Notice of supplier:
The product in the test called " unknown test product " is BeeVital HiveClean.
The testgroup called "VER" was the one in which BeeVital HiveClean was treated.

Test Report

By

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Test Period: 20.07.2002-15.03.2003

I. Analyses of the Varroacide Characteristics

Description of Test Procedure

The experiment was performed on 33 bee colonies. Exclusively young bees belonging to the race Apis mellifera carnica were used, kept in hives of type Hohenheimer for migratory beekeeping, of 20 Zander type frames each. The experiment was conducted in a location with as little bee population as possible in order to avoid impairing of the research due to mite infestation of exterior origin.

The experiment took place in two isolated locations about 3 km apart. All the bee colonies of both locations were involved in the research. 18 Colonies were used in location A and 15 in location B. Al colonies were placed on pallets in both locations.

At the beginning of the experiment Varroa control sheets were placed under each hive for three weeks. The Varroa sheets were checked every third day in order to obtain accurate information on the mite fall. The removal of dead mites by ants was avoided by smearing the sheet with a sticky substance. By several checking of the sheets it was proved that the ants carried not even the dead bees away.

Before the start of the test all the bee colonies were estimated using the LIEBEFELDER method which determines the number of bees and the extent of brood areas (open and sealed cells) for each colony.

At the beginning of the research the colonies were separated into groups corresponding to the different test variants in such a way that an equal distribution of the colony strength and level of infestation was assured.

The research procedure was based on the use of 11 hives for each of the 3 test variants and by only 3 applications. The applications were performed exactly one week apart.
The treatment concept guaranteed covering of the whole development period of the worker bee brood (21 days). Drone brood is not present any more at the time of application so that a longer treatment period (24 days) needs not to be taken into consideration. The reduction of the number of applications reduces the risk of residues in bee products and also produces less stress to the winter bee, which is of particular importance taking into consideration the use of oxalic acid.

The following variants were tested:

- Control Group (**Kon.**): 11 Colonies were not treated but the natural mite fall was measured at time intervals of 3-5 days for the whole duration of the experiment.

- Tested Product Treatment Group (**Ver.**): 11 colonies were treated with the unknown test product. The treatment procedure followed the application directions of the product At each application 15 ml of test substance was trickled in the gaps between comb frames in areas occupied by bees. The mite fall was determined every 3-5 days.

- Perizin Treatment Group (**Per**.): 11 colonies were weekly treated with Perizin during the experiment period according to the application directions of this product. In order to make possible the estimation and verification of the unknown test product efficiency the mite fall was measured every 3-5 days for this group.

All treatments were performed at average exterior temperatures of 20 degrees Celsius. The highest exterior temperature did not exceed 25 degrees Celsius.

At the end of the experiment all groups were treated with formic acid (85%). The application of formic acid followed the "medicine bottle without plate" method, according to the concept of Varroa control means used in state of Baden-Württemberg (Germany). In this way the application device was provided with a 2 mm thin wick (10x15 cm) located on the upper side of the frame. The treatment lasted one day and the quantity of formic acid used was 60 ml/colony.

As commonly known, the formic acid is efficient against the mites in the capped brood cells and thus the mite fall count had to continue for a longer time. After formic acid treatment the effect against the mites on the bees will be noticed first and as the brood continues to hatch the effect on mites in different stages of development will be seen.

The efficiency of the unknown tested treatment was determined at the end of this research.

Results

Table 1: Count of spontaneous mite fall after setting the Varroa sheets at 17/07/2002 during period 20/07/2002-10/08/2002.

Zeit Volk Nr.:	20.07. 2002	23.07. 2002	26.07. 2002	29.07. 2002	01.08. 2002	04.08. 2002	07.08. 2002	10.08. 2002	Summe
1	0	0	1	0	0	1	0	0	2
2	1	0	3	2	1	1	1	2	11
3	0	0	1	0	1	0	0	1	3
4	0	1	0	1	0	0	0	0	2
5	0	1	0	0	0	0	0	1	2
6	3	2	2	3	1	2	3	2	18
7	0	1	2	1	1	0	0	1	6
8	0	1	1	0	1	0	0	0	3
9	1	2	3	2	2	1	2	2	15
10	1	1	2	1	1	2	0	1	9
11	1	1	2	2	0	0	1	1	8
12	0	1	1	2	0	1	0	0	5
13	2	1	4	2	3	1	2	2	17
14	0	2	2	1	0	0	1	0	6
15	0	1	1	1	0	1	0	0	4
16	1	1	1	0	0	1	0	0	4
17	2	2	3	2	2	3	1	2	17
18	2	2	2	1	0	2	1	1	11
19	2	1	2	1	2	2	2	1	13
20	2	0	3	3	2	1	2	2	15
21	1	1	3	2	1	2	0	0	10
22	1	1	1	1	0	1	0	0	5
23	1	0	3	2	2	2	2	1	13
24	1	2	3	3	0	1	1	1	12
25	0	1	0	0	0	0	0	0	1
26	2	1	2	1	1	2	1	1	11
27	2	3	2	2	2	3	2	3	19
28	1	2	1	0	0	2	2	1	9
29	2	1	3	1	3	1	2	1	14
30	2	1	4	2	2	2	1	2	16
31	1	1	1	0	0	2	0	0	5
32	2	1	3	2	1	2	1	2	14
33	2	0	3	3	2	3	2	3	18

On 10/08/2002, after thorough estimation of the colonies they were divided into 3 groups, according to their strength (number of bees, extent of the brood areas) and mite fall. In this way the following groups resulted:

Kon.=Untreated, control group, for determination of the natural mite fall during the whole experiment

Ver.=Test group, treated with the (unknown) test product.

Per.=Perizin group, treated with Perizin.

Table 2: Mite fall after application of treatments during test period 10/08/2002-31/08/2002.

Zeit Volk Nr.:	10.8. 2002 Appl.	11.8. 2002	14.8. 2002	17.8. 2002 Appl.	18.8. 2002	21.8. 2002	24.8. 2002 Appl.	25.8. 2002	28.8. 2002	31.8. 2002	Summe
1 Kon.	---	1	1	1	0	2	1	1	0	1	8
2 Ver.	---	169	19	6	92	9	5	57	13	3	373
3 Per.	---	56	8	5	29	4	2	14	9	2	129
4 Per.	---	66	5	7	17	4	3	23	5	2	132
5 Kon.	---	0	0	2	1	0	0	1	1	1	6
6 Kon.	---	2	4	2	3	1	2	5	6	4	29
7 Ver.	---	87	13	9	47	11	5	30	9	2	213
8 Kon.	---	1	0	0	1	2	1	0	1	1	7
9 Kon.	---	2	2	1	3	1	2	2	3	4	20
10 Ver.	---	145	9	15	74	22	9	54	25	8	361
11 Kon.	---	0	2	1	3	2	2	1	3	1	15
12 Per.	---	126	35	21	82	18	11	49	26	4	372
13 Ver.	---	207	23	14	127	36	14	103	65	14	603
14 Ver.	---	45	6	9	19	7	2	22	5	0	115
15 Ver.	---	59	9	6	34	10	2	19	11	2	152
16 Ver.	---	76	16	4	40	14	4	51	17	4	226
17 Kon.	---	3	3	2	2	1	3	2	3	5	24
18 Per.	---	165	30	12	81	26	7	56	33	13	423
19 Per.	---	171	38	26	101	34	5	68	22	9	474
20 Ver.	---	132	24	15	73	21	3	52	27	11	358
21 Kon.	---	1	2	1	3	2	2	3	1	1	16
22 Per.	---	48	4	7	36	8	1	24	15	7	150
23 Kon.	---	2	3	1	1	3	2	4	2	2	20
24 Ver.	---	136	15	20	67	29	10	45	22	5	349
25 Ver.	---	15	5	2	12	4	0	15	1	0	54
26 Kon.	---	1	2	3	3	1	2	1	2	2	17
27 Per.	---	239	51	42	149	45	20	103	54	9	712
28 Per.	---	52	11	7	37	14	3	41	11	3	179
29 Ver.	---	170	36	21	71	56	15	82	38	16	505
30 Per.	---	189	31	39	89	37	5	68	21	5	484
31 Kon.	---	1	1	0	1	2	2	0	0	1	8
32 Per.	---	104	26	10	51	30	5	47	20	7	300
33 Per.	---	173	23	8	82	48	21	90	16	3	464

Kon.=Control (without any treatment)
Per.=Perizin treatment
Ver.=treatment with (unknown) test product.
Appl.=moment of treatment application.

After the 3 weeks test period a single final treatment of all colonies with formic acid (85%) was performed. The application of formic acid followed the Varroa control concept specific to Baden-Württemberg using the "medicine bottle" method.

Table 3: Mite fall after final application of 85% formic acid treatment

Zeit Volk Nr.:	31.8. 2002 AS-Appl.	01.9. 2002	04.9. 2002	07.9. 2002	10.9. 2002	13.9. 2002	17.9. 2002	Summe
1 Kon.	---	78	36	47	23	26	8	218
2 Ver.	---	22	4	5	1	0	0	32
3 Per.	---	13	3	5	2	0	0	23
4 Per.	---	5	1	0	0	1	0	7
5 Kon.	---	89	24	39	30	29	8	219
6 Kon.	---	262	103	125	139	87	21	737
7 Ver.	---	15	6	9	3	2	0	35
8 Kon.	---	109	23	34	18	22	3	209
9 Kon.	---	368	121	146	101	72	24	832
10 Ver.	---	13	2	1	0	0	0	16
11 Kon.	---	156	137	114	89	54	14	564
12 Per.	---	8	2	0	0	0	1	11
13 Ver.	---	17	5	2	0	0	1	25
14 Ver.	---	3	2	0	0	0	0	5
15 Ver.	---	3	0	1	0	0	0	4
16 Ver.	---	9	3	2	3	0	0	17
17 Kon.	---	301	107	149	124	82	11	774
18 Per.	---	15	6	4	1	0	0	26
19 Per.	---	22	9	11	5	3	0	50
20 Ver.	---	8	4	1	0	0	0	13
21 Kon.	---	184	57	62	41	13	4	361
22 Per.	---	4	0	0	1	0	0	5
23 Kon.	---	269	113	128	107	51	10	678
24 Ver.	---	11	7	8	2	1	0	29
25 Ver.	---	1	0	0	0	0	0	1
26 Kon.	---	268	71	86	50	57	13	545
27 Per.	---	23	11	8	13	5	1	61
28 Per.	---	8	3	1	0	0	0	12
29 Ver.	---	17	6	4	0	0	0	27
30 Per.	---	21	5	6	2	1	0	35
31 Kon.	---	76	31	55	46	35	6	249
32 Per.	---	10	6	3	3	1	0	23
33 Per.	---	25	4	5	2	1	0	37

Table 4: Calculation of efficiency for different treatments after final application of formic acid (85%)

Volk Nr.:	Summe (*)	Summe (**)	Wirkungsgrad (%)
1 Kon.	8	218	---
2 Ver.	373	32	91,4
3 Per.	129	23	82,2
4 Per.	132	7	94,7
5 Kon.	6	219	---
6 Kon.	29	737	---
7 Ver.	213	35	83,6
8 Kon.	7	209	---
9 Kon.	20	832	---
10 Ver.	361	16	95,6
11 Kon.	15	564	---
12 Per.	372	11	97,0
13 Ver.	603	25	95,9
14 Ver.	115	5	95,7
15 Ver.	152	4	97,4
16 Ver.	226	17	92,5
17 Kon.	24	774	---
18 Per.	423	26	93,9
19 Per.	474	50	89,5
20 Ver.	358	13	96,4
21 Kon.	16	361	---
22 Per.	150	5	96,7
23 Kon.	20	678	---
24 Ver.	349	29	91,7
25 Ver.	54	1	98,1
26 Kon.	17	545	---
27 Per.	712	61	91,4
28 Per.	179	12	93,3
29 Ver.	505	27	94,7
30 Per.	484	35	92,8
31 Kon.	8	249	---
32 Per.	300	23	92,3
33 Per.	464	37	92,0

(*) Mite fall after 3 fold application of different treatments during 10.8.2003-31.8.2003
(**) Mite fall after final formic acid (85%) application.
(%) percentage of efficiency

II. Research of Wintering Behavior

Period of Experiment: September 2002 to March 2003

Methodology

In order to prove the suitability of the treatment and the wintering behavior of the bee colonies after application of different varroacides the LIEBEFELDER method was used in the first stages of the project to estimate the colonies' strength by approximating the number of bees and the brood development (open and capped). A further evaluation was done after the Treatment performed on August the 30th.
During winter the colonies were checked every fortnight in order to prevent unusual situations like various sources of disturbance of the hives, high mortality, Nosema and/or dysentery infection.
After wintering period, in spring, a new estimation of the bee population followed, in order to evaluate the possible influence on the colony development as result of previous treatments.

Climatic Data

The autumn of year 2002 may be considered as bee-friendly. According to the meteorological records for Stuttgart-Hohenheim the average temperature in the autumn of year 2002 exceeded with 0.8 degrees Celsius the multi annual media, although the rainfall was very high (198% the normal quantities). There were 56 rainy days compared to the average of 41.5, which made the season fertile. And, with only 2 frost days a record was set.

September was wet and dull, October was stormy, November was the forth warmest since 1878 with only one frost day and although unusually rainy, reaching 238% of the multi annual average rain fall. The year ended with a very mild, foggy, and almost rainless December. The 30.12.2002, with 12.7 Celsius degrees was the highest temperature ever recorded and the bees had the chance of their first cleansing flight.
The winter 2002/2003 was relatively dry; the rain fall reaching only 87% of the normal and the sunshine time was 123% compared to usual. On 26.02.2003 the temperature rose to 14.9 Celsius degrees and a new cleansing flight followed. The February of 2003 was cold, dry and very sunny. The month of March was very mild,

unusually sunny and with only 24% of the normal rainfall, very dry. At the beginning of the month the temperatures reached in daytime 5-15 Celsius degrees but at nighttime frost prevailed. After a short period of a cold airwave in the middle of the month the temperature increased again considerably and reached its peak on March the 20th.

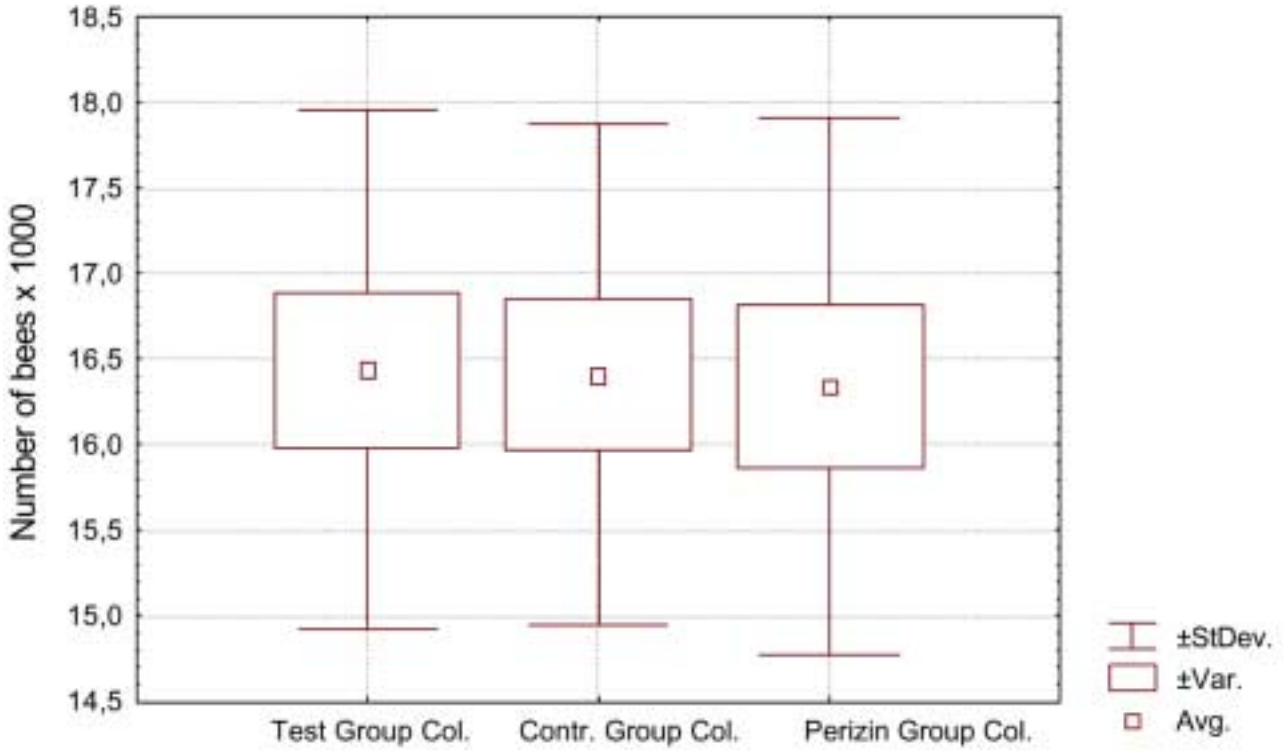
The blooming of snowdrops on the 1st of March announced the spring. The plants grew slowly as a consequence of dry weather. But the mild weather caused an early blooming of trees. It happened mostly to *Black Alder/Alnus Glutinosa* (01.03), *Dogwood/Cornus Mas)* (09.03), *Willow/Salix Caprea* (12.03), *Coughwort/Tussilago Farfara* (19.03), *Forsythia/Forsythia Intermedia* (24.03), *Blackthorn/Prunus Spinosa* (26.03), *Maple/Acer Platanoides* and *Gooseberry/Ribes Grossularia* (29.03), *Red currant/Ribes Sanguineum* (30.03), *Alder/Fraxinus Excelsior, Birch/Betula Pendula* and *Dandelion/Taraxacum officinale* (31.03). The mild weather and the abundant forage sources as well were worrying reasons due to the proper brood development conditions that could lead to a too early and rapid development of the bee colonies.

Results of the Colonies Evaluation in the autumn of the Year 2002

The LIEBEFELDER method was used in the first stages of the project in order to estimate the colonies' strength. The number of bees and the brood development (open and capped) were recorded separately for each colony. The colonies were organized in treatment groups in such a way that the smallest differences regarding the colony strength and Varroa infestation level (determined by natural mite fall count) among the subjects of each group were possible. All the colonies used in this test were young ones with queens produced in 2002.

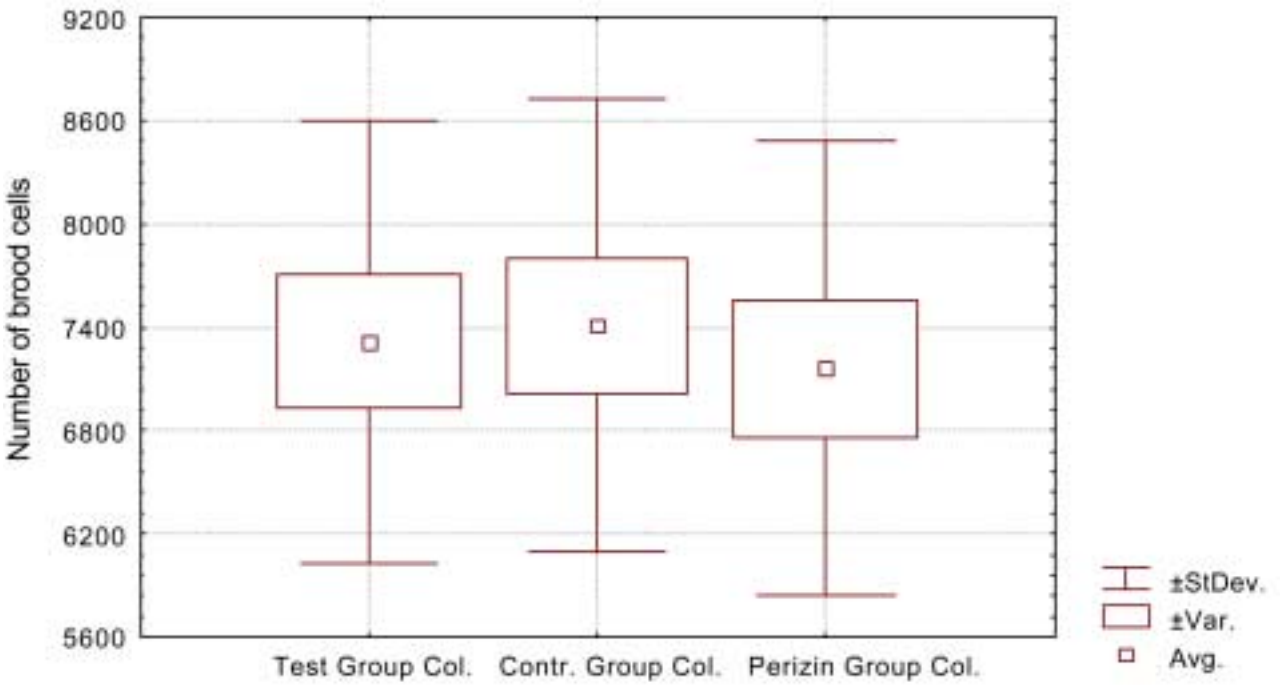
The graphic representation of the average strength of the colonies in the test groups (see graphic 1) shows that the 3 groups are almost identical in this respect. The average number of bees for the tested product group colonies is of 16436 +/- 1515 with a variation width from 14500 to 19000. The control group colonies were slightly weaker, with an average size of 16409 +/- 1459 bees and a fluctuation from 14750 to 18500 bees. The Perizin group was the weakest with an average count of 16341 +/-1570 and fluctuation spanning from 15250 to 19000 bees. The differences among the test groups were insignificant from statistical point of view. In conclusion the counting of bees demonstrated that the young test colonies were capable of good wintering. The number of bees leads to the assumption that very good wintering conditions for the young test colonies were provided.

Graphic 1. Number of bees of the test colonies before the start of the experiment. (n=33)



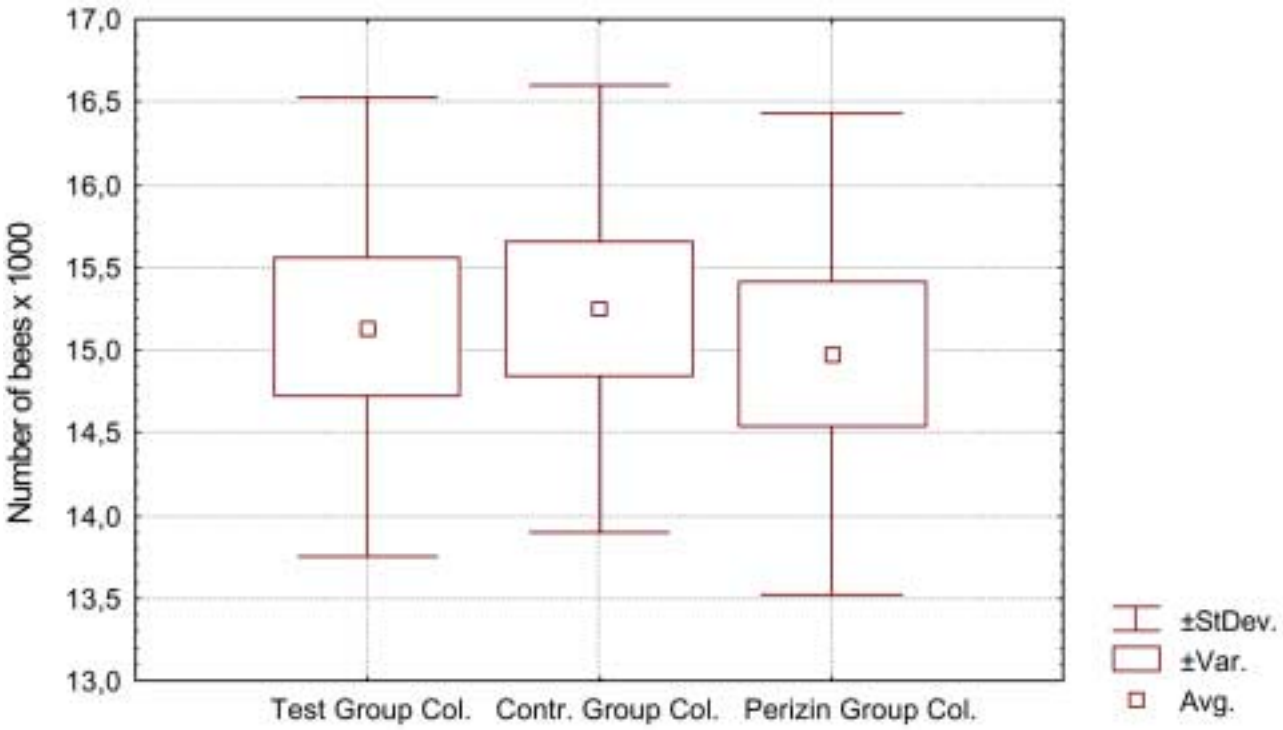
The measuring of the brood strength led to the following outcome (see graphic 2). For the tested product group the average number of brood cells reached 7318 +/- 1284 cells with a fluctuation varying from 6250 to 11000, the control group value was not essentially higher at 7409 +/- 1314 (variation width from 6000 to 11500), meanwhile the Perizin group, with 7159 +/- 1319 (variation width from 5750 to 10150) resulted as the weakest from the brood point of view. The average values for the number of brood cells are, at this time of the year, typical for strong young families. According to the standard deviation, the differences among the test groups are not significant. The application of the different varroacides was performed on a weekly basis at 10.08, 17.08 and 24.08, as previously explained. The behavior of all the colonies during treatments did not show any peculiar features. The bees stayed on the combs, there was not any proof of increased mortality in the tested colonies at any moment during the experiment, immediately after application within the period between applications.

Graphic 2. Number of brood cells (open or capped) of the test colonies before the start of the experiment. (n=33)



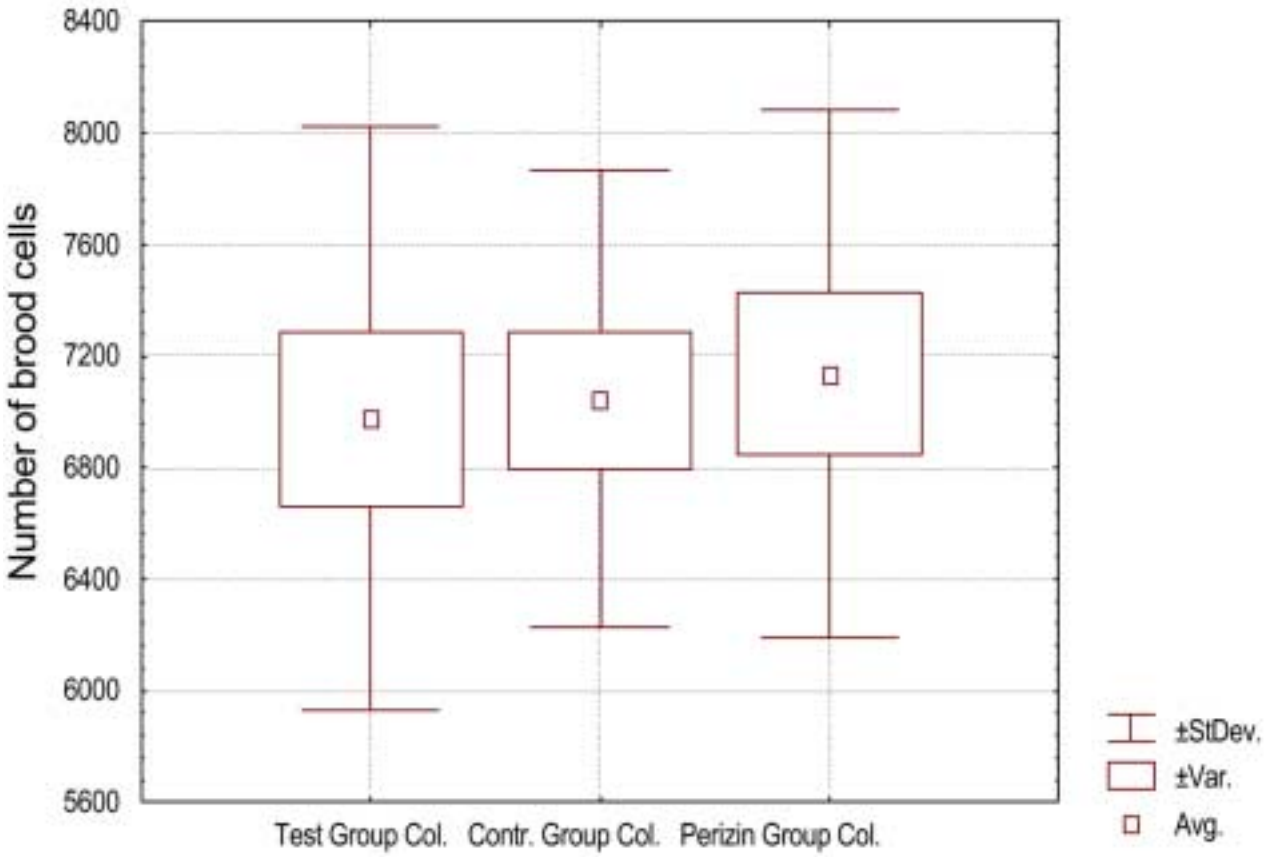
In order to reveal any possible impact of the various treatments on the tested colonies a new evaluation of all tested colonies was performed at the end of test on the 30th of August (see graphics 3 and 4). The measuring of the colony strength showed a slight decrease in comparison to the previous estimation performed on August the 9th. This decrease is caused by the different time of the year of the evaluation and corresponds to the natural development of the colony in autumn. For the tested product group the average number of brood cells was of 15136 +/- 1389, with a fluctuation varying from 13250 to 17500. The control group was slightly stronger with an average number of bees of 15250 +/- 1356 and a variation width from 13000 to 17500 bees. The Perizin treated colonies were the weakest with an average of 14977 +/- 1451 bees and the variation between 12500 and 17250 bees.

Graphic 3. Number of bees of the test colonies at the end of the experiment at 30.08.2002 (n=33).



A similar evaluation was performed for the brood cells (open and capped). For the tested product group the average number of brood cells was 6977 +/- 1045 with a fluctuation of 6000 to 8750, for the control group the average value was slightly higher, of 7045 +/- 820 (fluctuation from 5750 to 8750) and for the Perizin group the average was 7136 +/- 1026 (fluctuation from 5250 to 8750). The differences among groups regarding the bees and brood strength are not significant from statistical point of view due to large standard deviations.

Graphic 4. Number of brood cells (open and capped) of the test colonies at the end of the experiment at 30.08.2002 (n=33).

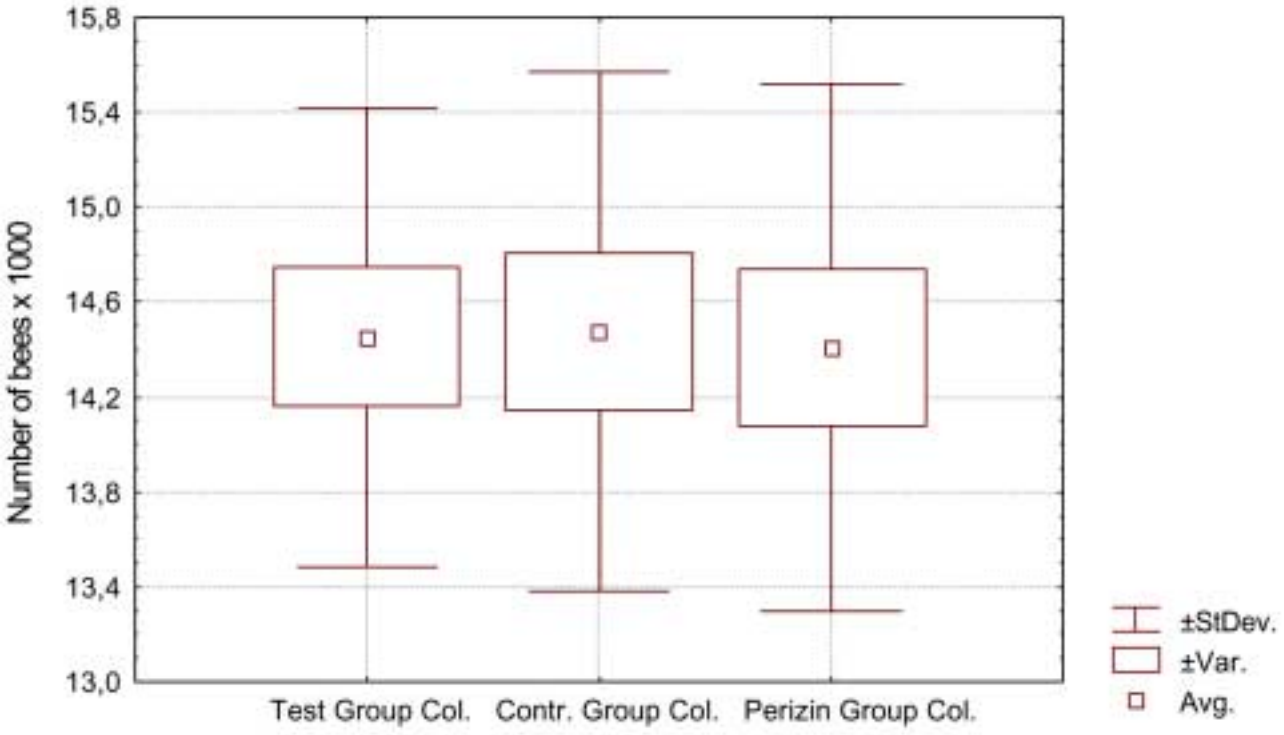


On the 31.08 the final control treatment with formic acid (85%) of all colonies followed. An additional evaluation of the colonies was not performed any more after this control treatment because from numerous publications and from own experience as well we know that after formic acid treatment the entire brood in uncapped stage will be removed.
During the winter the colonies were checked every fortnight in order to prevent unusual situations like various sources of disturbance to the hives, high mortality, Nosema and/or dysentery infection.
During the whole wintering period no anomaly or unusual situation was observed. All colonies were settled solid in winter cluster, no diseases like Nosema and/or dysentery infection were discovered.
Also concerning winter dead fall there was no difference to be seen between different treatment variations.
During all the winter months the natural mite fall was observed. By the occasion of the routine checking performed every fortnight the Varroa sheets were also checked.

The natural mite fall varied from 0 to maximum 3 mites/fortnight, which shows that the test colonies wintered virtually free of Varroa mites.
On 15.03.2003 a new evaluation of the test colonies was performed in order to obtain data regarding to their strength after wintering. In comparison with the number of bees in August, the size of the colonies determined on 15.03.2003 proved only an unessential decrease, which leads back to the conclusion of a good wintering. The average bee loss during the winter was lower than 5%. The colonies belonging to each test group wintered in comparable strength conditions as results from almost identical average number and standard deviation. In case of some of the test colonies even an increase in the number of bees during the wintering phase was obvious. This fact definitely leads back to the early brood development condition of the strong colonies in February.

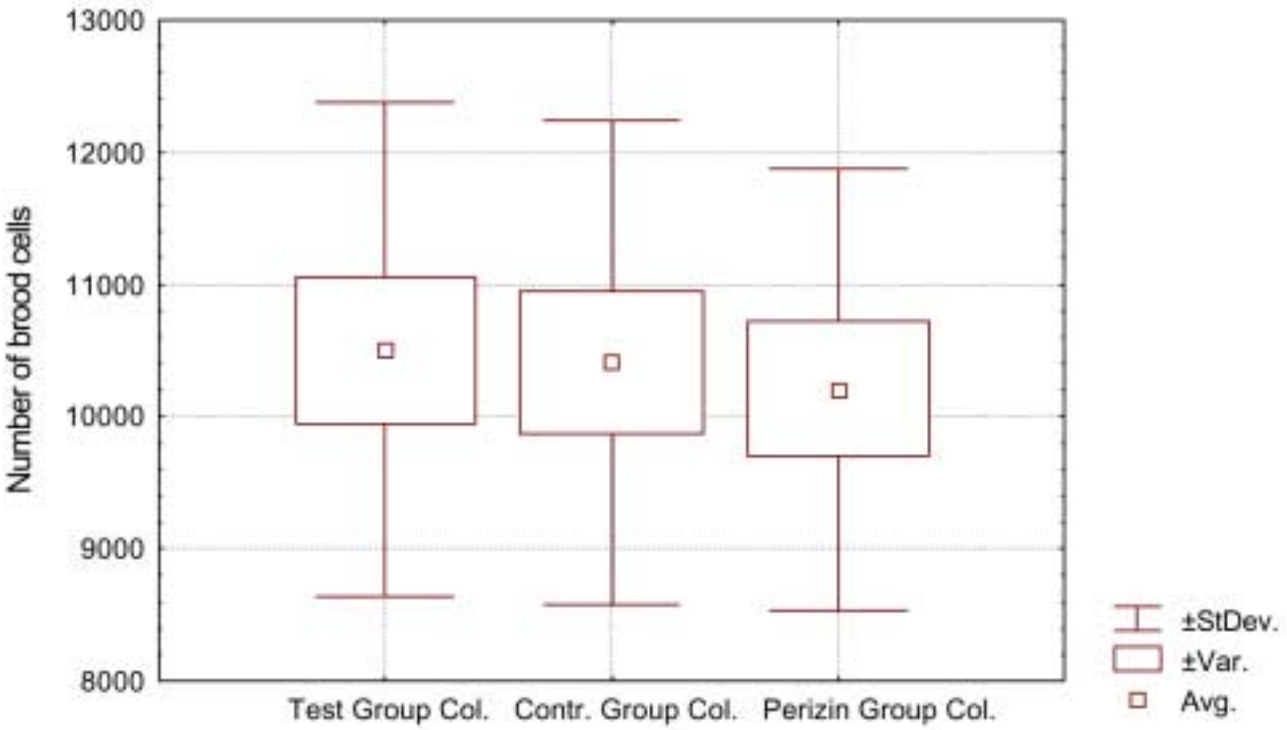
The average number of bees in the tested product group was of 14455 +/- 967, with a variation width from 12750 to 15500, the control group colonies counted 14477 +/- 1098 in the same size range (variation width 12000 to 15750), while the Perizin group colonies were only slightly weaker with 14409 +/- 1108 (variation width 13000 to 16500).

Grafic 5. (text and picture missing)



The evaluation of the brood strength (open and capped) performed after wintering on 15.03.2003 showed the following results (see Graphic 6). For the group of the product tested colonies the average number was of 10500 +/- 1867 cells with a fluctuation between 8500 to 15250, the control group had slightly lower figures with 10409 +/- 1828 (variation width from 8250 to 13500) while the Perizin group colonies were the weakest with an average of 10205 +/- 1676 (variation width 7750 to 13500). The differences among groups are not significant from statistical point of view due to large standard deviations.

Grafic 6 (text and picture missing)



Summary

The results of the Varroa treatments showed that the effectiveness of the tested unknown Varroa treatment (Ver) is about as high as the effectiveness of Perizin . The efficiency after three treatments (found out after a conclusive, one-time treatment with formic acid 85 %) was, for the test-substance between 83.6 and 98.1 %, for Perizin between 82.2 and 97.0 %. The calculated average efficiency for the test-substance is 93.9 +/- 4.1 %, and for Perizin it is 92.3 +/- 4.0 %. For the test-substance there were no greater differences in the efficiency between the single colonies except one (colony No. 7). Therefore the test-substance can be classified as good and reliably efficient. The average efficiency of nearly 94 % and the minor standard deviation within this group proves that the test-substance is good for anti-Varroa-treatment. The reasons for the minor success with the treatment of colony 7 (efficiency = 83.6 %) could not be found, but document that also other anti-Varroa-substances classified as "very efficient", for example Perizin, can sometimes show the same phenomena (look at colony No. 3, efficiency 82.2 %).

In the case of two colonies belonging to the test group there were attempts of requeening, during the treatment period in fall. These attempts were set to an end by removing these queen cells. It cannot be clearly stated if the requeening attempts were just a coincidence or a result of the treatment. At the end of wintering, on the 15th of March 2003, all colonies had not requeened yet. All (marked) young queens were laying eggs.
Perizin, as a systemic working medicine, is normally used (according to its application directions) only in brood free periods of a colony. Several applications normally cause no bee losses worth mentioning, during the winter as well. In contrast to Perizin oxalic acid containing anti-Varroa-substances after several applications seem to cause problems for the colonies in winter.
Therefore it was necessary to examine if three applications of the test-substance would cause any problems for the colonies in winter.
During or short after the application of the test-substance no higher fall of dead bees was noticed.
In contrast to this, Perizin colonies showed, shortly after the application, a slight fall of dead bees (about 30 to 50 bees). It might be that bees are directly contaminated with Perizin and get damaged by an "overdos".

During late fall and winter the colonies of the different treatment groups were checked every two weeks and no unusual situations were noticed. All colonies had built strong clusters. Disease like dysentery and Nosema infections did not occur. No differences in the winter losses between the different treatment groups were noticed. In direct comparison of the Perizin-colonies and the colonies of the test substance no significant differences in the number of bees and the brood-cells (open and capped brood) did occur, not in fall and not in spring.

The slight losses of bees between the population estimation of the 30th of August 2002 and the estimation in the spring on the 15th of March 2003 of less than 5 % is an indication for the very good efficiency and compatibility of the tested substances.

Due to the fact that the colonies withstood the treatment with the test substance without any negative effect and had no problems to winter it is expected (most likely) that the bees will not be damaged by several applications of the test-substance in tropical or subtropical regions, where almost the whole year bees fly and are brood active.

End.