(eficacia AB Var BIO)

Determination of the efficiency of a new varroacide product in *Apis Mellifera* beehives

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Introduction

Due to the severe sanitary and economic damage that varroa causes to beekeeping production and to the producers' growing need of having more efficient, economic and secure tools; trying to avoid homemade formulations and to improve the global situation of Argentinean honeys in regard to medicine residues, we try to show in this test the efficiency of a new product developed to fill these needs and get the official permission of the corresponding authority.

Properties of the product

The product is a varroacide based on thymol and presented in small cakes of 70 g, this last fact makes it completely novel and gives the product outstanding characteristics. The treatment is carried out in a single application over the combs of the brood chamber in the beehives. Once the product is placed, the bees, in their desire to clean the beehive, start crumbling the cake, leaving exposed its different layers with drug; so that the drug is distributed among the population, eliminating the mature varroa mites that are on the bees. The crumbling process lasts around 35 days.

In this way, the treatment covers at least two varroa reproductive cycles and ensures the elimination of the varroa mites that at the moment of applying were inside the sealed cells (protected from the chemicals action) so, a higher level of efficiency is got.
Regarding this brief explanation, the following benefits can be noticed:

- As it is a small cake, it is very easy and safe to use and reduces the risk of exceeding or lacking the dosage.
- The treatment is carried out in a single application, reducing highly the cost on trips and manpower.
- The product disappears from the beehive 35 days after the application, avoiding the contact of the mites with the varroacide, reducing the risk of generating resistance to the active ingredient.

Composition

Thymol	13 g
Excipients	70 g

Dosage
The dose used was a small cake (70 g) for each beehive (all the beehives had at least 5 brood combs at the beginning of the test).

Objectives
Determine the efficiency of the product in varroa control.

Materials and Methods

12 equal standard beehives were used for the test (each one had 5 brood combs), there was activity inside the brood nests of all the beehives at the beginning of the test. The beehives were tested for an estimation on varroa infestation through the method of brushing adult bees into a detergent solution[1]. Two groups of 6 beehives each were formed according to the infestation level. The first 6 beehives with the highest infestation level were included in the group "A" and the other 6 in the group "B". 3 Beehives of each group were treated with this new product (TR) and the others with OXAVAR, a varroacide approved by SENASA, to compare it with the efficiency of our product (TE, Pilot group). As it can be seen in the following table:

	High initial infestation "A"	**Low initial infestation "A"**
Treatment Thymol "TR"	**A-TR**	**B-TR**
Pilot group "TE"	**A-TE**	**B-TE**

The test began on September 29th 2004 when the beehives were divided into groups. The beehives were treated with slow release cakes or OXAVAR, depending on the group the beehive belonged, following the indications of use of these two products.
Every 3 days the fallen varroa mites were counted using a tray[2], specifically created for this use.
The treatment for the groups A-TR and B-TR was considered finished when 100% of the cakes was removed from the beehives. For the groups A-TE and B-TE, the treatment ended two days after the last application based on the application chronogram suggested by the maker.

When the treatment finished, all the fallen varroa mites were counted (VC-TR); then it was carried out a shock treatment in all the groups. This shock treatment consists of 4 applications of gasified Amitraz every 3 days during 12 days. The varroa mites fallen during the shock treatment were counted (VC-TCH). The frequency of this shock treatment is based on the cycle of life of the mite and on the capped-cell stage in the bee's growing cycle, in which the varroa mites are protected from the action of the active ingredient. The sum of varroa mites fallen during the first treatment and the varroa mites fallen during the shock treatment was considered as the total population of varroa (VC-T). In order to validate this assumption it was carried out a colony killing test counting the fallen varroa mites, through the De Jong's method, in one of the beehives of each group.

VC-T = VC-TR + VC-TCH
The efficiency of the treatments (ETR) was estimated in percentage base of the quotient among the varroa mites fallen during the treatment divided by the total population of varroa (VC-T)
ETR = VC-TR / VC-T x 100
The efficiency of the treatment in the pilot group (ETT) was estimated by the same way.

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Results
In Table 1 it can be seen the number of varroa mites fallen during the treatment with thymol cakes per beehive from the groups with high and low infestation (A-TR and B-TR), and the totals (VC-TR). In this table, it can also be seen the results of the shock treatment per beehive (VCTCH), and the efficiency of the thymol cakes treatment per beehive (ETR), per group (ETR "A" and "B") and the total of the treatment (ETR Total), in the bottom line of the table it is shown the standard deviation of the efficiency of the treatment among the beehives.

In Table 2 it can be seen the number of varroa mites fallen during the treatment with OXAVAR per beehive of the pilot group with high and low infestation (A-TE and B-TE), as well as the totals (VC-TE). On the other hand, in the same table it is shown the number of varroa mites fallen during the shock treatment per beehive (ETT) per group (ETT "A" and "B") and the totals of the treatment (ETT Total), in the bottom line of the table it is shown the standard deviation of the efficiency of the pilot treatment among the beehives of this group.

Table 1
Group of treatment with Thymol Cakes

Group	A-TR			B-TR		
Beehive No.	1	2	3	4	5	6
Date of Count						
Oct- 1- 04	164	397	104	67	51	62
Oct- 4 -04	363	547	224	327	97	221
Oct -6 -04	265	311	308	323	130	125
Oct -11 -04	407	420	593	497	57	218
Oct -18 -04	260	180	310	257	31	110
Oct-25 -04	219	84	198	95	58	77
Nov -3 -04	42	83	170	96	61	74
Varroa mites fallen during the treatment (VC-TR)	1720	2022	1907	1662	485	887
Nov -3 -04	**Start of shock treatment**					
Nov -6 -04	75	74	158	44	27	28
Nov -9 -04	17	14	110	13	26	18
Nov-12 -04	0	12	50	15	9	12
Nov-15- 04	0	0	15	6	2	10
Varroa mites fallen during the shock treatment (VC-T)	92	100	333	78	65	68
Total Varroa mites fallen	1812	2122	2240	1740	550	955
Efficiency of the Treatment per Beehive (ETR)	94,92%	95,29%	85,13%	95,52%	88,18%	92,88%
Efficiency of the Treatment per Group “A” y “B”	91,50%			93,50%		
Total efficiency of the treatment ETR Total	92,19%					
STD deviation	4,34%					

Table 2
Pilot Treatment group with OXAVAR

Group	A-TE			B-TE		
Beehive No.	13	14	15	16	17	18
Date of Count						
Oct – 1 - 04	27	27	30	28	39	8
Oct – 4 - 04	59	133	83	29	55	19
Oct – 6 - 04	44	114	24	60	36	39
Oct – 11 - 04	235	238	131	133	52	99
Oct – 18 - 04	150	99	69	53	81	57
Oct – 22 - 04	83	101	53	37	49	21
Varroa mites fallen during the treatment (VC-TT)	598	712	390	340	312	243
Oct – 22 - 04	**Start of shock treatment**					
Oct – 25 - 04	80	110	23	35	54	94
Oct – 28 - 04	245	313	258	179	345	144
Oct – 31 - 04	133	232	213	78	280	130
Nov – 3 - 04	119	179	170	67	259	76
Varroa mites fallen during the shock treatment (VC-TCH)	577	834	664	359	938	444
Total Varroa mites fallen	1175	1546	1054	699	1250	687
Efficiency of the Treatment per Beehive (ETR)	50,89%	46,05%	37,00%	48,64%	24,96%	35,37%
Efficiency of the Treatment per Group "A" and "B"	45,03%			33,95%		
Total efficiency of the treatment ETT Total	40,48%					
STD deviation	9,85%					

Results of the Colony Killing Test

In order to validate the effectiveness of the shock treatment, it was carried out a colony killing test after the last time of count (and later the varroa of all the adult bees and the brood were counted) in the beehive No. 2 for the group of treatment with thymol cake and the beehive No. 13 for the group of pilot treatment with OXAVAR, (in both groups the shock treatment was carried out with gasified Amitraz).
In Table 3 the results of the colony killing test can be seen:

Table 3
Colony Killing Test

Group	Thymol	OXAVAR
Beehive No.	2	13
Varroa mites fallen during the treatment (VC-TCH)	2022	598
Varroas fallen during the shock treatment (VC-TCH)	100	577
Total varroa mites fallen (VC-T)	2122	1175
Efficiency of the Treatment per Beehive (ETR)	95,29%	50,89%
Count of varroa mites after the colony killing Test	13	70
Total varroa mites + Varroa mites from the colony killing test	2135	1245
Efficiency of the Treatment Corrected	94,71%	48,03%
Diference %	0,58%	2,86%

According to the results obtained from the colony killing test, the total efficiency of the treatment with Thymol cakes (ETR) and the pilot treatment with OXAVAR (ETT) could be corrected in the following way:
ETR Thymol cakes = 97,99% - colony killing Dif. 0,71% = ETR corrected 97,28%
ETT OXAVAR = 40,48% - colony killing Dif. 2,86% = ETT corrected 37,62%

Life of Thymol Cakes
In Table 4 it can be seen, marked with an "X", the date when Thymol cakes disappeared completely from each beehive. It can also be seen the duration per beehive, the mean, the median, the mode and the standard deviation (days) for the group of beehives with this treatment.

Table 4

Beehive No.	1	2	3	4	5	6
Sep – 29 - 04						
Oct – 1 - 04						
Oct – 4 - 04						
Oct – 6 - 04						
Oct – 11 - 04						
Oct – 18 - 04						
Oct – 25 - 04	X				X	
Nov – 3 - 04		X	X	X		X
Duration (days)	26	35	35	35	26	35
Mean		32				
Median		35				
Mode		35				
STD deviation		4,648				

Conclusion
Thymol cakes proved to be more efficient than the acaricide OXAVAR approved by SENASA. In conclusion Thymol cakes - in regard to their efficiency and their practical use - could be used to control varroa in commercial beehives.

1. Method of brushing bees into detergent solution or David De Jong's Test
A vessel to strain bees is prepared. This vessel is made with a plastic bottle whose bottom is cut and a screen (of 4 mm) is put at the mouth. Then the bottle is corked, it is put upside down and filled up to the middle with a detergent solution. From the center of the beehive, 200 bees are brushed into the strainer as a sample and it is shaken during 3 or 5 minutes. The bottle is uncorked and the liquid is poured on a white piece of cloth that is placed on a wide-mouthed container. The bees will remain inside the bottle due to the screen, the liquid will go into the wide-mouthed container and the mites will end on the white piece of cloth where they would easily be seen and counted. The formula to evaluate infestation percentage is the following:

$$\% \text{ of infestation} = \frac{\text{No. of mites collected}}{\text{No. of bees from the sample}} \times 100$$

2 Tray method

For this method, it is necessary a sanitary bottom board or a tray of 33 by 45 cm (according to beehives measures) with an edge of 2 cm in width and 1 cm in height, opened up in one of its short sides, the tray is covered with a squared screen (8 squares per lineal inch), in such a way that a space is left between the screen and the tray bottom, there it is placed a white sheet of paper or cardboard, spread with vegetable or car oil.

The tray with the greasy piece of paper is placed between the brood chamber and the bottom board avoiding to block completely the entrance.

These methods were taken from the Beekeeping Pathology Manual, from the Secretary of Agriculture, Stock Breeding, Rural Development, Fishing and Feeding of the Argentine Republic, page 18.