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Original article

The development of HopGuard® as a winter treatment against *Varroa destructor* in colonies of *Apis mellifera*

Eva Rademacher, Marika Harz, Saskia Schneider

Institute of Biology/Neurobiology, Freie Universität Berlin, Königin-Luise-Str. 28-30, 14195, Berlin, Germany

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Abstract – The development of new veterinary medicinal products to control *Varroa destructor* is very important for the successful management of honey bee colonies. We tested natural plant compounds (hop acids) as active ingredients for a new veterinary drug for Varroa control. Tolerability of bees and efficacy against mites was tested in the laboratory and in the field. In laboratory tests, hop beta acids were proven to be safe for bees (5 % mortality) and effective against mites (93.3 % mortality); there is a six-fold safety margin for bees after individual dermal application compared to the lowest dosage highly toxic to mites. In a clinical study, these results were confirmed: bee colonies tolerated the treatment (<10 % mortality), while most of the mites were killed (efficacy up to 88 %). The treatment did not affect the adult bee population and winter survival of colonies.

veterinary drug / HopGuard® / clinical study / *Varroa destructor* / *Apis mellifera*

1. INTRODUCTION

The development of new veterinary products to control *Varroa destructor* (Anderson and Trueman) is very important for the management and future survival of honey bee (*Apis mellifera* L.) colonies. Over many years, the resistance of mites to synthetic acaricides has grown in European countries as well as the USA (Elzen et al. 1999; Lodesani et al. 1995; Milani 1999; Sammataro et al. 2005), probably by repeated use. Some products containing these chemicals as active ingredients are still available and used by beekeepers. The resistance of mites causes collapsing of colonies on a large scale. In Germany, the use of organic acids as an alternative control is very successful and recommended (Association of the German Bee Research Institutes 2007). Formic acid, oxalic acid dehydrate and lactic acid are registered as veterinary products that can be used legally by the beekeeper. Nevertheless, it is important to continue the search for other acaricidal substances to be used legally in practice as this is a process which takes many years. Honey bees are food producing animals, and every substance that is inserted into the bee colony to control a pest or pathogen must be registered as a veterinary medicine or in some cases as a pesticide. The drug registration process includes the safeguard of the target animal, efficacy against the parasite, as well as user safety and consumer protection (EMA 2010).

Jones et al. (1996) describe the effect of hop acids on the plant pest *Tetranychus urticae* Koch. We conducted a screening of natural plant compounds (especially alpha and beta acids) extracted from the resin of the hop plant *Humulus lupulus* L. The substance proven to be effective against *V. destructor* was later developed as a new acaricide, in the USA by DeGrandi-Hoffman et al. (2012).

To provide the data needed for registration as a veterinary drug in Europe, laboratory and field studies have to be conducted with the product for which marketing authorization is sought. The development process in the laboratory was

Corresponding author: E. Rademacher,
radem@zedat.fu-berlin.de
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completed by establishing the dose-response relationships of the active ingredient on bees and mites. We then tested its efficiency as a winter treatment in honey bee colonies. The bee tolerance as well as the efficacy against the Varroa mite was investigated in a clinical field study which also included the analysis of residues in bee products before and after the treatment and in spring of the following year.

2. MATERIAL AND METHODS

2.1. Laboratory study

In order to assess the tolerability and efficacy of alpha and beta acids, we conducted laboratory tests on bees and mites in a range of dosages as described by Rademacher and Harz (2011a). All test animals *Apis mellifera carnica* (Pollmann) and *V. destructor* were obtained from honey bee colonies at the apiary of the Institute of Biology/Neurobiology situated in the Botanical Garden, Berlin, Germany. The colonies are kept in polystyrene beehives and managed according to good beekeeping practice. Worker bees (ca. 5–10 days old) were brushed off combs from the central part of one hive throughout the experiment. Varroa mites feeding on adult worker bees were taken from colonies at least 8 weeks after the formic acid treatment to exclude combinatory effects of different treatment chemicals. BetaTec GmbH provided the hop alpha and beta acids.

2.2. Acute dermal toxicity on *A. mellifera*

The acute toxicity of both alpha and beta acids in different dosages (Tables I and II) was tested on *A. m. carnica*. Worker bees were treated individually by dermal application: trickling of 5-μL solution onto the ventral part of the abdomen. The toxicity tests were conducted with at least 30 bees per concentration (10 bees per cage, 3 cages) and one replicate (10 bees per cage, 3 cages) in the laboratory at a temperature of 22 °C and 65 % R.H. The cages were placed in shallow trays, kept in darkness, and draught-free. After application of the test solution, the bees were fed with sugar syrup ad libitum and additionally with sugar dough (a mixture of icing sugar, honey, and water). Control groups (10 bees per cage, 3 cages, one replicate) were handled identical to test groups. They were treated individually with 5 μL of distilled water. The bee mortality was monitored after 24, 48, and 72 h.

A small simulation was carried out in order to examine the aspect of dissemination of the tested substance in a small group of bees with mostly untreated individuals, thus simulating the natural situation at a colony treatment. In cage tests, 10 bees were treated (5-μL solution with 150 μg beta acids per bee), marked and then grouped with 90 untreated bees. The cages (n=2) were kept in the laboratory as described above.

2.3. Acute dermal toxicity on *V. destructor*

In order to evaluate the toxicity of the hop acids on *V. destructor*, we collected adult bees infested with phoretic mites from untreated colonies. The mites moved freely on the bees; they were undamaged and could be found at typical locations: on the thorax or ventral side of the abdomen between the sternites. These tests were conducted with 10 infested bees plus 10 noninfested bees per cage to ensure that the mites have the possibility to change host from infested to healthy bee. The beta acid formulation was applied to all test animals by trickling 5 μL onto the abdomen of the individual bees. In this way, we determined the mite mortality without handling the mites what usually

Table I. Application scheme—first screening dermal toxicity of alpha and beta acids.

Concentration % (w/w)	Amount/bee (μL)	Dosage/ bee (μg)	Alpha acids n		Beta acids n		Determination of mortality
			Bees	Mites	Bees	Mites	
0.00	5	0	140	45	140	45	24, 48, 72 h
0.50	5	25	90	30	80	45	24, 48, 72 h
1.00	5	50	90	30	80	47	24, 48, 72 h

Table II. Application scheme—dermal toxicity of beta acids on bees and mites.

Concentration % (*w*/*w*)	Amount/bee (μL)	Dosage beta acids /bee (μg)	*n*	Determination of mortality
0.00	5	0	60	24, 48, 72 h
0.125	5	6	60	24, 48, 72 h
0.25	5	12.5	60	24, 48, 72 h
0.50	5	25	60	24, 48, 72 h
0.75	5	37.5	60	24, 48, 72 h
1.00	5	50	60	24, 48, 72 h
3.00[a]	5	150	60	24, 48, 72 h

[a] This concentration was only tested with bees

causes around 50 % mortality (Schulz 1984). We used only adult female mites in their phoretic phase on older worker bees. The cages were kept in the laboratory as described above. The mite mortality was monitored after 24, 48, and 72 h; the mortality rate was calculated by the ratio of dead versus alive mites.

2.4. Clinical field study

The target animal safety and efficacy against *V. destructor* of a winter treatment using HopGuard® strips (active ingredient: beta acids) was tested in a clinical field study. The study was conducted on 60 bee colonies in two locations in Germany with differing climatic conditions. All colonies of *A. m. carnica* were kept in polystyrene hives and managed according to good beekeeping practice. They were naturally infested with *V. destructor*. During the 2011 season, colonies with a high Varroa infestation rate (natural mite mortality: >5 mites per day) were treated once with formic acid (Nassenheider Dispenser) after the honey harvest at the end of July to avoid colony collapse prior to the field tests in autumn 2011 (November). The trial locations were in Birkenwerder (location A, temperate climate) situated near Berlin and Celle (location B, maritime climate) north of Hannover, Germany. Both apiaries were located in sheltered woodland areas with sufficient distance (circa 3 km) to other local apiaries in order to reduce reinvasion of foraging bees with mites to a minimum. Natural mite mortality was recorded prior to the test treatment for up to 6 weeks to monitor the infestation: The colonies were in the range of a medium infestation (mean 0.3–3.0 mites/day). Each location comprised 30 colonies: 15 large colonies (two chambers) and 15 small colonies (one chamber) of which 10 were randomly chosen as the test group and the remaining 5 served as controls. In November 2011, all test colonies were treated with HopGuard®, the dosage depending on their size. HopGuard® consists of the active ingredient beta acids (16 % *w*/*w*) and two substances working as a carrier and thickening agent. The liquid formulation is soaked into cardboard strips which are placed into the bee colony. Large colonies with two chambers received four strips; small colonies with one chamber received two strips. The strips were placed vertically in the central part of the winter cluster. At the time of treatment, all colonies were brood free. The control groups were treated with Oxuvar® (3.5 % oxalic acid dehydrate solution) according to the package instructions for use. HopGuard® strips stayed in the colonies for 14 days.

To determine the bee mortality after the treatment, dead bees were counted every 2 days in dead bee traps (so called "Gary traps") fixed onto the flight entrance. To determine the mite mortality rate after the treatment, floor boards were placed at the bottom of the hive under the floor grid and lined with nonsticky paper. Dead mites were counted at 2-day intervals during the test period of 14 days. In order to calculate the relative amount of fallen mites after the test treatment, it is necessary to conduct a follow-up treatment with a drug of known efficacy (at least 95 % mite kill, in this case Oxuvar®). The dead mites on the floor boards were counted for further 3 weeks. Thus, it is possible to calculate the relative mite fall caused by the test treatment in relation to the follow-up treatment using Abbot's formula (EMA 2010):

Prior to the test treatment in November 2011 and then in March 2012, the colony strength was estimated using the "Liebefeld estimation method" (Imdorf et al. 1987), and overwintering of all colonies was evaluated.

Colonies with clinical symptoms other than Varroosis were examined in the laboratory by light microscopy for pathogens. Bee product samples (food, wax, and propolis) were taken from the test colonies prior to, and after, the test treatment in November 2011 and again in March 2012. Samples were analyzed at random for residues of beta acids by HPLC analysis in certified laboratories.

2.5. Statistical analysis

The statistical analysis was conducted using SigmaStat® 3.0 Software. Significant differences in acute dermal toxicity were determined with the Mann-Whitney *U* test in comparison to the respective control group. For determination of significant differences in mortality of bees and mites in the field study, a proportional *z* test was used. To compare the treatment groups for significant differences in number of bees and brood area, a paired *t* test was conducted. Differences were considered significant at $P \leq 0.05$.

3. RESULTS

3.1. Laboratory study

3.1.1. Screening of alpha and beta acids

Beta acids in dosages of 25 and 50 μg per bee had an acaricidal effect (mite mortality 77.8 and 85.1 %), significantly different to the controls with 15.6 % (MWU $P \leq 0.05$); the bee mortality rates were 13.8 and 7.5 % which are not significantly different to the controls with 10.0 % (MWU, $P \geq 0.05$). Dermal application of alpha acids resulted in bee mortality of 3.3 and 17.8 %, not significantly different to the controls with 10.0 % (MWU, $P \geq 0.05$); the mite mortality of 26.7 and 30 % was not significantly different to controls with 15.6 % (MWU $P \geq 0.05$) when applied at 25 and 50 μg per bee (Tables I and III).

3.1.2. Acute dermal toxicity on A. mellifera

In order to establish a dose response relationship for the acaricidal effective beta acids, they were tested by dermal application on bees in different dosages. Figure 1 shows the dose response graph: the bee mortality was in range of the corresponding control group; no significant differences occurred (MWU, $P \geq 0.05$).

To assess a safety margin for the application of beta acids for the target animal *A. mellifera* , bees were treated additionally with a dosage of 150 μg/bee which was well tolerated. The resulting bee mortality of 5 % was not significantly different from the control group (MWU, $P = 0.394$). When treated bees (150 μg/bee) were placed with large numbers of untreated bees (10:90), bee mortality did not occur.

The lethal effect of beta acids occurred shortly after application, normally within 24 h. The first damages like tumbling were observed within 1 h after the application of dosages leading at last to mortality.

3.1.3. Acute dermal toxicity on V. destructor

Dermal application of beta acids leads to high mite mortality rates (Figure 1). Significant differences started at a dosage of 12.5 μg/bee (73.3 % mortality). The minimum efficient dosage to kill ≥90 % of mites is 25 μg/bee. The efficacy of 37.5 and 50 μg/bee was 93.3 % and also significantly different to the control group (MWU, $P \leq 0.05$). A higher dosage did not lead to increased mite mortality. The acaricidal effect of beta acids can be observed shortly after application: the proportional highest mortality occurs within 24 h after application of 37.5 and 50 μg/bee with a mortality rate of 92.8 and 85.7 %, respectively.

4. CLINICAL FIELD STUDY

4.1. Location A

4.1.1. Tolerance of A. mellifera

The total bee mortality was scaled to the colony size prior to the treatment. The relative bee mortality of the small colonies 14 days after the start of the HopGuard® treatment in location A was 3.6 % (min. 1.22 %, max. 7.44 %). No significant differences in the bee mortality were found between the test (HopGuard®) and control (Oxuvar®) group (mean 0.5 %; min. 0.16 %, max. 0.67 %; *z*-test, $P = 0.117$). The mean bee mortality in the large colonies 14 days after the

Table III. Results—dermal toxicity of alpha and beta acids after 72 h.

Dosage µg/bee	Alpha acids				Beta acids			
	Mortality (%)				Mortality (%)			
	Bees	MWU test p	Mites	MWU test p	Bees	MWU test p	Mites	MWU test p
0	10.0	–	15.6	–	10.0	–	15.6	–
25	3.3	≥0.05	26.7	≥0.05	13.8	≥0.05	77.8	≤0.05
50	17.8		30.0		7.5		85.1	

HopGuard® treatment was 3.1 % (min. 0.88 %, max. 5.02 %). No significant differences in the bee mortality were found between the test and control group (mean 0.6 %; min. 0.21 %, max. 1.07 %; *z*-test, $P=0.132$). Table IV summarizes the relative bee mortality of all colonies 14 days after the treatment. Figure 2 illustrates the bee mortality: The range of maximum bee mortality is reached within 24 h; then, the curve progression is not increasing in respect to HopGuard® treated colonies; in comparison, the colonies of the control group show a slow rise to the maximum.

4.1.2. Efficacy against V. destructor

The total mite mortality during the test period was put into relation to the mite mortality after the follow-up treatment. The mean efficacy in the small colonies 14 days after the HopGuard® treatment was 83.8 % (min. 66.3 %, max. 100 %). No

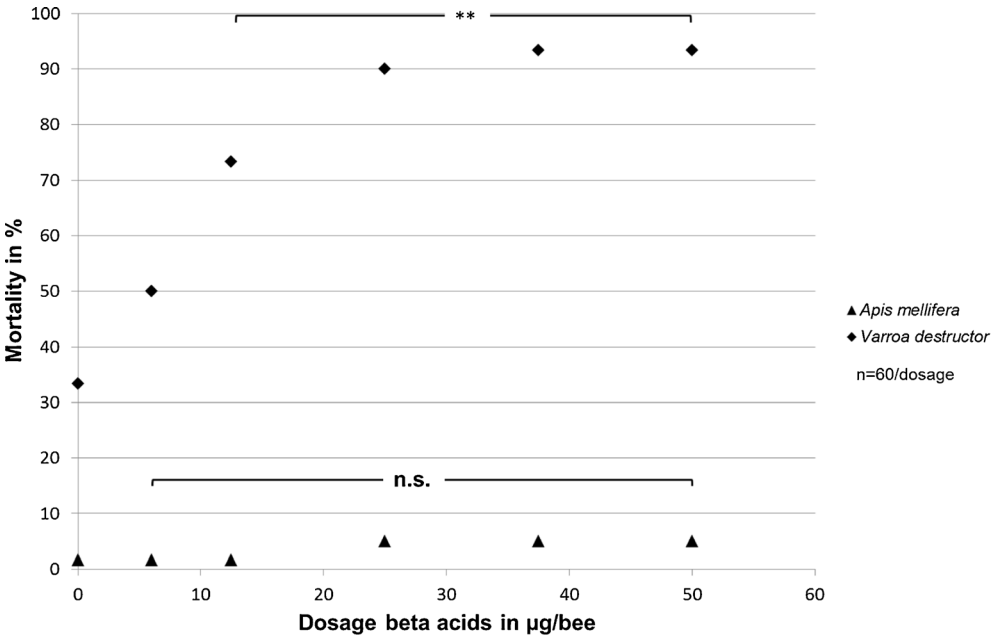

Figure 1. Dose-response graph for the dermal toxicity of beta acids, significant differences to the control group are indicated by *asterisks* ' (MWU, $P\leq0.01$), n.s. = not significant comparing test dosages versus control (0 µg/bee, MWU, $P\geq0.05$). The bee mortality was not considered different in any test dosage. Significant differences in mite mortality occurred at 12.5 µg beta acids/bee and continued with increasing dosage.

Table IV. Relative bee mortality in colonies 14 days after test treatment.

Group	*n*	Location A Colony size		Location B Colony size	
		small	large	small	large
		Mortality (%)±(SD)			
Test	10	3.6 (1.8)	3.1 (1.5)	5.5 (1.6)	12.3 (6.8)
Control	5	0.5 (0.2)	0.6 (0.4)	3.5 (1.2)	14.7 (10.9)
z-test *p*		0.117	0.132	0.247	0.394

Bee mortality was calculated in relation to the total number of bees in each colony

significant differences in the efficacy were found between the test and control group treated with oxalic acid (mean 94.2 %; min. 87.4 %, max. 98.3 %; *z*-test, $P=0.915$). The mean efficacy in the large colonies 14 days after the HopGuard® treatment was 83.0 % (min. 65.6 %, max. 95.2 %). No significant differences in the efficacy were found between the test and control group (mean 83.8 %; min. 43.8 %, max. 97.4 %; *z*-test, $P=0.706$).

The development of the acaricidal effect for location A is shown in Figure 3. The highest mite mortality occurred in the test colonies during the first 24 h (65.8 and 66.2 %). Up to 72 h after the application, the curve shows another considerable increase; afterwards, the curve rises gently to its maximum efficacy. Both test groups show similar curve progression. The curves of the control groups treated with oxalic acid show a slow mortality increase reaching the efficacy level of the

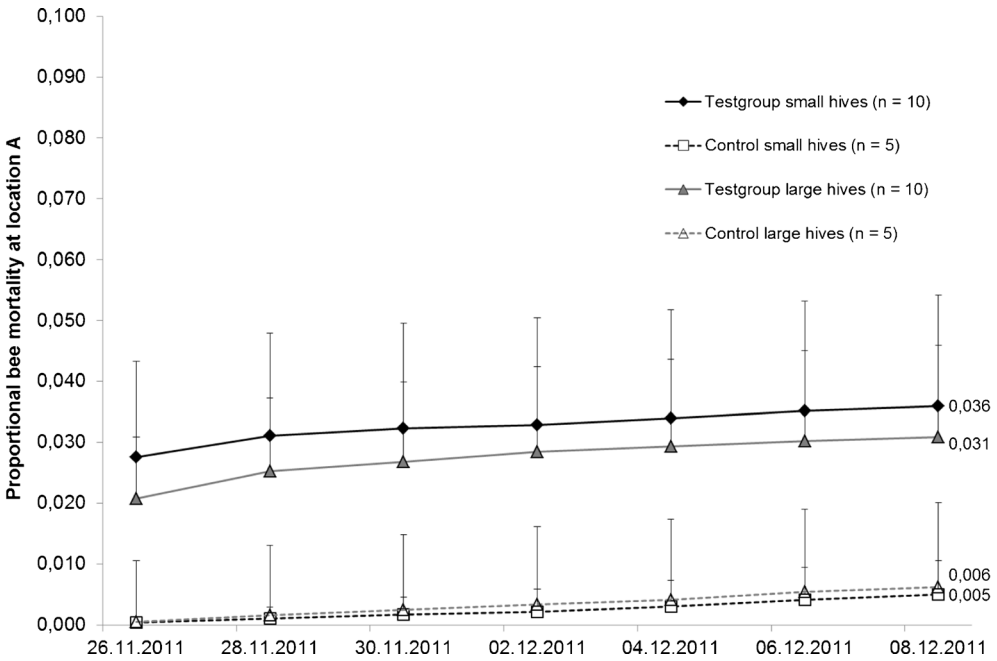

Figure 2. Bee mortality curve during the test period at location A, proportional bee mortality+SD, data accumulated for every test day, bee mortality is relative to the estimated colony size before the trial. There were no significant differences in any of the test groups compared to the control groups at the end of the trial (*z*-test, $P \geq 0.05$).

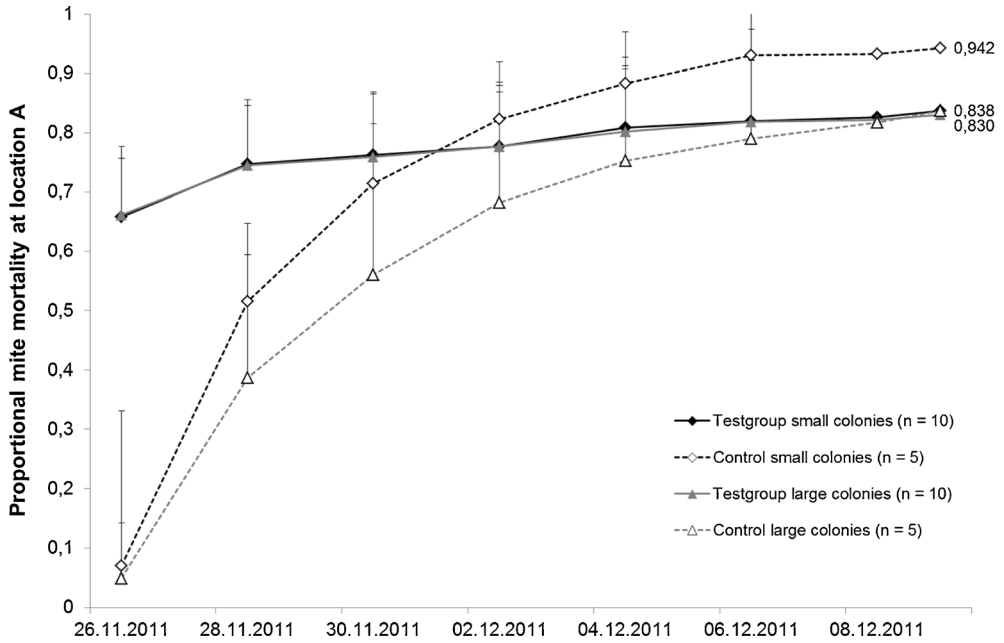

Figure 3. Mite mortality curve during the test period at location A, proportional mite mortality+SD, data accumulated for every test day, mite mortality is relative to the final follow-up treatment. There were no significant differences in any of the test groups compared to the control groups at the end of the trial (z-test, $P \geq 0.05$).

test colonies after 6 days (small colonies) and 13 days (large colonies), respectively.

4.1.3. Colony size estimation

The colony sizes were estimated in November 2011 prior to the treatment and in March 2012 to evaluate the colony overwintering. Table V summarizes the estimated colony size in the mean number of bees for the respective treatment and control groups. The mean number of bees in November 2011 did not differ significantly in the two groups (paired t test, $P=0.457$) of the small colonies. Also, in spring 2012, no significant differences in the mean colony size between the two groups could be detected (paired t test, $P=0.141$). In the test group, three colony losses occurred later in the spring. For the large colonies, the mean number of bees in November 2011 did not differ significantly in the two groups (paired t test, $P=0.782$). Also, no significant differences in the mean colony size between the two groups could be detected in spring 2012 (paired t test, $P=0.800$).

4.2. Location B

4.2.1. Tolerance of A. mellifera

The relative bee mortality in location B of the small colonies 14 days after the HopGuard® treatment was 5.5 % (min. 3.51 %, max. 8.70 %). No significant differences in the bee mortality were found between the test and control group (mean 3.5 %; min. 2.27 %, max. 5.23 %; z-test, $P=0.247$). The bee mortality in the large colonies 14 days after the HopGuard® treatment was higher, compared to the group small colonies, with a mean of 12.3 % (min. 5.38 %, max. 28.45 %), but no significant differences in the bee mortality were found between the test and control group (mean 14.7 %; min. 4.46 %, max. 30.24 %; z-test, $P=0.394$, Table IV). The bee mortality curve in respect to the small colonies after HopGuard® treatment reaches half of the maximum bee mortality 24 h after the application; afterwards, the curve runs shallower. The large colonies show a similar reaction after 24 h while at 72 h, the curves

Table V. Summary of the colony estimations at locations A and B.

Group	Location A Colony size mean # of bees (± SD)				Location B Colony size mean # of bees (± SD)			
	small		large		small		large	
	Nov 2011	March 2012	Nov 2011	March 2012	Nov 2011	March 2012	Nov 2011	March 2012
Test	7111	3297.78	11,146	7308	4166.4	2800	5846.4	2536.8
	(1772.6)	(1604.44)	(227.1)	(3628.5)	(1281.1)	(69.8)	(2313.8)	(1793.2)
Control	6395.6	5252.8	10,750	6809.6	4726.4	2979.2	6148.8	3752
	(1788.3)	(3112.5)	(3092.8)	(3257.1)	(1775.6)	(1083.3)	(326.7)	(1748.3)
t-test p	0.475	0.141	0.782	0.800	0.494	0.721	0.838	0.272

cross each other, and during the following days, the test colonies remain at a lower mortality rate than the controls (Figure 4).

4.2.2. *Efficacy against* V. destructor

The mean efficacy in the small colonies 14 days after the HopGuard® treatment was 75.3 % (min. 56.0 %, max. 94.5 %). No significant differences in the efficacy were found between the test and control group treated with oxalic acid (mean 93.4 %; min. 88.8 %, max. 96.1 %; z-test, P=0.844). The mean efficacy in the large colonies 14 days after the HopGuard® treatment was 88.2 % (min. 77.2 %, max. 96.6 %). No significant differences in the efficacy were found between the test and control group (mean 96.1 %; min. 93.6 %, max. 98.0 %; z-test, P=0.504). The development of the acaricidal effect for location B is shown in Figure 5. Both test groups showed similar curve progression. The highest daily mite mortality rate occurred within the first 24 h; afterwards, the curve rises gently to its maximum efficacy similar to location A; after 6 days, the curves of the control groups reach the efficacy level of the test colonies.

4.2.3. *Colony size estimation*

Table V gives a summary of the mean colony size for the respective treatment and control groups. In November 2011, the mean number of bees in the small colonies did not differ significantly in the two groups (paired t test, P=0.494). No significant differences in the mean colony size between the test and control group could be detected in the spring of 2012 (paired t test, P=0.721). Colony losses occurred in the test group: 2 colonies due to a *Nosema* spp. infection. For the large colonies, the mean number of bees in November 2011 did not differ significantly in the two groups (paired t test, P=0.838). Also, no significant differences in the mean colony size between the two groups could be detected in the spring of 2012 (paired t test, P=0.272). One colony was lost in the control group due to a *Nosema* spp. infection.

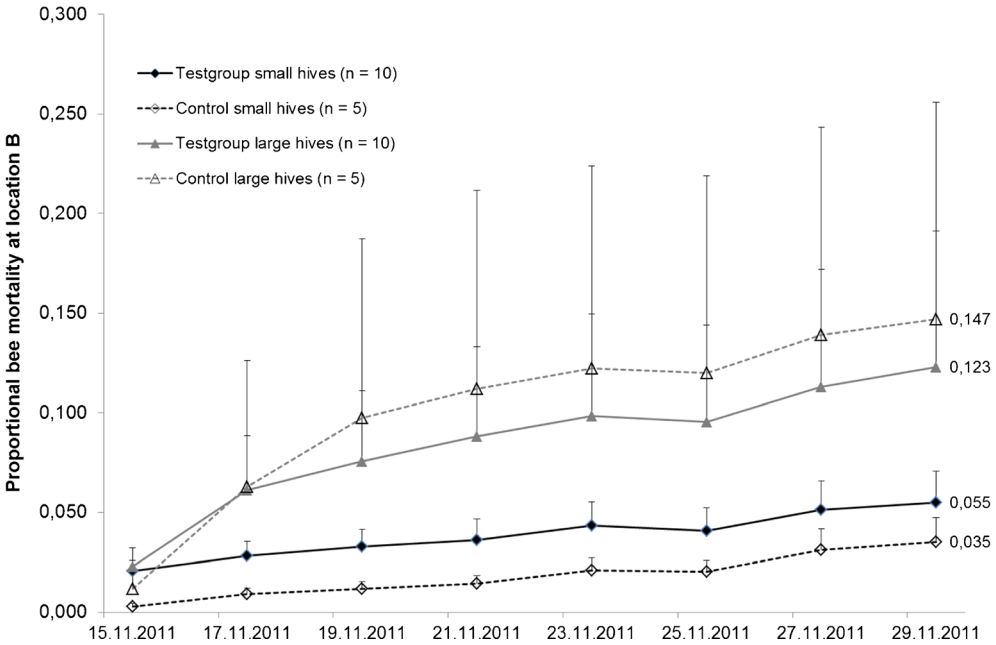

Figure 4. Bee mortality curve during the test period at location B, proportional bee mortality+SD, data accumulated for every test day, bee mortality is relative to the estimated colony size before the trial. There were no significant differences in any of the test groups compared to the control groups at the end of the trial (z-test, $P \geq 0.05$).

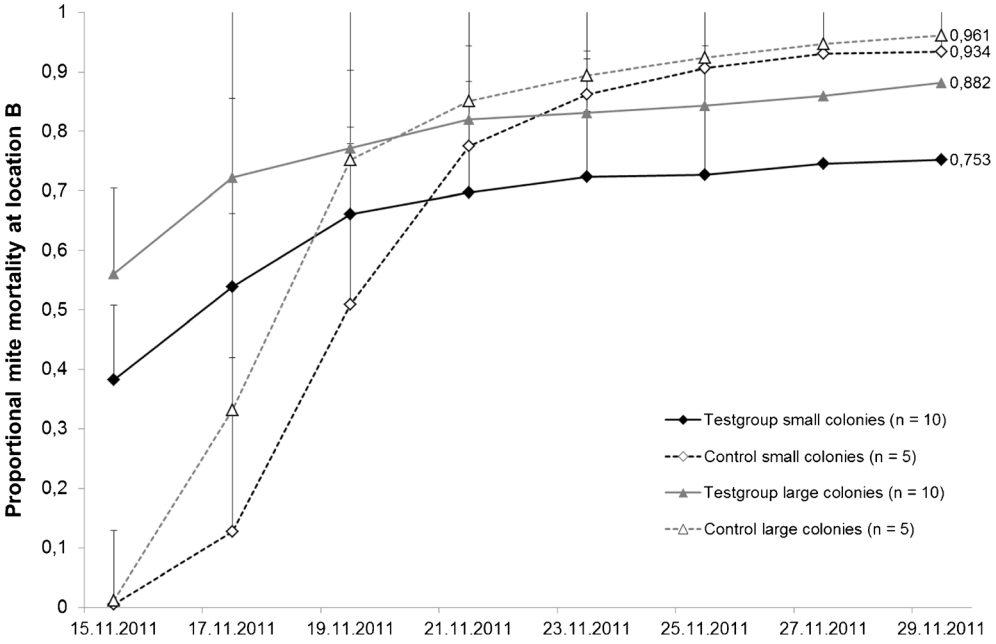

Figure 5. Mite mortality curve during the test period at location B, proportional mite mortality+SD, data accumulated for every test day, mite mortality is relative to the final follow-up treatment. There were no significant differences in any of the test groups compared to the control groups at the end of the trial (z-test, $P \geq 0.05$).

Table VI. Results—residue analysis in bee products.

Location	Group	Mean beta acids concentration in ppm (±SD) 14 days after treatment			Mean beta acids concentration in ppm (±SD) 3 months after treatment		
		Honey	Wax	Bees	Honey	Wax	Bees
A	*small*	n.d.	n.d.	n.a.	n.d.	n.d.	n.a.
	sample n	8	8		7	7	
A	*large*	n.d	5.21 (10.10)	n.a.	0	0	n.a.
	sample n	8	8		5	5	
B	*small*	n.d.	4.20 (5.17)	n.d.	n.d.	n.d.	n.a.
	sample n	5	5	2	2	2	
B	*large*	n.d.	21.23 (24.12)	n.d.	n.d.	n.d.	n.a.
	sample n	5	5	2	2	2	

n.d. not detected, *n.a.* not analyzed

4.2.4. Analysis of residues in bee products—locations A and B

Wax samples taken 14 days after the application of HopGuard® had low amounts of beta acids averaging 5.21 ppm (±10.1) and 21.23 ppm (±24.12) for large colonies and 4.2 ppm (±5.17) in small colonies (Table VI). Honey samples were free of beta acids in all groups. There were no residues of beta acids found in any tested bee product samples 3 months after the application of HopGuard®.

5. DISCUSSION

5.1. Laboratory study

The first screenings of hop acids indicated that only beta acids have the potential as an active ingredient in a veterinary drug. Alpha acids did not yield efficient mite mortality and were not further developed. The results of the cage tests with beta acids showed that all tested dosages were well tolerated by the bees. There is a safety margin of at least 6 (i.e., a six-fold difference in mite mortality versus bee mortality) considering the minimum efficient dosage of 25 μg/bee to achieve ≥90 % mite mortality. The cage tests with treated bees showed that even a dosage of 150 μg/bee is tolerated without any bee mortality when placed to a group of untreated bees. Treated bees tolerate high dosages; presumably they pass on the substance in a short time period thus reducing the dosage for themselves and the other bees. This was shown by Rademacher et al. (2012) with computed tomography on treated bee colonies. We expect that bee-to-bee contact distributed this substance because oral administration of beta acids led to high bee mortality in corresponding dosages (Rademacher and Harz 2011b). The effect of beta acids occurs shortly after application, with possible damage to bees as well as mite mortality. The highest efficacy against *V. destructor* occurs within the first 24 h.

5.2. Clinical field study

The 14-day treatment with HopGuard® strips was well tolerated by the bees in both trial locations. In location A, the bee mortality was clearly under the 5 % level and not different from the controls. In location B, the bee mortality was generally higher than that in location A, but yet again, there was no difference to the respective control colonies. This means that the bee mortality after the

HopGuard® treatment was acceptable. The mortality rate of bees in the large colonies (test and control colonies) could be attributed to the relatively high Varroa infestation in the weeks prior to the treatment and the impact on the health of these colonies.

The use of varroacides is always linked to side effects like bee mortality. The bee mortality after HopGuard® treatment can be tolerated by small and large colonies in beekeeping practice. It was shown by Rademacher and Schneider (2012) that the vitality of treated honeybees was positively influenced by a HopGuard® treatment.

The 14-day treatment with HopGuard® strips resulted in a moderate efficacy against *V. destructor*. In both trial locations, the mean efficacies did not exceed 90 %. In location A, the efficacy in the small colonies and the large colonies was not different from the respective control colonies treated with oxalic acid. In location B, the efficacy in the small colonies was lower, but neither in this group, nor in the large colony group, was there a difference to the respective control colonies treated with oxalic acid.

The acaricidal effect (mean efficacy) can be observed soon after the application: within 24 h up to 65 % of the mites are killed. The effect of oxalic acid is gradually slower; it takes about 5 to 10 days longer for oxalic acid to reach the same efficacy as HopGuard®. The main advantage of HopGuard® in comparison to other winter treatments is this fast acaricidal effect. Similarly, DeGrandi-Hoffman et al. (2012) found the highest mite drop during the first 12 days after hop beta acid strips were placed in colonies.

Varroa control has to be conducted several times during the bee season. Control measures include biotechnical methods and the use of acaricides. The mite mortality and resulting efficacy after HopGuard® autumn/winter treatment are sufficient within an integrated Varroa control concept as recommended by the Association of the German Bee Research Institutes (2007).

In location A, the colony sizes at the beginning of the experiment in November 2011 and at the end date in March 2012 did not differ significantly in the test and control groups; this was the case for the small colonies and large colonies. The reduction in the number of bees from autumn to spring reflects the normal situation after the winter. Three colony losses occurred in the small hive test group. No colony losses occurred in the large colonies group, but the reduction in number of bees meant that many colonies were reduced to one super which is common beekeeping practice after the winter. Winter colony losses were lower than described for many regions of Germany (Otten 2012).

In location B, again, the colony sizes at the beginning of the experiment in November 2011 and at the end date in March 2012 did not differ significantly between the test and control groups; this was the case for the small and large colonies. The reduction in the number of bees from autumn to spring in location B reflects also the normal situation after the winter. Three colonies were lost, two of the small colonies test group and one of the large colonies control group. These losses are 10 % of all colonies at this location and are lower than the overall colony losses in Germany in the winter of 2011/2012 (Otten 2012). Considering that the large colonies in location B were impaired by high bee mortality probably due to the Varroa infestation, the overwintering was very successful.

The residue analysis indicates that the quality of bee products in both locations was not affected by the HopGuard® treatment. The consumer safety is assured by the food grade quality of additional ingredients.

On the basis of these results, there is no indication of negative effects of the treatment on the colony winter survival and strength in the following spring. The climatic differences of the two test locations did not seem to have any effect on the treatment result.

The commercial product HopGuard® (containing 16 % beta acids as active ingredient) is approved in the USA under Section 18 emergency use exemptions in selected states to combat Varroosis. A European registration procedure has been initiated for the approval of HopGuard® as a drug for honey bee colonies.

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Le développement de HopGuard® comme traitement d'hiver contre *Varroa destructor* dans des colonies d'*Apis mellifera*

médicament vétérinaire / étude clinique / efficacité du traitement / composé d'origine végétal

Die Entwicklung von HopGuard® als Winterbehandlung gegen *Varroa destructor* in Bienenvölkern (*Apis mellifera*)

Tierarzneimittel / klinische Studie / *Varroa destructor* / *Apis mellifera*

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