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REVIEW

The efficacy of neem seed extracts (Tre-san®, MiteStop®) on a broad spectrum of pests and parasites

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Abstract The paper summarizes the acaricidal and insecticidal effects of a patented neem seed extract when diluted 1:10 with shampoo or 1:20, 1:30, 1:33, 1:40, respectively, 1:66 with tap water. It was shown that a broad range of pests and parasites, such as house dust mites, poultry mites, harvest mites, *Ixodes* and *Rhipicephalus* ticks, cat fleas (adults, larvae), bed bugs (all stages), head lice and mallophaga, cockroaches (genera *Blatta*, *Blattella*, *Gomphadorhina*), raptor bugs (*Triatoma*), and even food-attacking beetle (*Tenebrio molitor*) might be controlled with this extract, which is available as Tre-san® (against house dust mites) and MiteStop® (against mites, ticks, insects of any kind) to become water diluted or as Wash Away Louse® or Picksan LouseStop® being diluted in a shampoo. Tests on skin compatibility proved that there are no skin irritations during or after use. However, some target species are less sensible (beetles, *Triatoma* stages, fly maggots), while the specimens of the other species cited above were successfully killed even at low concentrations of the extract.

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Introduction

Pests and parasites are attracted by human households and occur in stables of farm animals, since there they easily find food and a broad range of places for shelter, where they may live and reproduce. Of course, many insecticides and acaricides have been developed that are highly active. However, many of them can only be used with several precautions, since they may harm health of humans and animals or may produce residuals inside the body of farm animals, respectively, inside eggs (Mehlhorn 2008). Therefore, in recent times, many plant extracts have been tested, whether they work against endo- or ectoparasites and/or against pests, which may contaminate food and/or rooms with agents of diseases (Schmutterer 2002; Athanasiadou et al. 2007; Bäumler 2007; Brown 1996; Fajimi and Taiwo 2005; Leung 1985; Mehlhorn et al. 2005, Mehlhorn 2008, 2010; Mehlhorn et al. 2005, 2006, 2010; Michaelakis et al. 2009; Oladimeji et al. 2000; Amer and Mehlhorn 2006; Abdel-Ghaffar and Semmler 2007; Abdel-Ghaffar et al. 2008a, b, 2009; Heukelbach et al. 2006; Kim et al. 2007).

Apart from several failures depending on the mode of extraction, several plant extracts turned out to be highly effective against important pests and parasites living in the surroundings of humans and their animals. Especially the so-called neem tree *Azadirachta indica* (syn. *Melia azadirachta*, *Antelaea azadirachta*) offers various possibilities of protection against the attacks of ectoparasites and pests besides many described applications against internal dis-

eases of humans and animals (Schmutterer 2002; Lundh et al. 2005; Mulla and Su 1999; Semmler et al. 2009). The present review summarizes own experiments with a patented extract of neem seeds that is used after dilution with tap water or shampoo against Acari (mites, ticks) and insects (fleas, flies, lice, mallophages, bugs, cockroaches, beetles, etc.). Four products (Tre-san®, MiteStop®, Wash Away Louse®, Picksan LouseStop®) had been launched being based on data obtained in the experiments summarized in the present paper.

Material and methods

Parasites and pests

1.1. *Dermatophagoides pteronyssinus*: These house dust mites were cultured in the institute since more than 18 years.
1.2. *Dermanyssus gallinae*: This blood-sucking so-called red poultry mite was cultured in the institute. Examples were also treated in French, German, and Egyptian farms (Mul et al. 2009).
1.3. *Rhipicephalus sanguineus*: These brown ticks of South Europe, which are often imported into human dwellings, were reared in the institute and obtained from Prof. Dr. E. Schein (Berlin).
1.4. *Ixodes ricinus*: This very common tick, which can be kept only with difficulties inside houses, although it often drops down from dogs or cats, was always freshly caught in nature before the experiments.
1.5. *Neotrombicula autumnalis*: This so-called grass or summer itch mite was treated when the larvae crawled out of the soil in the early summer until the end of autumn in German private houses or in public parks leading to severe itching.
1.6. *Ctenocephalides felis*: Adults and larvae of this so-called cat flea were obtained from own in vitro-rearing facilities in the institute (using a so-called artificial dog system).
1.7. *Cimex lectularius*: The bed bugs (larvae and adults) were obtained for different purposes from the Institute of Parasitology of the Free University of Berlin (Prof. Dr. E. Schein).
1.8. *Triatoma infestans*: These so-called rapture bugs originated from the Department of Entomology of the University Los Andes at Bogota (Colombia) and were reared since more than 30 years in the institute.
1.9. Cockroaches (*Gomphadorhina* sp., *Blatta orientalis*, *Blattella germanica*): The large Madagascar cockroaches and the two smaller species were obtained from Bayer AG (Leverkusen, Germany) and were reared for 15 years in the institute.
1.10. *Pediculus humanus capitis*: These blood-sucking head lice were obtained by combing and/or washing the heads of children in Cairo, Egypt.
1.11. *Werneckiella equi equi*: These mallophages (=biting lice) were treated after they had been diagnosed on more than 100 horses in Western Germany.
1.12. *Tenebrio molitor*: These so-called flour beetles were reared in the institute to be used as intermediate hosts for the tapeworm *Hymenolepis microstoma*.
1.13. *Calliphora erythrocephala* maggots: These flies were kept in permanent culture in the institute.

Material

The neem seed extracts were produced using nature-safe ester solutions. They were diluted 1:10 with shampoos to be included in anti-lice products (Wash Away Louse®, Picksan LouseStop®) or were used as dilutions with tap water in the following series:

- 1:20 dilutions to be distributed on horses to kill mallophages (MiteStop®)
- 1:33 dilutions to kill mites, ticks, and insects (MiteStop®)
- 1:40 dilutions to kill mites and ticks insects (MiteStop®)
- 1:40 or 1:66 dilutions to kill dust mites (Tre-san®)

These products were delivered by Fa. Alpha-Biocare GmbH (Düsseldorf, Germany).

Test designs

1. Head lice: The specimens were obtained by combing children in Egypt. The motile lice were incubated in vitro for 3 and 10 min into the Wash Away Louse® or Picksan® product, then washed intensively three times with tap water, placed onto white filter paper and controlled for signs of life by help of a stereo microscope at intervals of 1 h for 24 h. Furthermore, 20 children were treated with Wash Away Louse® for 10–20 min. After treatment their hair were just washed with tap water, and the death of the lice was controlled after combing.
2. Mallophaga: About 100 horses were washed using a 1:20 water diluted solution which was brought onto the hair by help of a brush. For treating one horse, a 100 ml bottle of the extract (MiteStop®) had been diluted in 2 l of tap water. The product was left on the hair, and after drying, the dead mallophages were brushed away from the tips of hair, where they had climbed up from their normal place close at the skin.
3. Bugs, fleas, cockroaches, beetles, ticks, and mites: All stages (larvae and adults) were treated in two ways: (a)

Table 1 Test of the acaricidal activity of MiteStop® against *Ixodes ricinus* ticks

Ixodes ricinus					
Experimental design	1 h	2 h	3 h	4 h	5 h
MiteStop® 1:40 sprayed directly on ticks	4 motionless, stiff legs	4 motionless, stiff legs	2/4 supine; 2/4 motionless, stiff legs	3/4 supine, slight movements; 1/4 dead	4/4 dead
MiteStop® 1:40 ticks on treated filter paper	4 motionless, stiff legs	4 motionless, stiff legs	4 motionless, stiff legs	4 motionless, stiff legs	2/4 dead; 2/4 motionless, after 6 h all dead

They were placed onto dry white filter paper and sprayed with the 1:33 or 1:40 water dilutions of MiteStop® until they were wet (5× spraying from a distance of about 15–20 cm). Then their fate was followed for 24–48 h depending on the species. (b) The specimens were placed onto wet filter paper containing the product MiteStop® at dilutions of 1:33, 1:40, or 1:66 and controlled for 24–48 h.

4. Red poultry mites: Floors and/or resting places of chickens in stables in Germany and Egypt were sprayed with a 1:33 or 1:40 dilution of MiteStop® two, three, or four times at intervals of 7 days (depending on the amount of the blood-sucking poultry mites and/or on the fact, whether some hidden places of the mites could be reached during the spraying or not). Furthermore, in vitro tests were done either by direct spraying onto the mites or by placing them onto filter paper containing the product.
5. House dust mites: The mites were placed onto filter papers in plastic Petri dishes and sprayed with Tre-san® containing the neem seed extract (test A) in a concentration of 1:40 or 1:66. In a second approach (test B), the mites were placed onto wet filter paper containing the Tre-san®–water solution. In both experiments, the fate of the mites was followed for the next hours by help of a stereo light microscope, and the results were documented in protocols.
6. Harvest mites: These *N. autumnalis* mites were treated in the evenings of summer days at intervals of 3–4 days by application of the 1:60 diluted extract onto the soil or grass. About 2–3 h later, the treated soil/grass was sprayed with fresh water.

Results

The results of the several times repeated experiments were documented as examples in the Tables 1, 2, 3, 4, 5, 6, 7, and 8. It can be seen that the 1:40 dilution kills ticks of the genera *Ixodes* and *Rhipicephalus* within 5 h when sprayed on the surface or when the ticks get in contact just with their feet to the compound (Tables 1 and 2). Even a dilution of 1:66 kills *Ixodes* ticks; while using this dose, *Rhipicephalus* die only after direct spraying onto their backside (Table 3).

House dust mites (*D. pteronyssinus*) are killed within 1 h when coming into contact either directly or indirectly (on filter paper) with the 1:40 water diluted product (Table 3). Bed bugs (*C. lectularius*) are killed when getting into contact with the 1:40 dilution, while the 1:66 dilution leaves some survivors (Tables 3 and 5). Fly maggots (genus *Calliphora*) are more resistant, since even contacts with a dilution of 1:20 leave some survivors (Tables 3 and 4).

Cockroaches turned out to be sensitive to the extract. However, the killing rate varied according to the species (Tables 3 and 6). *B. germanica* (a small-sized species) finally died when coming into contact with the 1:40 dilution, which worked not very well with the larger species (*Gomphadorhina* sp. or *B. orientalis*), which needed a treatment using the 1:20 dilution (Tables 3 and 6).

Cat fleas (*C. felis*) died as larvae even when having contact to the 1:33 dilution already within 1 h, while in adult fleas, it took longer. Some of them died only after 24 h (Table 7), but remained at limited motility until then.

Table 2 Test of the acaricidal activity of MiteStop® against *Rhipicephalus sanguineus* ticks

Rhipicephalus sanguineus					
Experimental design	1 h	2 h	3 h	4 h	5 h
MiteStop® 1:40 sprayed directly on ticks	4 walking around	4 motionless, stiff legs	3/4 supine, slight movements; 1/4 dead	3/4 dead; 1/4 supine, slight movements	4/4 dead
MiteStop® 1:40 ticks on treated filter paper	4 walking around	3/4 motionless, stiff legs; 1/4 supine	2/4 motionless, stiff legs; 2/4 supine, stiff legs	3/4 motionless, stiff legs; 1/4 supine	4/4 dead

Table 3 Trials on the efficacy of MiteStop®, respectively, Tre-san® (Fa. Alpha-Biocare) against, two species of ticks (*Ixodes ricinus* and *Rhipicephalus sanguineus*), house dust mites (*Dermatophagoides pteronyssinus*), Tre-san®, bed bugs (*Cimex lectularius*), cockroaches (*Gomphadorhina portentosa*) subadults, maggots of *Calliphora* fly larvae; two concentrations: water diluted 1:40, 1:66; experimental design A, sprayed directly on targets and experimental design B, mites or insects were placed on sprayed filter paper in plastic Petri dishes

Species/number of objects	Dilution	Experimental design	Observations after... hours							
			1 h	2 h	3 h	4 h	5 h	6 h	24 h	48 h
Ixodes (3)	1:66	A	3/3 motionless, legs are sensitive to contact	3/3 motionless, legs are sensitive to contact	3/3 motionless, legs are sensitive to contact	3/3 motionless, legs are sensitive to contact	All dead			
Ixodes (4)	1:66	B	4/4 motionless, legs are sensitive to contact	4/4 motionless, legs are sensitive to contact	4/4 motionless, legs are sensitive to contact	4/4 motionless, legs are sensitive to contact	1/4 dead; 3/4 motionless, legs are sensitive to contact	3/4 dead; 1/4 motionless, legs are sensitive to contact	All dead	
Rhipicephalus (4)	1:66	A	4/4 alive, weak movements	4/4 alive, weak movements	4/4 alive, weak movements	4/4 alive, weak movements	4/4 alive, weak movements	4/4 alive, weak movements	All dead	
Rhipicephalus (3)	1:66	B	3/3 alive, weak movements	3/3 alive, weak movements	3/3 alive, weak movements	3/3 alive, weak movements	3/3 alive, weak movements	3/3 alive, weak movements	3/3 alive, weak movements	2/3 alive, 1/3 dead
Dermatophagoides (ca. 300)	1:40	A	All dead							
Dermatophagoides (ca. 300)	1:40	B	All dead							
Dermatophagoides (ca. 300)	1:66	A	All dead							
Dermatophagoides (ca. 300)	1:66	B	All dead							
Cimex (20)	1:40	A	20/20 alive	20/20 alive	20/20 alive	7/20 dead, 13/20 alive	11/20 dead, 9/20 alive	13/20 dead, 7/20 alive	All dead	
Cimex (5)	1:40	B	5/5 alive	5/5 alive	5/5 alive	1/5 dead, 4/5 alive	2/5 dead, 3/5 alive	2/5 dead, 3/5 alive	All dead	
Cimex (4)	1:66	A	4/4 alive	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	3/4 dead, 1/4 alive	
Cimex (7)	1:66	B	7/7 alive	7/7 alive	7/7 alive	7/7 alive	1/7 dead, 6/7 alive	2/7 dead, 5/7 alive	3/7 dead, 4/7 alive	
Gomphadorhina (2)	1:40	A	1/2 alive, 1/2 dead	1/2 alive, 1/2 dead	1/2 alive, 1/2 dead	1/2 alive, 1/2 dead	1/2 alive, 1/2 dead	1/2 alive, 1/2 dead	1/2 alive, 1/2 dead	All dead
Gomphadorhina (1)	1:40	B	1/1 alive	1/1 alive	1/1 alive	1/1 alive	1/1 alive	1/1 alive	dead	
Gomphadorhina (1)	1:66	A	1/1 alive	1/1 alive	1/1 alive	1/1 alive	1/1 alive	1/1 alive	1/1 alive	1/1 alive
Gomphadorhina (1)	1:66	B	1/1 alive	1/1 alive	1/1 alive	dead				

			1 h	2 h	3 h	4 h	5 h	6 h	24 h	48 h
Maggots of flies (10)	1:40	A	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	1/10 dead, 9/10 alive	2/10 dead, 8/10 alive	2/10 dead, 8/10 alive
Maggots of flies (10)	1:40	B	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive, 3 of them pupated
Maggots of flies (10)	1:66	A	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive, 4 of them pupated
Maggots of flies (10)	1:66	B	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive, 3 of them pupated

The raptor bugs (*T. infestans*) were very resistant even against the 1:20 dilution (Table 8), since several specimens survived even after 24 h. The knock-out ratio after 24 h was rather similar after contacts to 1:20, 1:33, or 1:40 dilutions (Table 8).

The beetle (*T. molitor*), a common food pest, turned out to be rather resistant to the neem extract. Even in dilutions of 1:20, a larger number of survivors occurred in the same range as after contacts to dilutions of 1:33 or 1:40.

The common red poultry mite (*D. gallinae*) was also tested intensively (Abdel-Ghaffar et al. 2008a, 2010; Locher et al. 2010). It was found in any case that the 1:40 dilution of the extract MiteStop® is able to kill the developmental stages of this species within 1–2 h as well in vitro as in vivo. Furthermore, it was shown that the two times spraying at an interval of 7 days with a 1:33 dilution inside chicken stables in France and Germany eliminated the mites or reduced their quantity enormously.

A 1:10 dilution of the neem extract within fine shampoos was able to kill head lice (*P. humanus capitis*) within 3 min (in vivo) or within 10–15 min within wet hair of children (Abdel-Ghaffar and Semmler 2007; Heukelbach et al. 2006; Abdel-Ghaffar et al. 2010).

Horses (100) being heavily infested with Mallophaga (biting lice) of the rather common species *W. equi equi* (Mehlhorn 2010) were treated with a 1:20 dilution. Starting 1–2 h after the product had been brought onto the hair by help of a brush, hundred thousands of dead mallophages became visible at the tips of the hair. The complete surface of the hair seemed to be covered with “fine wool” representing a layer of dead mallophages. The horse owners also reported that blood-sucking and/or other molesting insects were repelled for at least 4 h, when treated horses were grazing on the meadow.

After spraying of the 1:66 diluted extract three times onto a mite-containing soil or grass at intervals of 3–4 days, the numbers of mites were drastically reduced. A repetition of this treatment after 4 weeks made the mites disappear in the next year or afforded then only a single treatment series at the beginning of the season. This repetition was needed due to the fact that mites do not leave daily the soil and thus some might not get into contact with the extract.

Discussion

Thousands of older and recent publications show that plants contain thousands of components that have either an insecticidal/acaricidal activity or possess a potential property of repellency. These compounds had been developed by the plants during their evolution in order to protect them against feeding attacks of various organisms. Some of those components have a very strong activity (e.g., pyrethrum)

Table 4 Trials on the efficacy of MiteStop® (Fa. Alpha-Biocare) against maggots of flies (*Calliphora erythrocephala*) after spraying four times, dilution 1:40 and 1:20 on filter paper in plastic Petri dishes

Number of maggots	Dilution	Observations after... hours				
		1h	2h	6h	24h	48h
10	1:40	10/10 alive	10/10 alive	10/10 alive	10/10 alive, 1 of them pupated	2/10 dead, 8/10 alive, 1 of them pupated
20	1:20	20/20 alive	20/20 alive	20/20 alive	20/20 alive	20/20 alive

and consequently were already used by humans as insecticides against a broad spectrum of pests attacking plants, animals, and/or humans. Many other compounds are present in plants only in small amounts, so that they have to become enriched via extraction methods before use. However, many of the acaricidal and insecticidal compounds cannot be used since they induce severe side effects or are harmful to skin. Thus, it must be considered that natural compounds may also be dangerous for humans or may remain ineffective, in case the active compounds are only present in low concentrations in the plants. Therefore, only intense tests may show the capacity of different plant extracts. In addition, it must be considered that the potential target organisms have an often strongly varying sensitivity against plant extracts.

Such a different sensitivity was shown in the present paper reviewing the activity of an extract of seeds of the neem tree. While *Calliphora* fly maggots, stages of the rapture bug *Triatoma*, and adult *Tenebrio* beetles showed practically no sensitivity against the extract, on the other hand ticks, mites, cockroaches, lice, mallophages, bed bugs, and fleas were killed by the extract (Tables 1, 2, 3, 4, 5, 6, 7, and 8). The efficacy, however, varied depending on the target species or on the concentration of the extract. Therefore in order to develop a useful product in any case intensive tests have to be done to evaluate the range of efficacy against each target species. Further tests are needed to find out the lowest effective dose and the highest security level for humans, animals, and environment before the product is launched. The present review showed the high efficacy of the products MiteStop®, Tre-san®, Wash Away Louse®, and Picksan LouseStop®.

For lice, it was clearly shown that the activity of the neem extract is based on the fact that the shampoo with the extract covers the terminal ends of the tracheoles, thus blocking mechanically the oxygen transfer through the fine water layer into the cell. This blocking has the effect that the cells of the body muscles, heart, etc. are disrupted from oxygen uptake. Thus, a rather quick knock-out effect occurs within short periods. Apparently, the different needs of oxygen, the size of the internal systems of tracheoles, the size and structure of the openings of the tracheoles, the

Table 5 Test on the insecticidal activity of MiteStop® against adult bed bugs (*Cimex lectularius*); experiment A, five bed bugs were placed onto filter paper which had been sprayed with MiteStop® (1:40 diluted) twice from a distance of 20 cm and experiment B, bed bugs had been sprayed once directly (from a distance of 20 cm, dilution 1:40) with MiteStop®

Experimental design	0 h	1 h	2 h	3 h	4 h	5 h	6 h	8 h
A	Moving with stiff legs, trying to stay away from the filter paper with their ventral side	Motionless, legs are sensitive to contact	Motionless, legs are sensitive to contact	Motionless, legs are sensitive to contact	2/5 supine, no reaction; 3/5 weak reflexes of legs	3/5 supine, no reaction; 2/5 weak reflexes of legs	5/5 supine, no reaction; 1 weak reflexes of legs	All dead
B	Moving intensely	Motionless, legs are sensitive to contact	Motionless, legs are sensitive to contact	1/4 supine, dead; 2/4 motionless; 1/4 weak reflexes	3/4 supine, dead; 1/4 weak reflexes	3/4 supine, dead; 1/4 weak reflexes	All dead	All dead

Table 6 Trials on the efficacy of MiteStop® (Fa. Alpha-Biocare) against juvenile and adult stages of cockroaches (*Blattella germanica* and *Blatta orientalis*), dilution of MiteStop® 1:20, 1:33, and 1:40, being sprayed four times from a distance of 20 cm; the test objects were sprayed directly from above, on filter paper in plastic Petri dishes

Species (number of test objects)	Dilution	Observations after... hours										
		1h	2h	3h	4h	5h	6h	7h	8h	18h	24h	48h
B. germanica juv. (4)	1:33	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	2/4 dead, 2/4 alive	3/4 dead, 1/4 alive	All dead			
B. germanica juv. (4)	1:40	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	2/4 dead, 2/4 alive	3/4 dead, 1/4 alive	All dead		
B. germanica adult (7)	1:33	7/7 alive	7/7 alive	7/7 alive	7/7 alive	7/7 alive	7/7 alive	1/7 dead, 6/7 alive	All dead			
B. germanica adult (6)	1:40	6/6 alive	6/6 alive	6/6 alive	6/6 alive	6/6 alive	6/6 alive	2/6 dead, 4/6 alive	3/6 dead, 3/6 alive	All dead		
B. orientalis juv. (8)	1:20	8/8 alive	8/8 alive	8/8 alive	8/8 alive	5/8 dead, 3/8 alive	All dead					
B. orientalis juv. (4)	1:20	4/4 alive	4/4 alive	4/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	3/4 dead, 1/4 alive	All dead	
B. orientalis juv. (4)	1:33	4/4 alive	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	3/4 dead, 1/4 alive
B. orientalis juv. (4)	1:40	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive
Species (number of test objects)	dilution	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	18 h	24 h	48 h
B. orientalis adult (6)	1:20	3/6 dead, 3/6 alive	All dead									
B. orientalis adult (4)	1:20	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	1/4 dead, 3/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive	3/4 dead, 1/4 alive	All dead		
B. orientalis adult (4)	1:33	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	1/4 dead, 3/4 alive	2/4 dead, 2/4 alive
B. orientalis adult (4)	1:40	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	4/4 alive	2/4 dead, 2/4 alive	2/4 dead, 2/4 alive

Table 7 Trials on the efficacy of MiteStop® (Fa. Alpha-Biocare) against larvae and adult stages of cat fleas (*Ctenocephalides felis*); dilution of MiteStop®, 1:33 and 1:40, being sprayed four times from a distance of 20 cm; the test objects were sprayed directly from above

Species (number of test objects)	Dilution	Observations after... hours							
		1h	2h	3h	4h	5h	7h	8h	24h
Ctenocephalides larvae (10)	1:33	All dead							
Ctenocephalides larvae (10)	1:40	All dead							
Ctenocephalides adult (30)	1:33	30/30 alive	22/30 dead, 8/30 alive	All dead					
Ctenocephalides adult (30)	1:40	30/30 alive	30/30 alive	11/30 dead, 19/30 alive	18/30 dead, 12/30 alive	21/30 dead, 9/30 alive	26/30 dead, 4/30 alive	28/30 dead, 2/30 alive	All dead

thickness of the body cuticula, and the size of the internal cuticle layers induce the variations in the speed of the knock-down effects seen in the experiments with different pests presented here.

Conclusions

Again, it has been proven that natural extracts may have high insecticidal and acaricidal activities. These effects depend, however, on the dosage/concentration used and on the sensitivity of the target organisms. Since it is known that plant extracts may lose their activity when having a too long contact with water, the products used in the present study were stored in a complete water-free version or were used in a composition with different fine shampoos. In the case of the fresh preparation of the solution prior to use an extreme high efficacy was reached against a broad spectrum of very important parasites and pests of humans and animals. Since tests of skin irritations remained negative (Pittermann et al. 2008) and such neem extracts may even be used as food, the described products offer a considerable support to human and animal health.

Table 8 Trials on the efficacy of MiteStop® (Fa. Alpha-Biocare) against two insect species (*Triatoma infestans* and *Tenebrio molitor*); dilution of MiteStop®, 1:33 and 1:40, being sprayed four times from a distance of 20 cm; the test objects were sprayed directly from above, on filter paper in plastic Petri dishes

Species (number of test objects)	Dilution	Observations after... hours							
		1h	2h	3h	4h	5h	6h	18h	24h
Triatoma subadult (6)	1:20	6/6 alive	6/6 alive	6/6 alive	6/6 alive	6/6 alive	1/6 dead, 5/6 alive	All dead	
Triatoma subadult (10)	1:33	10/10 alive	2/10 dead, 8/10 alive	4/10 dead, 6/10 alive	4/10 dead, 6/10 alive	6/10 dead, 4/10 alive	6/10 dead, 4/10 alive	6/10 dead, 4/10 alive	8/10 dead, 2/10 alive
Triatoma subadult (10)	1:40	10/10 alive	10/10 alive	10/10 alive	1/10 dead, 9/10 alive	2/10 dead, 8/10 alive	2/10 dead, 8/10 alive	2/10 dead, 8/10 alive	3/10 dead, 7/10 alive
Tenebrio adult (10)	1:20	10/10 alive	10/10 alive	6/10 dead, 4/10 alive	8/10 dead, 2/10 alive	All dead			
Tenebrio adult (10)	1:33	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	3/10 dead, 7/10 alive	3/10 dead, 7/10 alive
Tenebrio adult (10)	1:40	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	10/10 alive	1/10 dead, 9/10 alive	4/10 dead, 6/10 alive

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