ПЧЕЛАРСТВО

EVALUATION OF OPTIMAL TIME OF HONEYBEE (*APIS MELLIFERA*) TREATMENT WITH ANTI-MITE PREPARATIONS

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The first sources of *Varroa jacobsoni* mites in Lithuania were discovered in 1979, and despite taken drastic measures (the honeybee families were put to sleep or burned), varroatosis spread wider. Supposedly, the area of honeybee varroatosis enlarges 10-15 km each year (**Stevenson et al.,** 2005), but the rate of contagion is influeanced by climatic conditions (**Harris et al.,** 2003). Only in 2000 it was found that *Apis mellifera* kept in Europe was assailed by another mite *Varroa destructor* which also came from Asia (**Anderson et al.,** 2000). The level of honeybee disturbance depends on the honeybee species too (**de Guzman et al.,** 2007). Africanised honeybees are less sensitive to varroa mites, and at the same time the honeybee colonies are also more resistant to them (**Martin,** 2004). The mites might be spreaders of honeybee deseases (**Zhang et al.,** 2007; **Nordstrom,** 2003), and furthermore, honeybees infested with mites become less resistant to contagious deseases (**Shen et al.,** 2005; **Celle et al.,** 2008), their behaviour changes, they become aggressive (**Caroline et al.,** 2001). It is considered that the critical limit of *Varroa destructor* mites per one honeybee family is 5000, and besides, the viral infections have a negative impact too.

A number of chemical substances are suggested to fight honeybee varroatosis, but there always remain a possiblity that they will get into the honey (**Bogdanov et al.,** 1998) or wax (**Korta et al.,** 2003), and besides, the mites become resistant to the preparations used against them (**XIE et al.,** 2008; **Lipinski and Szubstarski,** 2007). It is assumed that biological devices could be also used to fight *Varroa destructor* mites (**Chandler et al.,** 2001). The effect of treatment is influenced not only by the efficiency of preparations but also by the time they are used and by the meteorological condition (**Bacandritos et al.,** 2007). It is supposed that tropical climate does not have either negative or positive effect on the efficiency of some preparations (**Sammatoro et al.,** 2008). If the strips impregnated with Gabon, Apistan or another medicine are used after the honeybee feeding for winter, when the activity of honeybees is low, the active substances of these preparations do not penetrate honeybees colony and their efficiency to strive against *Varroa destructor* mites decreases (**http://www.bitininkas.lt/ligos/varoze.htm**).

The aim of this study was to investigate the dynamics of mite distribution in honeybee colonies and the efficiency of chemical substances used to prevent honeybee varroatosis before and after the autumn feeding. The number of mites fallen from honeybees was chosen to be a criterion of efficiency of substances used against mites.

MATERIAL AND METHODS

The study was carried out in the apiaries of northern Lithuania in 2009. The dynamics of *Varroa destructor* mite distribution in honeybee colonies was investigated in May, the variation dynamics of *Apis mellifera* honeybee infection with mites was investigated in the active period after the spring treatment of honeybees and the efficiency of the applied chemical substances was investigated before and after the autumn feeding.

Six honeybee colonies with 9 combs squated by

honeybees were used for the investigation of the dynamics of mite distribution in honeybee families. The level of colony infection awas aproximately 11.3 ± 2.17 % (the honeybee colonies were not treated for *Varroa destructor* mites in the autumn). The catchers of mites were put under the combs. The bottoms of the catchers were lined with paper sheets smeared with vaseline and divided into 72 squares. A box with Tymovaroi (VET - AGRO, Poland) preparation was put on the frames of the nest. The cover of the box was turned half of the winding so that there would be a gap of 5 mm left. The preparation was composed of Thymol and medical filling. The preparation was kept there for 14 days and afterwords the subframes were taken out. The place of the highest mite concentration in the honeybee colony and the preparation efficiency during the spring treatment was estimated according to the number of mites fallen on the subframe bottom.

The residue level of *Apis mellifera* honeybee infection with mites was estimated after the treatment with Tymovaroi preparation in the same honeybee colonies. The infection dynamics with *Varroa destructor* mites was investigated after the treatment till the end of the honey gathering season.

In order to estimate the level of honeybee infection with mites, the honeybees were taken from three places of the hive (from the middle comb and from both edge combs). The honeybees were shaken down in to a vessel with a gauze pad attoched to the cover and moistened in ether. Then the honeybees and the mites on them were counted up. The level of the mites infestation was expressed in percentage and is equal to the number of mites divided by number of investigated honeybees and multiplied by 100. On the purpose to estimate the level of the mite infestation, no less than 200 insects were taken from every honeybee colony. The number of mites fallen from honeybees has been chosen to be a efficiency criterion of substances used against mites. Also the the number of naturally fallen mites per day in the family was estimated before the treatment with preparations. The number of fallen mites was examined every third day, then this number was divided by the number of the days from the last examination.

The standard mite catchers with paper sheets smeared with vaseline (because part of the mites are still alive after the treatment) were put under the frames to estimate the number of fallen mites. The catchers were fastened with rubber strips because the bottom often separates from the top while putting them into the hive. The wire was fastened to the first mite catcher put under the nest comb, other subframes were laid down on the wire and moved under the frames. After the treatment the subframes were pulled out with the help of the wire, and the fallen mites were calculated.

The honeybees were treated chemical by in spring after the first flights, then after the honey gathering and in autumn after the expansion of the brood. The substances used in fighting *Varroa destructor* mites were Thymol, Tymovaroi (VET - AGRO, Poland, composed of thymol and medical filling), Apistan (VITA Europe Ltd, England) and oxalic acid. These substances were used in springo, besides 2 % aqueous solution of oxalic acid was used as an ecological device before and after autumn honeybee feeding. Honeybee families of average strength (9 combs fully squated with honeybees) were put to treatment.

Tymovaroi preapration was kept for 14 days put on the frames of the hive in the middle of the colony. After taking out the subframes the fallen mites were counted on the bottom of the subframes. The number of naturally fallen mites was found to be about 19.3 ± 4.7 per day, the average level of infestation in the families was 11.3 ± 2.17%.

When treating honeybees with Thymol in spring, the powder of Thymol was poured into gauze bags of 5g in each, placed on the top of the nest, covered with cerecloth and kept warm. There were three bags on the top of each nest. The preparation was kept there for 14 days, then the subframes were taken out and the fallen mites were counted. The number of naturally fallen mites was found to be about 23.7 ± 3.3 per day, and the average level of infestation was 6.26 ± 0.73%.

Honeybees colonies were treated in spring with 2% aqueous solution of oxalic acid although it is recommended to use the mixture of 2.9 % oxalic acid and 31.9 % sugar syrup (**Gregorc,** 2002). If the sugar syrup is applied to prepare the oxalic acid solution, these is a greates possibility for oxalic acid to

get into the body of honeybee and combs with honey when the honeybee cleans its body surface. The number of naturally fallen mites was found to be approximately 21.5 ± 0.72 per day in the honeybee colony and the average level of family infestation was 7.4 ± 0.73%. A manual sprayer was used for aerosol covering of the surface of honeybee bodies. At the time of treatment the temperature was about 14°C.

When treating honeybees with Apistan, two stripes with 0.8 g active substance Fluvalinate were put in to each honeybee family. The preparation was kept for 14 days. The number of naturally fallen mites was found to be about 11.3 ± 1.31 per day in honeybees colony, and the average level of family infestation was 9.3 ± 0.27 %.

Two honeybees colonies group, with 10 families each were treated with 2 % oxalic acid before and after autumn feeding by spraying it twice every seven days. The level of the infection with Varroa destructor mites in the colonies was approximately from 17.0 ± 1.38 % to 16.2 ± 1.41 %.

Statistical analysis. The investigation data were processed using statistical package Statistica for Windows version 6.0 (**StatSoft,** 2001) and following the basic guide to the statistical analysis of biological data by **Tucker** (2003). The difference was considered significant when $P< 0.05$.

RESULTS AND DISCUSSION

Six hives with 9 combs squated with honeybees were used for investigation of the dynamics of mite distribution in each honeybee colony. The infestation level of colonies was approximately 11.3 ± 2.17%. The bottoms of the catchers were lined with paper sheets smeared with vaseline and divided into 72 squares. There was a box with Tymovaroi (VET - AGRO, Poland) preparation put on the frames of the nest and kept there for 14 days. When the subframes were taken out, according to the amount of fallen mites, the place of the highest mite concentration in the honeybee colony was estimated. The number of mites fallen from the 8th comb was chosen to be control (table 1).

The bee-entrance of the hive of 2.5 cm width was left beetween the 4th and 5th comb. The highest mite concentration was found in 5th comb. Approximately 35.2 ± 12.87 mites were found in each of eight squares of this comb though in separate squares of this comb the number of mites differed significantly. The highest amount of mites was found in D5 square - 95.3 ± 4.63 mites. It was the highest number not only in the 5th comb, but in the whole the honeybees colony. In square A5 near the bee-entrance 23.3 ± 0.88 fallen mites were found, while in the square near the backside of the hive about 2.3 ± 0.33 fallen mites were found, that is 90.1% ($P< 0.001$) less. About 282 mites were found in the all the squares (from A to H) of the 5th comb. The mite concentration is significantly lower on the edge combs. 33 mites were found in the squares A to H under the first comb, 20 mites were found in the squares A to H under the 9th comb. Despote the fact that it is easier and simplier to put impregnated strips into the outside combs of the hive it is advisable to put strips with remedial preparations between 4-5 and 5-6 combs. While treating honeybee colonies with aerosol, special attention must be paid to the honeybees in the central part of the hive.

The main aim of honeybee colony treatment is to plevent them from reaching the critical infection line and to save honeybee colonies from the negative impact of mites. The level of the honeybee infection with Varroa destructor mites after the spring treatment of honeybee families with Tymovaroi preparation decreased from 11.3 ± 2.17% to 1.7 ± 0.22% ($P<0.001$; Fig. 1). In 17 days of May the honeybee infection with mites increased from 1.7 ± 0.22% to 3.9 ± 1.16% (about 2.2%). In June infestation increased about 3.0% but this increase is quite conditional, because in June honeybee families grew up intensively and this reduced the number of mites fallen to one grown up indivual. Moreover, part of the female mites might be not fertilized as the mortality of male mites increases (**Martin,** 2001). The honeybee infection with mites increase in about 1.4% in July and 2.3% in August. From May 14th to September 1st the honeybee infestation level increased from 1.7 ± 0.22% to 10.6 ± 1.08 % ($P <0.001$) and reached the spring level.

The spring treatment is advisable only if the honeybee families were not treated in autumn and if there is a danger to their survival.

Table 1. **The dynamics of mite distribution in honeybee colonies (*n* = 6)**

*	Comb number								
	1	2	3	4	5	6	7	8 (control)	9
A	3.3±0.33	5.0±0.58	14.0±0.58	22.0±0.58	23.3±0.88	12.7±0.44	12.3±1.2	2.3±0.33	3.0±0.33
B	0.7±0.33	12.0±0.58	28.0±0.33	28.7±2.9	44.3±2.9	31.3±0.67	18.3±1.2	2.8±0.78	1.7±0.33
C	1.3±0.3	3.3±0.33	31.0±0.33	31.0±1.15	81.7±4.8	64.0±2.65	30.7±1.2	3.3±0.67	2.7±0.33
D	10.7±0.33	11.7±0.33	29.7±1.76	40.3±6.69	95.3±4.63	71.3±0.88	51.3±0.88	5.3±0.88	2.0±0.58
E	3.0±0.58	19.0±0.58	29.0±3.61	26.7±4.8	30.0±1.15	16.3±0.88	8.0±0.58	8.0±0.58	0.7±0.33
F	10.3±0.33	5.0±0.58	7.0±2.08	7.0±1.15	3.7±1.2	4.3±0.88	3.7±1.2	4.3±0.88	4.7±0.88
G	2.3±0.33	4.0±0.58	13.0±1.73	0.3±0.33	1.0±0.58	11.0±0.58	12.0±1.15	5.3±1.2	2.7±0.33
H	1.0±0.0	0.67±0.33	7.33±1.14	0.67±0.33	2.3±0.33	12.7±0.33	1.7±0.78	2.0±0.33	2.3±1.44

*horizontal lines A,B,...H dividing the mite catcher into 8 parts.
Vertical lines were the lines betwen the combs.

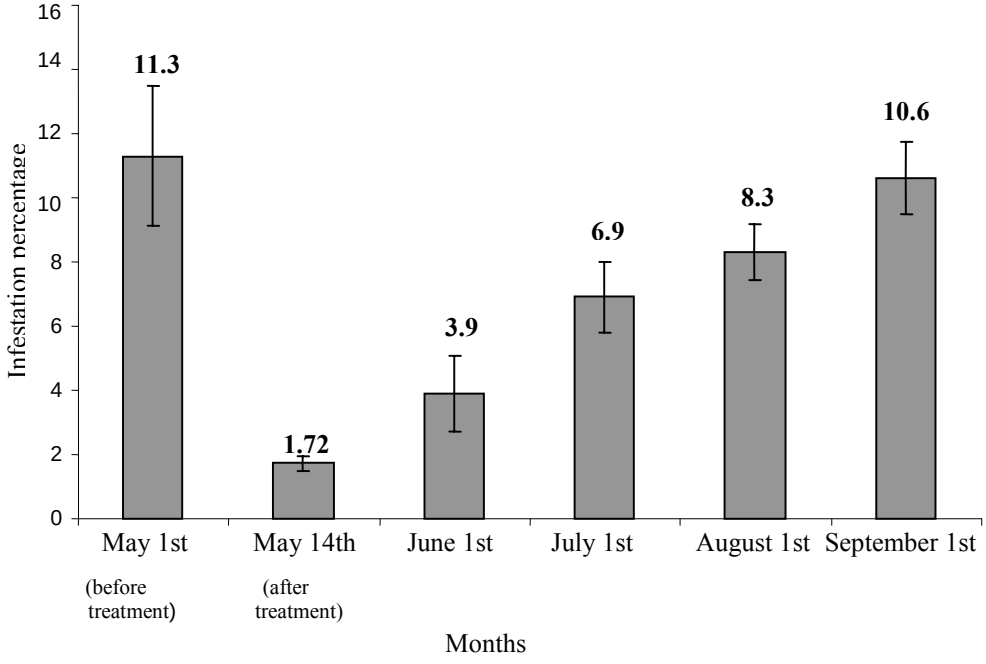

Fig. 1. **The infestation dynamics in honeybee colonies in the active period after the spring treatment (*n*= 6)**

In the spring treatment of honeybees with Apistan, Tymovaroi preparation, Thymol and oxalic acid these was no direct dependency beween the level of honeybee infection and the number of naturally fallen mites established (Table 2).

When the level of honeybee infection with *Varroa destructor* mites was minimal (6.3 ± 0.73%), the number of the fallen mites was the highest (23.7 ± 3.3). 1105 ± 85.6 fallen mites were found when the honeybee infection was 11.3 ± 1.31% and Tymovaroi preparation was kept for 14 days. After the treatment with Thymol, when the honeybee infection was 6.3 ± 0.73%, the number of fallen mites was found to be 252 ± 16.7 ($P < 0.001$). No mites on the adult

Table 2 **Efficiency of preparations after the spring treatment of *Apis mellifera* honeybee colonies for *Varroa destructor* mites ($n = 6$)**

Name of preparation	Infection of colonies with *V.destructor* mites, %	Naturally fallen mites	Mites fallen after the treatment	Infection of colonies with *V. destructor* mites after the treatment, %
Apistan	9.3 ± 0.27	11.3 ± 1.31	717 ± 53.3	1.6 ± 0.23
Tymovaroi	11.3 ± 2.17	19.3 ± 4.7	1105 ± 85.6	1.7 ± 0.22
Thymol	6.3 ± 0.73	23.7 ± 3.3	252 ± 16.7	*
2% Oxalic acid ($2^{\times}$ a week)	7.4 ± 0.53	21.5 ± 0.72	432 ± 14.6	*

* mites on adult individuals were not found.

honeybees were found after the treatment with Thymol, though **Bacandritsos and Papanastasiou** (2006) indicated that the efficiency of Thymol was about 90.5 %. When the level of honeybee relatively low and accounts 7.4 ± 0.53% the treatment with 2% oxalic acid (twice a week) resulted in 432.7 ± 14.6 fallen mites and 2.17 ± 0.4 honeybees. This result shows a relatively low toxicity of the oxalic acid for honeybee colonies and this is agreement with the data of **Aliano et al.** (2006). 717 ± 53.3 fallen mites were found after the treatment with Apistan which was kept for 14 days when the level of honeybee infection was 9.3 ± 0.27 %. This number of mites was by 607 units lower ($P<0.05$) in comparison to the number comparing with the number of fallen mites after using Tymovaroi preparation, but then the level of honeybee family infection was 11.3 ± 2.17%.

There was no expected result afte the spring treatment of honeybee colonies. The main reason was that when the treatment of honeybee colonies was prolonger and honeybee families became stronger with the increased number of brood, and the mites infested the brood. This is an explanation why no mites were found on adult honeybees after the treatment with Thymol and oxalic acid. Due to these reasons it is advisable to treat in spring only those honeybee families that were not treated in autumn.

The acaricidal efficiency of preparations mostly depends on the time they were used. Two groups of honeybees colonies with 10 families in each, where the level of infection with mites was respectively 17.0 ± 1.38% and 16.2 ± 1.41%, were treated with 2% aqueous solution of oxalic acid before and after the autumn feeding (Table 3).

The honeybees were treated with 2 % oxalic acid at the end of August, and after the first treatmenton on the average 921 ± 29.99 mites fell down, after the second treatment 894 ± 33.39 fallen mites were found, or 2.9% less ($P>0.5$). After the two treatments on the average 1816.1 ± 43.33 mites ful down. When the honeybees were treated after the autumn feeding, after the first treatment 22483 ± 138.43 fallen mites were found and after the second there were 251.8 ± 22.97 of them, or is 8.9 times less ($P<0.001$). This result shows that after the first treatment with oxalic acid the number of mites in the colony decreased to minimum. In two treatments 2500 ± 138.36 fallen mites were found in total or 37.7% ($P<0.001$) more comparison with two treatments before autumn feeding and 5.8 ($P<0.001$) times more in comparison with the spring treatment of the honeybees. After the autumn use of oxalic acid the samples of on the average 200-350 honeybees were taken from each family, but no mites were found on them.

The treatment of honeybees with anti-mite preparations is advisable to apply in autumn as late as possible. This is in agrement with the data of **Gro et al.** (2004). Howeve the use of oxalic acid in the late season, October or November, as it is recommended by **A. Gregorc and I. Planinc** (2002) is restricted by the climatic conditions of northern Lithuania.

Table 3. **The relationship betwen the efficiency of 2 % aqueous solution of oxalic acid and the time of treatment of honeybee families (*n* = 10)**

Treatment time	Infection with mites, %	Fell down on average after the first treatment		Fell down average after the second treatment		Total number of fallings	
		mites	honeybees	mites	honeybees	mites	honeybees
Before autumn feeding (treated 24 Aug and 01 Sept	17.0 ± 1.38	921± 29.99	0.3 ± 0.21	894± 33.39	0.2± 0.13	1816.1±43.33	0.5±0.22
After autumn feeding (treated 0.4 Sep And11 Sept)	16.2 ± 1.41	2248.3 ±138.43	0.8 ± 0.0	251.8±22.97	0.4± 0.16	2500± 138.36	1.0±0.42

CONCLUSIONS

The highest concentration of mites was found on the honeybees in the central part of the hive. It is advisable to place anti-mite preparations in this part of the hive.

The spring treatment of honeybee colonies is inexpedient because honeybee infection with mites after the treatment in autumn increased from 1.7 ± 0.22% to 10.6 ± 1.08% ($P<0.001$). The spring treatment of honeybees colonies is advisable only if the honeybee families were not treated in autumn and there is a danger to their survival.

In autumn it is advisable to treat honeybees in the second half of September or later if meteorological conditions alow.

Oxalic acid is a sufficiently effective substance it is advisable to use it for the autumn treatment of honeybee colonies.

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SUMMARY

The purpose of the study was to investigate the changes of mite distribution in the honeybee colonies and to assess the efficiency of chemical measures used to prevent the varroatosis of honeybees. The study was carried out in 2009, in the apiaries of Northern Lithuania. The changes of *Varroa destructor* mite distribution in the hive were investigated in May, the variation of *Apis mellifera* honeybee infection with mites was investigated after the spring treatment of honeybees and the efficiency of chemicals was investigated before and after the autumn feeding. Honeybee colonies with 9 combs squated with honeybees were used for the investigation of the changes in mite distribution in honeybee families. The level of infistation was approximately 11.3 ± 2.17%. The mite catchers were put under the combs and the bottoms of the catchers were lined with paper sheets smeared with vaseline and divided into 72 squares. A box with Tymovaroi (VET - AGRO, Poland) preparation was put on the frames of the nest and kept there for 14 days. When the subframes were taken out it was estimated by the number of fallen mites that the highest place of the mite concentration was in the central part of the hive.

The level of honeybee infection with *Varroa destructor* mites after the spring treatment of honeybee families with Tymovaroi (VET - AGRO, Poland) preparation decreased from 11.3 ± 2.17% to 1.7 ± 0.22% ($P<0.001$), but the level of infection in September was 10.6 ± 1.08%. At the end of August the honeybee were treated with 2% oxalic acid aerosol ($n = 10$). About 921 ± 29.99 mites fell down after the first treatment and after the second there were 894 ± 33.39 fallen mites ($P>0.5$). About 1816.1 ± 43.33 mites in total fell after the two treatments. In the second half of September the honeybees (n = 10) were treated again. About 2248.3 ± 138.43 mites fell down after the first treatment and about 251.8 ± 22.97 after the second one ($P< 0.001$). About 2500 ± 138.36 mites in total fell after the two treatments or 37.7% ($P<0.001$) more in comparison with the number of fallen mites at the end of August.

Key words: *Apis mellifera, Varroa destructor, oxalic acid, treatment, Tymovaroi, Apistan, Thymol*