

## METICAN AGAINST VARROATOSIS AMONG BEES

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### ABSTRACT

*The acaricidal activity of Metican (Ecostop), a product of "Primavet – Sofia" Ltd. composed of 5 g menthol and 2 ml peppermint oil, has been tested on honey bee colonies in Dadant-Blat hives. The preparation is designed for prevention and treatment of varroatosis in honey bee colonies and is offered as solid aerosol with long emission of the active substances.*

*Metican (Ecostop) has been applied during the autumn of 2001 and the spring of 2002 in the area of the town of Stara Zagora, Bulgaria at 1 to 3 doses depending on the strength of the bee colonies for a period of 45 days.*

*In the autumn, an acaricidal effect of  $93.34 \pm 1.21\%$  has been determined after application of 2 doses to a colony on 7-8 frames with infestation level of  $8.43 \pm 1.13\%$  for the bees and  $14.80 \pm 2.15\%$  for the brood. An effectiveness of  $92.24 \pm 2.46\%$  has been recorded after application of 3 doses to a colony on 10-12 frames with infestation level of  $6.53 \pm 2.86\%$  for the bees and  $27.61 \pm 8.54\%$  for the brood.*

*In the spring, Metican (Ecostop) has shown an effect of  $90.59 \pm 1.83\%$  against varroatosis at application of 2 doses to colonies with 6-8 combs and infestation level of  $4.98 \pm 1.40\%$  for the bees and  $46.80 \pm 21.17\%$  for the brood.*

*It has been ascertained that Metican (Ecostop) has a good acaricidal activity against the agent of varroatosis – *Varroa jacobsoni*.*

### INTRODUCTION

The alternative means for fighting against varroatosis have been known a long time ago. Now they have become especially pressing because in many countries *Varroa jacobsoni* developed resistance toward various conventional acaricidal agents.

In alternative therapy and prophylaxis no resistance to the natural products, such as essential oils, acids, etc. is created. The risk of contamination of bee products is reduced because the alternative substances are of natural origin, nontoxic for bees and humans, and are contained in bee honey.

The natural climatic conditions and the beekeeping technology in the Republic of Bulgaria allow for the successful application of the alternative means and methods for fighting against varroaosis. These circumstances allow for development and testing of the effectiveness of new preparations based on acids, essential oils and other natural substances under the conditions of our country and to register them as veterinary medicinal preparations.

The present investigation aims at clinical trying of the effectiveness of Metican (Ecostop). Metican (Ecostop) is a preparation manufactured at Primavet – Sofia, Ltd. for fall 2001 and spring 2002 treatments against varroaosis in bees.

## MATERIALS AND METHODS

The investigations were carried out during June – September 2001 and April – May 2002 on 40 bee colonies at a test apiary in the region of the city of Stara Zagora. The bee colonies were treated with Metican (Ecostop) /active substance containing 5.0 grams of thymol and 2.0 ml of mint oil/, that was offered as solid aerosol with prolonged release of the active substances.

Control and trial groups were set up. In the trial groups Metican (Ecostop) was applied according to the manufacturer's instructions, and the results obtained were compared to the results obtained from a group that was treated with Varrostop, as well as to the results obtained from a control group that remained untreated. Observations looking for manifestations of adverse side effects of Metican (Ecostop) upon the bee colonies, such as death among the queen-bees, bees, and brood, as well as "robberies", have been carried out.

The spring treatment was conducted in the following way:

- Group I: 10 bee colonies with strength of  $1,72 \pm 0,04$  kg were treated with 2 plates of Metican (Ecostop) for 14 days. In 2001 5 of these colonies were treated with 3 plates, and 5 of them were treated with Varrostop.
- Group II: 10 bee colonies with strength of  $1,72 \pm 0,04$  kg were treated with 2 plates of Metican (Ecostop) for 45 days. In 2001 5 of these colonies were treated with 2 plates, and 5 of them were treated with Varrostop in 2001.
- Group III /K<sub>1</sub>/: 10 bee colonies with strength of  $1,82 \pm 0,05$  kg were treated with 2 strips of Varrostop (3.6 mg of flumetrin in a single strip) for 45 days. In 2001 these colonies were treated with 1+1 plates of Metican (Ecostop) at an interval of 15 days.
- Group IV /K<sub>2</sub>/: 10 bee colonies with strength of  $1,77 \pm 0,06$  kg remained untreated. In 2001 these colonies constituted a control group as well.

The changes in the numbers of the mites fallen were determined within the trial groups on the 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup>, 35<sup>th</sup>, 42<sup>nd</sup>, and 45<sup>th</sup> days.

In order to obtain a more comprehensive and complex evaluation of the effectiveness of Metican (Ecostop) the EI /Extensinvasion/ of trial and control groups before and after the treatment was determined.

The acaricidal activity of the preparation was established via control treatment of the trial and control groups with Varrostat – R /Rodovarr/ by determination of the number of the mites fallen. The preparation contained Amitraz 12,5%, and was applied on the 14<sup>th</sup> day for Group I and on the 45<sup>th</sup> day for Groups II, III, and IV respectively.

The results represented undergone variation – statistical work-up via the routine methods with the help of a computer program.

## RESULTS

From the investigations carried out during 2001 fall, it was established that the treatment of the bee colonies with two plates manifested good effectiveness. After the application of two or three plates no reliable difference in effectiveness was observed -  $93.34 \pm 1.21\%$  effectiveness with parasite infestation rate of  $8.43 \pm 1.13\%$  among bees and  $14.80 \pm 2.15\%$  among brood, and  $92.24 \pm 2.46\%$  effectiveness with parasite infestation rate of  $6.53 \pm 2.86\%$  among bees and  $27.61 \pm 8.54\%$  among brood. Bee colonies spent the winter without losses of bees and queen-bees.

At the initiation of the spring experiment the development of the colonies was within the normal ranges for the season /strength of  $1.72 \pm 0.04$  to  $1.82 \pm 0.05$  kg/. After the completion of the experiment the strength of the colonies in the trial and the control groups was increased /from  $2.00 \pm 0.06$  to  $2.60 \pm 0.07$  kg/,  $p \leq 0.05$  /Table 1/.

**Table 1.** Condition of Bee Colonies

\* 14 days duration of the experiment (April 21st – May 5th, 2002)

\*\* 45 days duration of the experiment (April 21st – June 5th, 2002)

n = 10

Groups	Beginning – April 21 <sup>st</sup> 2002				End – June 5 <sup>th</sup> 2002			
	Strength – kg		Brood – number of kg		Strength – kg		Brood – number of kg	
	x ± Sx	C	x ± Sx	C	x ± Sx	C	x ± Sx	C
Group I (2 plates of Ecostop)*	1.72 ± 0.04	8,23	8660 ± 642.25	23.45	2.00 ± 0.06	10.21	8170 ± 387.02	14.98
Group II (2 plates of Ecostop)*	1.72 ± 0.04	8.23	8310 ± 514.34	19.57	2.32 ± 0.07	10.20	10620 ± 729.96	21.74
K <sub>1</sub> (2 bands of Varrostop)**	1.82 ± 0.05	9.25	7650 ± 308.13	12.74	2.42 ± 0.05	6.96	10760 ± 474.50	13.94
K <sub>2</sub> (untreated)**	1.77 ± 0.06	10.39	8860 ± 453.19	16.17	2.60 ± 0.07	8.11	12080 ± 509.86	13.35
Reliability			K <sub>1</sub> -K <sub>2</sub> (P ≤ 0.05)		II – K <sub>2</sub> (P ≤ 0.05)			

The values of the parasite infestation rate /extensinvasion, EI/ of the mites before and after the treatment that were obtained pointed out that the randomly chosen bees and larvae were infested with parasites at a rate that correlated with the strength of the colony /p ≤ 0.001/. The percentage of EI among the bees before the treatment was from 3.71 ± 1.08% to 4.98 ± 1.40% in the trial groups and from

1.47±0.14% to 1.59±0.18% in the control groups. Among the brood the following values were obtained: from 23.60±10.45% to 46.80±21.17% in the trial groups and from 10.00±2.00% to 11.20±2.21% in the control groups respectively /Table 2/. Thus the mites were concentrated within the sealed brood-cells that manifested higher values of EI, bearing in mind that EI was relatively less in spring than in fall.

**Table 2.** Extensinvasion among Bee Colonies

\* 14 days duration of the experiment (April 21<sup>st</sup> – May 5<sup>th</sup>, 2002)

\*\* 45 days duration of the experiment (April 21<sup>st</sup> – June 5<sup>th</sup>, 2002)

Signs	Groups			
	I*	II**	K <sub>1</sub> **	K <sub>2</sub> **
	(2 plates of Ecostop)	(2 plates of Ecostop)	(2 bands of Varrostop)	(untreated)
	x ± Sx	x ± Sx	x ± Sx	x ± Sx
1. Extensinvasion among bees				
- Beginning (April 21 <sup>st</sup> , 2002), %	3.71 ± 1.08	4.98 ± 1.40	1.47 ± 0.14	1.59 ± 0.18
- End (June 5 <sup>th</sup> , 2002), %	-	0.90 ± 0.11	0.80 ± 0.56	5.94 ± 1.42
Reliability				
Beginning	II – K <sub>1</sub> (P≤0.05)	II – K <sub>2</sub> (P≤0.05)		
End	II – K <sub>2</sub> (P≤0.01)	K <sub>1</sub> – K <sub>2</sub> (P≤0.001)		
2. Extensinvasion among brood				
- Beginning (April 21 <sup>st</sup> , 2002), %	23.60 ± 10.45	46.80 ± 21.17	10.00 ± 2.00	11.20 ± 2.21
- End (June 5 <sup>th</sup> , 2002) - %	-	2.80 ± 0.90	0.00 ± 0.00	14.40 ± 1.43
Reliability				
Beginning	-			
End	II – K <sub>2</sub> (P≤0.001)	K <sub>1</sub> – K <sub>2</sub> (P≤0.001)		

After the plates have stayed in the beehives for 45 days, the EI of the mite in the trial group that was treated with two plates was  $0.90 \pm 0.11\%$  among bees and  $2.80 \pm 0.90\%$  among larvae. The control group that was treated with Varrostop ( $K_1$ ) showed the lowest EI rate –  $0.80 \pm 0.56\%$  among bees and  $0.00 \pm 0.00\%$  among brood. In the control (untreated) group ( $K_2$ ) EI rate was increased from  $1.59 \pm 0.18\%$  to  $5.94 \pm 1.42\%$  among bees and from  $11.20 \pm 2.21\%$  to  $14.40 \pm 1.43\%$  among larvae / $p \leq 0.001$ /. This was due to the breeding of the mite and pointed out that the parasite was still found in a higher percentage within the sealed brood-cells.

When monitoring the number of the mites fallen the following data were obtained in the trial groups treated with Meican (Ecostop). In Group I /treatment lasted for 14 days/ the number of the mites that have fallen was  $379.6 \pm 70.94$ . In Group II /treatment lasted for 45 days/  $820.2 \pm 216.32$  mites have fallen, no matter that there was no reliable difference in the value of the parasite infestation rate in Groups I and II. In the group that was treated with Varrostop  $311.7 \pm 68.52$  mites have fallen.

The number of the mites fallen after the control treatment in the trial groups was  $257.7 \pm 41.38$  in the group that was treated with 2 plates for 14 days, and  $62.60 \pm 8.95$  in the group that was treated with 2 plates of Metican (Ecostop) for 45 days respectively / $p \leq 0.001$ /. In the control group that was treated with Varrostop  $1.3 \pm 0.27$  mites have fallen and in the control group that remained untreated, a reliably greater amount of mites have fallen, i.e.  $259.89 \pm 33.45$  mites in comparison with Group II and  $K_1$  ...  $p \leq 0.001$  /Table 3/.

**Table 3.** Mites Fallen (Number) Due to the Treatment\* 14 days duration of the experiment (April 21<sup>st</sup> – May 5<sup>th</sup>, 2002)\*\* 45 days duration of the experiment (April 21<sup>st</sup> – June 5<sup>th</sup>, 2002)

Periods of determination	Groups			
	I*	II**	K <sub>1</sub> **	K <sub>2</sub> **
	x ± Sx	x ± Sx	X ± Sx	x ± Sx
1. Total number of mites fallen due to treatment with Ecostop	379.6±70.94 (81 – 799)	820.2±216.32 (163 – 2117)	311.7±68.52 (53 – 670)	142.6±41.21 (38 – 437)
- April 28 <sup>th</sup>	263.1±50.47 (22 – 536)	346.9±99.49 (67 – 1010)	194.3±39.39 (28 – 409)	28.3±10.97 (4 – 114)
- May 5 <sup>th</sup>	116.5±22.86 (32 – 263)	134.3±37.43 (44 – 415)	76.9±24.49 (7 – 222)	15.5±6.88 (1 – 69)
- May 12 <sup>th</sup>		169.4±65.76 (24 – 634)	16.1±2.57 (3 – 25)	21.7±8.43 (1 – 83)
- May 19 <sup>th</sup>		76.5±17.05 (7 – 157)	6.9±1.43 (1 – 15)	16.1±5.87 (1 – 55)
- May 26 <sup>th</sup>		44.2±9.02 (6 – 88)	6.6±0.64 (4 – 10)	20.9±2.72 (12 – 38)
- June 2 <sup>nd</sup>		37.5±9.71 (1 – 104)	6.1±1.03 (3 – 12)	30.1±6.76 (7 – 68)
- June 5 <sup>th</sup>		11.4±2.26 (3 – 26)	4.8±1.12 (1 – 12)	10.0±2.73 (1 – 26)
2. Control treatment (2 Bands of Rodovarr) - June 5 <sup>th</sup> (for Group I)  - June 6 <sup>th</sup> (for the other groups)	257.7±41.38 (72 – 463)	62.6±8.95 (26 – 108)	1.3±0.27 (0 – 2)	259.89±33.45 (129 – 406)
3. Total number of mites fallen (1 + 2)	637.3±86.70 (223 – 1117)	882.8±222.82 (193 – 2225)	313.0±68.49 (55 – 672)	406.11±77.14 (167 – 843)

The established effectiveness of Metican (Ecostop) manifested /Table 4/ that the preparation possessed a relatively high acaricidal activity during spring treatment with 2 plates for 45 days of colonies with strength of  $1.72 \pm 0.04 - 90.59 \pm 1.83\%$ . Its acaricidal activity was lower when 2 plates for 14 days were applied, namely  $57.45 \pm 6.65\%$  -  $p \leq 0.001$

**Table 4.** Effectiveness of Treatment of Bees with Ecostop (2002)ž

Signs determined	G r o u p s			
	I*	II**	K <sub>1</sub> **	K <sub>2</sub> **
	x ± Sx	x ± Sx	x ± Sx	x ± Sx
1. Total number of mites fallen after treatment with Ecostop	379.6±70.94 (81 – 799)	820.2±216.32 (163 – 2117)	311.7±68.52 (53 – 670)	142.6±41.21 (38 – 437)
2. Total number of mites fallen after control treatment (2 bands of Rodovarr)	257.7±41.38 (72 – 463)	62.6±8.95 (26 – 108)	1.3±0.27 (0 – 2)	259.89±33.45 (129 – 406)
3. Total number of mites fallen (1 + 2)	637.3±86.70 (223 – 1117)	882.8±222.82 (193 – 2225)	313.0±68.49 (55 – 672)	406.11±77.14 (167 – 843)
4. Effectiveness, %	57.45±6.65 (14.89– 82.22)	90.59±1.83 (79.41 – 95.74)	99.15±0.39 (96.36 – 100.0)	31.33±3.92 (17.16 – 51.84)

During the entire course of the experiment no manifestations of adverse side effects upon the queen-bee, the bees, and the brood were observed to the preparations tested.

## DISCUSSION

The development of resistance to the acaricidal preparations belonging to various chemical groups represents a problem in many countries. When using Apistan or its analogs for a long time it has been established that *Varroa* has got accustomed to it in various European countries. Trouller (1998) established in his investigations that were carried out in 1995 – 1997 the presence of resistant offspring of the *V. jacobsoni* mite in Italy, Slovenia, Switzerland, France, Belgium, Austria, etc.



According to Moosbeckhofer (1994) pyrethroids were accumulated in bee products after their usage for 7 or more years.

Thymol and essential oils are widely used as acaricidal agents in the alternative therapy. Besides acaricidal activity, they act against the bee-louse (*Braula caeca*) and the tracheal mite (*Acarapis woodi*). Different concentrations and doses of thymol were tested independently or with various rate frequency, intervals and duration of treatment. The effectiveness established varied from 82% to 98.8% (Naneti, 1996). The application of thymol in combination with other natural substances, such as essential oils and acids, enhanced its effect (Bogdanov et al. 1999). The Swiss preparation Api-Life-Var that contained 75% thymol, 16.4% eucalyptol, 3.8% menthol, and 3.8% camphor manifested good acaricidal effectiveness within the range 95% to 98.6% (Abou-Zaid et al. 1995; Imdorf et al. 1995; Moosbeckhofer 1994).

No resistance to natural products was established as well as no adverse influence of essential oils upon bee colonies under proper conditions of application (within the 14°C to 25°C temperature range) was seen. The risk of contamination of bee products was reduced as well (Imdorf et al. 1999).

It was established that the remaining amounts of the natural substances in the bee products disintegrated in the course of one week, and their amounts measured in honey were small and did not harm the consumer (Imdorf et al. 1999).

The new requirements for the production of pure bee products, including honey, direct the investigators to test acaricidal agents based on natural substances.

During the course of the experiments conducted by us in order to determine the effectiveness of Metican (Ecostop) (active substance 5.0 grams of thymol and 2.0 ml of mint oil) against varroaosis in bees, a greater number of mites fallen in the group that was treated for 45 days in comparison with the one that was treated for 14 days, was established inspite of the similar parasite infestation rates in both groups. This fact showed that the Metican (Ecostop) plates should remain in the bee nest at least for 45 days in order their acaricidal effect to be manifested. The smaller number of mites fallen among the bee colonies that were treated with Varrostop (in comparison with the other groups) can be explained with the relatively lower parasite infestation rate in the beginning of the experiment.

The effectiveness of Meticane (Ecostop) in the second group (90.59±1.83%) that was observed confirmed our statement that the treatment should continue for at least 45 days in order to eliminate a sufficient percentage of the mites so they could not cause harm to bee colonies. These results confirm the data of other authors who got 95–98% effectiveness during treatment with Api-Life-Var (Abou-Zaid et al. 1993; Imdorf et al. 1993, 1994; Moosbeckhofer 1993).

The good acaricidal effect in the group that was treated with Varrostop (99.15±0.39 %) proved that in the Republic of Bulgaria no resistance to flumetrin has been created yet.

The observation of the group that was treated showed that Metican (Ecostop) did not have any adverse side effects upon the bee colonies, such as death of queen-bees,

bees and brood, and no robberies were observed during the treatment with this preparation.

The results obtained by us provide good reasons to consider this preparation a suitable means for fighting against varroatosis in bees under the conditions in the Republic of Bulgaria, due to its proven effectiveness when applied during fall and spring treatments. Metican (Ecostop) will supplement the limited spectrum of preparations for fighting against varroatosis at the apiaries with organic apiculture and it will provide the apiarists the opportunity to produce bee honey without remnants of chemically based acaricidal agents that are harmful to humans.

Metican (Ecostop) manifested its greatest effect against varroatosis /90.59±1.83%/ when applied in a dose of 2 plates to colonies with strength of 1.72±0.04 kg and parasite infestation rate of 4.98±1.40% among the bees and 46.80±21.17% among the brood.

57.45±6.65% effectiveness was achieved via 2 plates of Metican (Ecostop) in colonies with strength of 1.72±0.04 kg and EI of 3.71±1.08 among the bees and 23.60±10.45 among the brood when the duration of the treatment was 14 days.

Varrostop in a dose of 2 strips applied in colonies with strength 1.82±0.05 kg manifested 99.15±0.39% effectiveness.

When Metican (Ecostop) was properly applied no adverse influences upon bee colonies were observed.

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