damage to stored foodstuffs and for allergy problems in people who handle the infested products (Candura et al. 1961; Cuthbert et al. 1979, 1984; Green and Woolcock 1978; Korsgaard et al. 1985; Revsbech and Anderson 1987). In storage areas the most widespread species are *Acarus siro* L. on flour and cereals, *Tyrophagus putrescentiae* (Shrank) on seasoned pork products and cheeses, and *Glycyphagus domesticus* (be Geer) on various vegetable and animal products (Cantoni et al. 1970; Rota 1972; Domenichini et al. 1972; Galli et al. 1977, 1980; Domenichini 1978; Lozzia and Ottoboni 1987; Lozzia and Rota 1992). The damage caused consists of the consumption of the substrates and of physical and chemical modifications (an increase in humidity and in the temperature of the substrates, spreading of mould and micro-organisms, pollution by means of deposits and despoiling, appearance of bad smells), in such a way to make the product useless as human and animal food (Braude et al. 1980; Curtis et al. 1981; Süss 1988).

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Pyroglyphidae (*Dermatophagoides pteronyssinus* (Trovessart), *D. farinae* (Hughes), *Euroglyphus maynei* (Fain) which are mainly responsible for allergies to house dust, are however more common in houses (Bronswijk and Sinha 1971). Chemical pesticides and hygienic measures are necessary in the control of the mites in storage areas and houses. A great deal of research has been carried out to identify the active acaricide principles to use both on foodstuffs and in houses (Wilkin and Hope 1973; Bowley and Bell 1981; Anderson and Wilkin 1982; Pagani 1987; Pagani and Ciampitti 1990, 1992; Koren 1993; Shono at al. 1993).

In this study we have evaluated the acaricide activity 'in vitro' of 3 formulations with a synthetic pyrethroid base, of 9 'ex novo' synthetic molecules and 16 essential oils, on the principal species of mites which infest foodstuffs and houses.

Material and Methods

Various species of mites were used during the tests: Pyroglyphidae, *Dermatophagoides farinae* (DF) and *D. pteronyssinus* (DP); Acaridae, *Acarus siro* (AS) and *Tyrophagus putrescentiae* (TP); Glycyphagidae, *Blomia kulagini* (BK), *Glycyphagus domesticus* (GD) and *Lepidoglyphus destructor* (Shrank) (LD); Labidophoridae *Gohieria fusca* (Oudemans) (GF); and Chortoglyphidae, *Chortoglyphus arcuatus* (Troupeau) (CA). The strains were bred in the laboratory on a substrate of mouse feed, yeast and wheat germ at 25°C and 75% r.h.

The efficacy of the new molecules and of the essential oils was evaluated on *D. pteronyssinus* and *G. domesticus*, while that of the pyrethroids was evaluated on all species.

The different active principles were tested using the 'Potter Tower' (Burkard, England) on 30 adult mites of the species being examined, taken from breeding flasks by means of a fine needle. The specimens were placed on a piece of adhesive tape of 1 cm^2 which was placed on a glass slide and then in a petri dish (diameter 15 cm). For each test 3 repeat tests were carried out at the same time, placing 3 slides on the same plate. The plate of the Potter Tower was kept at a distance of 2 cm from the lower end of the sprinkler tube during the direct sprinkling, and the air pressure was 34.5 kpa (5 lb/in^2). The same procedure was used for the control tests.

After the sprinkling, the capsules with the slides were placed in climatic cells and maintained at a temperature of 25°C and at 75% r.h.

The number of dead mites was evaluated by means of a microscope after 1, 4 and 24 hours from the sprinkling, delicately touching each mite with a small hair brush.

The formulations of synthetic pyrethroids studied were Pertrin E® (pure Permethrin 5%), Pertrin S® (permethrin 5% with pyrethrum extract 3%) and Cipertrin T® (cypermethrin 2.5% with tetrametrin 1%), (Copyr, Milan). The products were prepared following the manufacturers instructions in an aqueous solution of 2%: 1 mL of emulsion was used for the

tests with Pertrin E and Pertrin S, while for the treatment with Cipertrin T, 1.5 mL was used. The control was treated with water.

The nine new synthesis molecules (Fig. 1) were prepared by the Institute of Organic Chemistry of the Pharmacy Faculty of the University of Milan in the form of a pure, active principle, made up of a powder dissolved in acetone (1% weight). The tests were carried out with 1 mL of solution in acetone at different concentrations: 0.05%, 0.1%, 0.2%, 0.4% and 0.6%, while the control was treated with pure acetone.

The commercial essential oils of anise, black pepper, caraway, eucalyptus, eugenia, garlic, geranium, juniper berry, lavender, lemon, mint, nikkel, origanum, Peru balsam, rosemary and thyme were used at 1% in acetone. The control was treated with pure acetone.

The statistic analysis of the results were carried out with ANOVA (LSD test) on the mortality rate with the formula $\arcsin \sqrt{\text{Proportion of dead mites}}$ to evaluate the effect of the single factors (products, species, time) and of their interactions. The differences with $P \leq 0.05$ were considered significant.

Results

Pyrethroids

The results show a fairly good acaricide action of the pyrethroids towards the house and storage mites. The average mortality rates at 1, 4 and 24 hours from the beginning of the treatment are reported in Table 1 and those of the relative statistical analysis in Table 2.

Cipertrin T® was significantly more efficacious and had a greater speed of action on all the species (Table 3). Pertrin S® and Pertrin E®, on the other hand, did not differ in either of the two factors. The highest mortality rate was always reached after 24 hours from the beginning of the treatment. The sensitivity to the 3 pyrethroids varied considerably from family to family (Table 4) increasing from Pyroglyphidae to Acaridae, Chortoglyphidae, Labidophoridae and Glycyphagidae. There are also great differences in sensitivity even in the same family, i.e. between DF and DP. The most sensitive species to Cipertin T® were GF (72–93%) and BK (88–100%), and the most tolerant were TP (10–64%) and DF (5–22%).

The analysis of the mortality rate by means of the 3 way ANOVA testing to establish which of the factors involved were significant— species, time, products and their interactions— showed that the interactions product-species, product-time and species-time were all significant ($P \leq 0.05$).

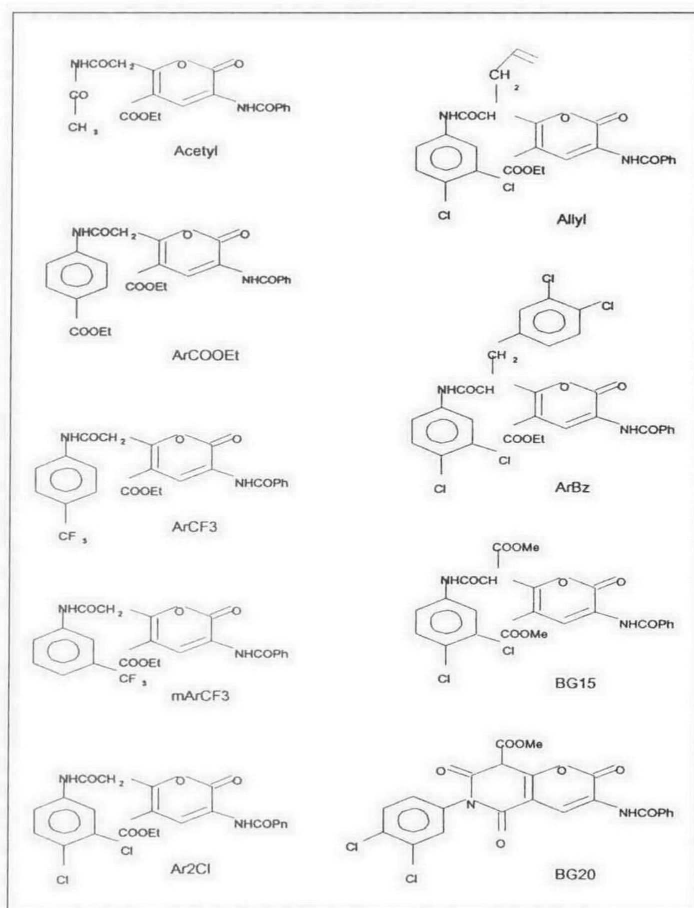

Fig. 1. New synthesis molecules.

New molecules

The new active ingredients were not very effective in the control of GD and DP, as shown by the average mortality rate regarding the different concentrations and in different times (Table 5). A fairly good difference was observed between the 2 species. In the case of DP, the mortality rate caused by the 9 molecules, at all concentrations tested, was little different from that observed in the controls. The most effective molecules proved to be mArCF3, Allyl and Acetyl (Table 7). GD was more sensitive and the higher mortality rate was obtained by the molecule marked as Ar CF3, followed by Ar2Cl, BG15 and BG20 (Table 8). In all situations the speed of action is clearly lower compared with that of pyrethroids.

Table1. Average mortality rate (percentage) of house mites and foodstuff mites at 1, 4 and 24 hours after the treatment with pyrethroids.

Species	Pertrin S			Pertrin E			Cipertrin T			Control		
	1	4	24	1	4	24	1	4	24	1	4	24
BK	97.7	100.0	100.0	64.2	83.9	88.4	97.7	100.0	100.0	3.4	3.4	23.3
GD	33.8	58.9	69.5	43.4	53.3	66.9	86.1	90.2	97.6	0.9	0.9	0.9
LD	10.0	16.1	68.0	13.3	54.5	71.8	75.8	91.9	96.2	1.0	1.0	2.7
CA	6.2	13.1	42.0	4.5	21.4	44.8	15.9	38.2	90.6	11.1	13.4	22.1
GF	53.4	68.4	93.5	21.7	38.9	72.3	58.5	65.9	91.9	4.4	7.9	16.9
AS	19.0	22.9	40.0	13.3	18.3	25.0	11.5	54.9	83.0	1.3	1.3	1.3
TP	4.4	5.1	13.7	0.7	4.0	9.9	5.2	6.0	64.1	1.1	1.1	2.3
DF	1.3	1.3	12.0	0.9	0.9	5.3	5.9	11.4	22.3	0.0	0.0	0.0
DP	6.4	17.0	40.0	5.3	16.0	38.9	38.3	43.0	44.0	2.2	2.2	4.5

Table 2. Three-way ANOVA relative to pyrethroids testing.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F	Sign. of F
Main effects	54.106	13	4.162	536.908	0.00 *
Species	5.479	2	2.740	353.417	0.00 *
Pyrethroids	23.937	3	7.979	1029.31	0.00 *
Time	24.690	8	3.086	398.130	0.00 *
2-way interactions	9.914	46	0.216	27.820	0.00 *
Species-pyrethroids	0.851	6	0.142	18.291	0.00 *
Species-Time	0.707	16	0.044	5.702	0.00 *
Pyrethroids-Time	8.356	24	0.348	44.913	0.00 *
3-way interactions	1.801	48	0.038	4.840	0.00 *
Explained	65.820	107	0.615	79.355	0.00 *
Residual	1.674	216	0.008		
Total	67.495	323	0.209		

*Significantly different (P 0.05).

Table 3. House mites and foodstuff mites: LSD tests relative to the efficacy of pyrethroids. Different letters indicate significant differences in mortality rate at 24 hours after the treatment ($P \leq 0.05$).

Pyrethroid	Mortality rate	Standard error (±)	Homogeneous subsets
Blomia kulagini			
Pertrin E	88.44	5.80	a
Pertrin S	100.00	0.00	b
Cipertrin T	100.00	0.00	b
Glycyphagus domesticus			
Pertrin E	66.93	15.14	a
Pertrin S	69.55	2.04	a
Cipertrin T	97.66	1.34	b
Lepidoglyphus destructor			
Pertrin E	71.80	3.51	a
Pertrin S	68.03	1.79	a
Cipertrin T	96.18	2.72	b
Chortoglyphus arcuatus			
Pertrin E	44.83	6.45	a
Pertrin S	41.98	3.49	a
Cipertrin T	90.58	1.27	b
Gohieria fusca			
Pertrin E	72.35	7.77	a
Pertrin S	93.55	0.67	b
Cipertrin T	91.81	1.29	b
Acarus siro			
Pertrin E	25.00	1.44	a
Pertrin S	40.00	5.77	a
Cipertrin T	82.97	5.40	b
Tyrophagus putrescentiae			
Pertrin E	9.86	0.93	a
Pertrin S	13.66	2.55	a
Cipertrin T	64.12	4.63	b
Dermatophagoides farinae			
Pertrin E	5.29	1.52	a
Pertrin S	12.04	3.40	a b
Cipertrin T	22.35	3.52	b
Dermatophagoides pteronyssinus			
Pertrin E	38.88	5.59	a
Pertrin S	40.01	0.36	a
Cipertrin T	45.25	6.47	a
All the species			
Pertrin E	47.04	5.78	a
Pertrin S	53.20	5.91	a
Cipertrin T	76.77	5.13	b

Table 4. House mites and foodstuff mites; LSD tests relative to the sensitivity of the species. Different letters indicate significant differences in mortality rate at 24 hours after the treatment ($P \leq 0.05$).

Species	Mortality rate	Standard error (±)	Homogeneous subsets
Pertrin E			
BK	88.44	3.35	e
GD	66.93	15.14	d
LD	71.80	3.51	d
CA	44.83	6.45	c
GF	72.35	7.77	d e
AS	25.00	1.44	b c
TP	9.86	0.93	a b
DF	5.29	1.52	a
DP	38.88	5.59	c
Pertrin S			
BK	100.00	0.00	e
GD	69.55	2.04	c
LD	68.03	1.79	c
CA	41.98	3.49	b
GF	93.55	0.67	d
AS	40.00	5.77	b
TP	13.66	2.55	a
DF	12.04	3.40	a
DP	40.01	0.36	b
Cipertrin T			
BK	100.00	0.00	f
GD	97.66	1.34	f
LD	96.18	2.72	e f
CA	90.58	1.27	d e
GF	91.81	1.29	d e
AS	82.97	5.40	d
TP	64.12	4.63	c
DF	22.35	3.52	a
DP	45.26	6.47	b

From three-way ANOVA testing the effects of the three principal factors and their interactions 2 by 2 result in being significant, except for the interaction molecule–time in group A (Table 6) and species–molecule in group B (Table 6), while the interaction of the three factors is not significant (Table 6).

Essential oils

Table 9 shows the mortality rate at 1, 4 and 24 hours after the treatment. All the essential oils tested were ineffective against DP (Table 11) and partially ineffective against GD (Table 12). In fact on the latter, eugenia and garlic caused mortality rates of about 41% and 36%, respectively, after 24 hours. The effect of the single factors and their interactions on the mortality rate is significant, with the exception of those of oils–time and of the 3 factors at the same time (Table 10).

Discussion and Conclusions

The pyrethroids seem to have a good acaricidal effect on almost all the species tested, together with a fairly rapid speed of action. Cipertrin T® in particular could be used against Glycyphagidae, Chortoglyphidae and Labidophoridae in the protection of foodstuffs, while it seems to be less useful in the disinfestation of dust-mites in houses.

As for the molecules of new synthesis, the partially negative results are inconclusive, having tested only pure active ingredients, not formulated with inert ingredients such as antioxidants etc. Further experiments regarding this are being carried out.

The essential oils do not seem to be possible for the control of *Dermatophagoides pteronyssinus* in houses because they could damage the furnishings and fabrics. Nevertheless, their use could be interesting in the protection of some foodstuffs where Glycyphagidae and Acaridae mites proliferate considering that the smell or taste of the product could be altered.

A further consideration regards the sensitivity of the different species of mites towards pyrethroids. It can be clearly seen that those which are strictly mycophagous (BK, GF, GD, AS), and need a high relative humidity, are more sensitive to the products used.

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Table 5. Average mortality rate of *G. domesticus* and *D. pteronyssinus* at 1, 4 and 24 hours after the treatment with new synthesis molecules.

New synthesis molecules %	*Glyciphagus domesticus*			*Dermatophagoides pteronyssinus*		
	1 hour	4 hours	24 hours	1 hour	4 hours	24 hours
Group A (molecules synthesised at 22 February 1993)						
ArCOOEt 0.05	1.15	3.37	5.52	0.98	1.99	5.12
ArCOOEt 0.1	3.26	4.34	5.45	0.00	0.93	4.53
ArCOOEt 0.2	0.00	1.04	1.04	2.02	4.17	5.18
ArCOOEt 0.4	0.93	1.85	1.85	0.93	0.93	0.93
ArCOOEt 0.6	0.00	1.85	2.71	1.08	2.27	2.27
Acetyl 0.05	1.11	2.09	6.12	1.01	1.01	4.32
Acetyl 0.1	1.01	2.09	7.30	1.19	2.14	3.33
Acetyl 0.2	0.00	0.00	7.89	2.26	2.26	3.37
Acetyl 0.4	1.75	3.77	4.65	3.90	3.90	8.01
Acetyl 0.6	0.00	0.90	4.55	2.06	2.06	6.07
ArCF3 0.05	2.80	2.80	11.27	3.30	4.41	6.59
ArCF3 0.1	2.02	3.57	16.52	1.08	1.08	5.06
ArCF3 0.2	0.98	0.98	8.91	0.00	1.04	1.04
ArCF3 0.4	8.01	21.15	42.19	0.00	2.02	4.14
ArCF3 0.6	4.94	22.60	46.25	1.01	1.01	4.24
Ar2Cl 0.05	3.06	5.12	15.22	2.19	2.19	2.19
Ar2Cl 0.1	2.98	6.87	9.02	0.00	0.00	3.19
Ar2Cl 0.2	7.24	12.84	15.66	0.00	0.00	1.08
Ar2Cl 0.4	5.94	10.21	21.08	0.00	2.15	3.03
Ar2Cl 0.6	3.51	6.85	14.28	2.73	2.73	3.61
Control	0.00	1.94	1.94	0.00	0.00	1.08
Group B (molecules synthesised at 14 September 1993)						
ArBz 0.05	0.88	1.92	9.05	0.00	2.01	4.08
ArBz 0.1	0.00	3.23	7.51	0.00	1.08	1.08
ArBz 0.2	3.41	4.60	15.64	0.00	1.67	1.67
ArBz 0.4	3.10	6.67	10.06	0.00	0.00	3.75
ArBz 0.6	1.11	4.00	5.93	1.11	3.33	4.41
mArCF3 0.05	1.01	3.06	10.23	0.85	2.73	3.56
mArCF3 0.1	0.90	1.85	14.39	2.51	2.51	5.40
mArCF3 0.2	1.83	2.71	16.40	1.11	2.30	11.19
mArCF3 0.4	2.44	7.33	15.56	2.17	2.17	5.70
mArCF3 0.6	2.89	7.04	16.32	3.23	4.38	16.81
Allyl 0.05	0.00	1.90	14.85	0.88	2.76	3.81
Allyl 0.1	0.83	5.99	22.45	2.02	2.02	11.54
Allyl 0.2	1.63	4.23	13.51	0.00	0.00	5.45
Allyl 0.4	0.00	1.11	14.26	0.00	3.89	12.68
Allyl 0.6	0.79	1.72	14.31	1.93	1.93	3.84
BG15 0.05	3.00	4.85	11.00	1.11	2.06	6.43
BG15 0.1	5.48	7.63	14.33	0.00	0.00	10.33
BG15 0.2	2.55	6.47	15.82	1.88	1.88	9.55
BG15 0.4	4.93	6.89	18.21	0.00	0.00	5.61
BG15 0.6	3.38	5.56	18.92	2.22	2.22	8.57
BG20 0.05	3.91	7.30	17.81	0.93	1.85	3.70
BG20 0.1	3.95	5.97	25.27	1.04	1.04	2.15
BG20 0.2	5.07	11.46	24.54	2.09	2.09	4.59
BG20 0.4	4.99	4.99	25.04	1.23	2.38	4.57
BG20 0.6	4.69	11.35	28.46	0.00	0.90	4.31
Control	4.31	4.31	7.57	0.79	0.79	0.79

Table 6. *G. domesticus* and *D. pteronyssinus*: three-way ANOVA relative to new synthesis molecules testing.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F	Sign. of F
Group A (molecules synthesised at 22 February 1993)					
Main effects	3.814	23	0.166	19.211	0.00 *
Species	1.481	2	0.741	85.798	0.00 *
Molecules	1.518	20	0.076	8.795	0.00 *
Time	0.814	1	0.814	94.343	0.00 *
2-way interactions	2.477	62	0.040	4.629	0.00 *
Species–molecules	0.395	40	0.010	1.143	0.26
Species–time	0.173	2	0.087	10.046	0.00 *
Molecules–time	1.909	20	0.095	11.058	0.00 *
3-way interactions	0.332	40	0.008	0.961	0.54
Explained	6.623	125	0.053	6.138	0.00 *
Residual	2.175	252	0.009		
Total	8.798	377	0.023		
Group B (molecules synthesised at 14 IX 1993)					
Main effects	6.428	28	0.230	30.090	0.00 *
Species	1.658	1	1.658	217.365	0.00 *
Molecules	3.965	2	1.982	259.852	0.00 *
Time	0.804	25	0.032	4.218	0.00 *
2-way interactions	1.443	77	0.019	2.456	0.00 *
Species–molecules	0.228	2	0.114	14.931	0.00 *
Species–time	0.783	25	0.031	4.104	0.00 *
Molecules–time	0.432	50	0.009	1.133	0.26
3-way interactions	0.358	50	0.007	0.938	0.59
Explained	8.228	155	0.053	6.958	0.00 *
Residual	2.380	312	0.008		
Total	10.609	467	0.023		

*Significantly different ($P \leq 0.05$).

Table 7. *D. pteronyssinus*: LSD tests relative to new synthesis molecules. Different letters indicate significant differences in mortality rate at 24 hours after the treatment ($P \leq 0.05$).

New synthesis molecule	Mortality rate	Standard error (±)	Homogeneous subsets
Group A (molecules synthesised at 22 February 1993)			
ArCOOEt 0.4%	0.93	0.93	a
ArCOOEt 0.6%	2.27	1.14	a b
Acetyl 0.4%	8.01	1.23	b
Acetyl 0.6%	6.07	1.71	b
ArCF3 0.4%	4.14	2.66	a b
ArCF3 0.6%	4.24	0.91	a b
Ar2Cl 0.4%	3.03	1.87	a b
Ar2Cl 0.6%	3.61	0.83	a b
Control	1.08	1.08	a
Group B (molecules synthesised at 14 September 1993)			
ArBz 0.4%	3.75	1.91	a b
ArBz 0.6%	4.41	2.95	a b
mArCF3 0.4%	5.70	2.51	b
mArCF3 0.6%	16.81	2.37	d
Allyl 0.4%	12.68	0.95	c d
Allyl 0.6%	3.84	0.94	b
BG15 0.4%	5.61	0.95	b c
BG15 0.6%	8.57	1.43	b c d
BG20 0.4%	4.57	1.43	b
BG20 0.6%	4.31	1.20	b
Control	0.79	0.79	a

Table 8. *G. domesticus*: LSD tests relative to new synthesis molecules. Different letters indicate significant differences in mortality rate at 24 hours after the treatment ($P \le 0.05$).

New synthesis molecule	Mortality rate	Standard error (±)	Homogeneous subsets
Group A (molecules synthesised at 22 February 1993)			
ArCOOEt 0.4%	1.85	1.85	a
ArCOOEt 0.6%	2.71	0.08	a
Acetyl 0.4%	4.65	1.04	a
Acetyl 0.6%	4.55	1.78	a
ArCF3 0.4%	42.19	7.81	c
ArCF3 0.6%	46.25	5.77	c
Ar2Cl 0.4%	21.08	2.49	b
Ar2Cl 0.6%	14.28	4.76	b
Control	1.94	0.98	a
Group B (molecules synthesised at 14 September 1993)			
ArBz 0.4%	10.05	4.25	a b
ArBz 0.6%	5.93	2.62	a
mArCF3 0.4%	15.56	2.94	b c d e
mArCF3 0.6%	16.32	6.39	b c d e
Allyl 0.4%	14.26	1.65	b c d
Allyl 0.6%	14.31	2.89	b c d
BG15 0.4%	18.21	0.97	c d e f
BG15 0.6%	18.92	3.51	d e f
BG20 0.4%	25.04	0.43	e f
BG20 0.6%	28.46	5.85	f
Control	7.57	0.91	a b

Table 9. Average mortality rate of *G. domesticus* and *D. pteronyssinus* at 1, 4 and 24 hours after the treatment with essential oils.

Essential oils	*Glycyphagus domesticus*			*Dermatophagoides pteronyssinus*		
	1 hour	4 hours	24 hours	1 hour	4 hours	24 hours
Anise	9.7	13.2	27.3	1.0	2.0	3.0
Black pepper	4.5	13.3	22.3	1.7	1.7	1.7
Caraway	6.4	14.8	21.3	2.4	3.3	4.9
Eucalyptus	5.3	17.7	29.5	0.0	0.9	1.8
Eugenia	8.3	12.9	41.2	0.0	0.9	0.9
Garlic	3.3	6.7	36.5	0.0	1.1	1.1
Geranium	3.8	6.4	16.7	0.0	0.0	0.8
Juniper berry	4.6	6.7	23.5	0.0	2.9	3.9
Lavender	2.7	10.9	25.0	0.9	1.9	3.0
Lemon	3.0	6.4	18.1	1.0	1.0	4.7
Mint	3.0	5.0	11.5	0.0	1.7	3.5
Nikkel	1.8	5.7	20.5	1.7	2.5	3.4
Origanum	5.1	5.1	15.8	1.7	2.6	4.5
Peru balsam	1.6	4.1	14.7	1.7	1.7	3.4
Rosemary	1.9	3.8	8.5	2.0	2.0	3.0
Thyme	3.0	4.0	14.5	0.0	0.9	2.1
Control	1.0	2.0	3.1	0.0	0.8	1.8

Table 10. *G. domesticus* and *D. pteronyssinus*: three-way ANOVA relative to essential oils testing.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F	Sign. of F
Main effects	5.758	19	0.303	37.232	0.00 *
Species	3.230	1	3.230	396.740	0.00 *
Oils	0.685	16	0.043	5.257	0.00 *
Time	1.844	2	0.922	113.279	0.00 *
2-way interactions	1.369	50	0.027	3.363	0.00 *
Species-oils	0.750	16	0.047	5.759	0.00 *
Species-time	0.459	2	0.229	28.173	0.00 *
Oils-time	0.160	32	0.005	0.614	0.95
3-way interactions	0.257	32	0.008	0.986	0.495
Explained	7.384	101	0.073	8.981	0.00 *
Residual	1.661	204	0.008		
Total	9.045	305	0.030		

*significantly different ($P \le 0.05$).

Table 11. *D. pteronyssinus*: LSD tests relative to essential oils. Different letters indicate significant differences in mortality rate at 24 hours after the treatment ($P \leq 0.05$).

Essential oil	Mortality rate	Standard error (±)	Homogeneous subsets
Anise	3.07	0.08	a b c d
Black pepper	1.73	0.87	a b c d
Caraway	4.89	0.14	d
Eucalyptus	1.85	1.85	a b c
Eugenia	0.88	0.88	a
Garlic	1.11	1.11	a b
Geranium	0.81	0.81	a
Juniper berry	3.87	0.84	b c d
Lavender	2.98	1.81	a b c d
Lemon	4.68	0.87	c d
Mint	3.49	0.86	a b c d
Nikkel	3.41	0.14	a b c d
Origanum	4.53	1.10	c d
Peru balsam	3.42	1.07	a b c d
Rosemary	3.00	1.75	a b c d
Thyme	2.14	1.11	a b c d
Control	1.82	0.93	a b c d

Table 12. *G. domesticus*: LSD tests relative to essential oils. Different letters indicate significant differences in mortality rate at 24 hours after the treatment ($P \leq 0.05$).

Essential oil	Mortality rate	Standard error (±)	Homogeneous subsets
Anise	27.26	5.39	e f g
Black pepper	22.27	2.07	d e f
Caraway	21.33	2.36	c d e f
Eucalyptus	29.49	3.77	f g h
Eugenia	41.20	2.04	h
Garlic	36.48	4.13	g h
Geranium	16.66	3.39	b c d e
Juniper berry	23.51	1.43	d e f g
Lavender	25.03	6.93	d e f g
Lemon	18.14	4.78	b c d e f
Mint	11.55	1.59	b c
Nikkel	20.56	0.85	c d e f
Origanum	15.77	3.21	b c d e
Peru balsam	14.67	2.54	b c d
Rosemary	8.55	2.67	b
Thyme	14.56	6.83	b c d
Control	3.13	1.86	a

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