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A COMPARATIVE STUDY OF DIAGNOSTIC METHODS FOR DETECTION OF *VARROA DESTRUCTOR* INFESTATION LEVEL IN HONEY BEE (*APIS MELLIFERA*) COLONIES

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ABSTRACT: The main methods for detection of infestation level of parasitic mite *Varroa destructor* in honey bee colonies were studied. The methods using *vivo* evaluation are more sparing for bees but less precise; those using evaluation with the killing of bees or brood are the most precise but less sparing for bees. The indirect method of evaluation of mite infestation by sampling the natural mortality of mite is the most sparing but not as precise as the previous ones, though the correlation between the rate of infestation of adult bees and natural mortality of mites is strong. The natural mortality of 1mite/24-hours corresponds to the presence of about 160 mites on bees per colony.

KEY WORDS: honey bee, mite, *Varroa destructor*, infestation, diagnostic methods

INTRODUCTION

The discovering of *Varroa destructor*

The mite *Varroa destructor* Anderson et Trueman, 2000 is the species of ecto-parasitic mites (Acari: Varroidae) that feeding on the hemolymph cause serious disease of larvae, pupae and adults of honey bee *Apis mellifera* L. — varroosis (varroatosis). The mite is also known to transfer pathogenic viruses into the bee (Allen et al. 1986) and is suspected to be one of the agents causing colony collapse disorder (Borrello et al. 2009). Currently, due to the damage caused the disease represents one of the most important problems of the world beekeeping and is attributed by the International Epizootic Bureau to the list 'B' of quarantine diseases of bees along with American foulbrood and acariosis. Therefore, varroosis must be regularly controlled to predict colony loss.

For a long time, the species was being classified as *Varroa jacobsoni* Oudemans, 1904. The author of the species described in detail the female mites collected from the body of adult Asiatic or Eastern honey bee (*Apis cerana* Fabricius, 1793) at the island of Java by entomologist Edward Jacobson (Oudemans 1904). The generic name was obviously given in honor of Roman scholar Varro known for his scientific works in agriculture, including beekeeping, and the species name — by the name of the scientist who was the first to find this parasite.

However, subsequently the species originally described as *V. jacobsoni,* appeared to be not the species that attacks *Apis mellifera*. In 2000 during the study of mitochondrial DNA of the mite, Anderson and Trueman (2000) found that *V. jacobsoni* in fact consists of two species: actual *Varroa jacobsoni* and also *Varroa destructor*, which was isolated in a separate species. It was also found that *V. jacobsoni* parasitizes only *A. cerana* and its natural habitat covers the Indo-Malayan zoogeographic region, while *V. destructor* is able to parasitize both *A. mellifera* and *A. cerana* (Anderson and Fuchs 1998).

The species *V. destructor* was first found on *A. cerana* in South Korea, but subsequently it switched to *A. mellifera* (Anderson and Trueman 2000). The expansion of host range of *Varroa destructor* occured evidently in the end of 1950s — beginning of 1960s in southern and southeastern Asia, when European strains of *A. mellifera* (or Western honey bee) were introduced by beekeepers in the places of original habitat of *A. cerana* in Asia (Martin 1995). On *A. mellifera* the mite *V. destructor* was first registered in China in 1958 (Yan Tsin-khe 1965).

It seems like human activity was the main factor contributing to *V. destructor* transition from Asiatic honey bee to the European one. Having transited on *A. mellifera* the mite got the wide opportunities for breeding and dramatic expansion of its geographic distribution. This was facilitated by the absence of the specific defense mechanism in *A. mellifera* against the parasite because this couple had not had a long enough period of coevolution like *A. cerana* and *V. destructor* had (Rosenkranz et al. 2010). It was noted that *V. destructor* caused much less damage to *A. cerana,* its natural host, as compared to *A. mellifera* (Boot et al. 1997).

As a consequence, the mite *V. destructor* has turned into a cosmopolitan invasive species, having spread through the trade or exchange of stock material among beekeepers almost all over the

world, except Australia (Holland 2012), causing serious damage to beekeeping. The spread of varroosis in the world has had a character of panzooty, each time destroying tens of thousands of honey bee colonies during assimilation of a new space by the parasite (Maslennikova 2002).

In Russia the first cases of infestation of *A. mellifera* were registered in 1964 at the Primorsky Territory (Far East) by Salchenko (1965), Poltev et al. (1965), who proposed the term "varroatosis", and Kulikov (1965) called this disease "varroosis". Unfortunately, the problems encountered in beekeeping with the spread of varroosis in Russia in 1960–1970s have not got their adequate solution to date.

In Italy *Varroa destructor* appeared in the beginning of 1980s (Milani 1990). Since the arrival, the mite spread rapidly across the country, which led to some 10 to 20% reduction in bee colonies (Sanford 1989). Now Varroa destructor is considered the most serious parasite of honey bees for European (Imdorf 2003) and Italian beekeepers (Mitzman 2012) with the losses of numerous colonies of bees annually. Studies on the nature, biology and control of the mite are constantly increasing here (Lodesani et al. 1995; Colombo et al. 2001; Colombo et al. 2002; Lodesani 2004; Mitzman 2012 and others), but a necessary key ingredient to an effective integrated pest management program against Varroa mite is still missing.

Therefore, in 2010 in Italy a new international research project on control of the main honey bee diseases (varroosis and nosemosis) "STRANOVA" was launched. The project provides a comprehensive participation of scientists of different specialties: entomologists, parasitologists, microbiologists, geneticists and professional beekeepers, under the guidance of Prof. Mario Colombo (Dobrynin et al. 2011). The objective of the project is to deepen the knowledge of the diseases to develop control measures. For mite *Varroa destructor* the focus will be on elaboration of a set of measures aimed both at improving the systems already in use and the development of innovative methodologies of the pest management that combine practicality, low environmental impact and high efficiency.

The main methods for detection of *Varroa destructor* infestation

One of a major problem of *Varroa* control is still that of detecting the beginning of an infestation. Usually Varroa mites are difficult to observe within a bee colony, because they are not always located on the back of bees. Most often, they are either hidden between the segments of abdomen of adult bees or within capped cells in a bee brood. Thus, it is very important for beekeepers to have the most accurate and simple diagnostic methods for the detection of Varroa mite infestation level in bee colonies.

Currently there are several methods for detection of *Varroa destructor* infestation level in bee colonies. The most widely utilized methods for evaluation of *Varroa* infestation of honey bee colonies can be combined into the following three groups.

Evaluation of live samples (*Vivo* evaluation)

Evaluation of adult bees collected from the hive for the presence of mites on their body can be carried out by inspection in transparent diagnostic device (Polishchuk and Pilipenko 1990). After counts, the bees are returned into their colony. Examination of preimaginal bee stages (larvae and pupae of workers and drones) in newly capped brood combs for the presence of mites can be carried out by looking through a strong light (Ibid).

Both adult and brood examination, according to Webster and Callaway (1992), may be the most sensitive methods, but they are laborious ones, and brood examination can be done only when it is available.

Evaluation with the killing of adult bees or brood

Evaluation of the mite infestation of adult bees taken from the hive can be carried out by treatment in different ways: washing with different liquids (the most common — ethanol) and detergents (De Jong et al. 1982), rotating in a jar with little amount of ether (Shabanov et al. 1980) to separate and count the mites. However, the latter procedure can show overrated values in a survey with higher infestation levels and is unsafe because of the high flammability of ether vapors (Fakhimzadeh 2001). The use of dishwashing detergent is an effective and economical alternative to washing with alcohol for mite detection on adult bees and shaking the bees with the use of a mechanical shaker makes the method more accurate (Rinderer et al. 2004).

Evaluation of infestation of preimaginal bee stages can be carried out directly by taking them from a brood comb and inspecting for the presence of mites (De Jong 1979). However, accord-

ing to Herbert et al. (1989), brood examination is a protracted labor-consuming procedure and can be implemented only during the presence of brood in a hive.

Evaluation of the natural mortality of the mite

The evaluation of natural mortality of the mite is carried out with the use of sticky sheet on the hive bottom for retaining the V*arroa* mites fallen from the body of bees (Parkman et al. 2002). This method is sparing for bees because it does not require disruption of the colony while detection of mite infestation. However, according to Branco et al. (2006), the method can be considered reliable only if there is an adequate amount of brood and on the early stages of the infestation.

Close to the above method, is the analysis of the debris collected from the bottom of a hive and examined for the presence of the fallen Varroa mites (Calatayud and Verdu 1993). According to Fries et al. (1991), hive debris is more effective at detecting mites than sampling brood, which is more effective than sampling adult bees during the summer months. But this method is efficient only at a low-level infestation (ibid).

To stimulate the falling of mites for evaluation procedure the toxic vapor chemicals placed in a hive are used (Ellis et al. 1988). However, there are some evidences that chemicals can contaminate honey (Atienza et al. 1993) and other products of hive (Chauzat and Faucon 2007).

There were also published analytical works on comparison of different methods of evaluation of the mite infestation of honey bee population, but with contradictory conclusions (Herbert et al. 1989; Calderone 1999; Branco et al. 2006; Barlow and Fell 2009).

Therefore, there is a need for evaluation and development of different diagnostic methods of *Varroa destructor* infestation to find more precise, suitable and sparing ones, which could be the essential part of successful integrated pest management strategy for mite control.

MATERIALS AND METHODS

The testing of different methods of evaluation of *Varroa* mite infestation of honey bee (*Apis mellifera* L.) colonies was conducted in the experimental apiary in 2011–2012 near Voronezh, Russian Federation. The hives were placed so that drifting of foragers was reduced. Availability of nectar sources was sufficient. The colonies were not remarkably affected by diseases other than varroosis. The colonies were inspected to note their conditions and the presence of the queen at the beginning and at the end of the experiment.

All colonies in the experimental apiary were treated against *Varroa* mite in September 2011 after infestation counts and hatching of the last brood with Bipin (active ingredient — Amitraz), 0.00625% water emulsion, which was injected into between-comb space of bee nest with the help of syringe-gun at a dose of 10 ml per space.

Testing of all five methods to value the rate of Varroa infestation was carried out three times per season: in the beginning (April 25), in the middle (July 8 — just before the main honey flow collection) and at the end of the season (September 6 — before the acaricide treatment) on the basis of counting the number of female mites per 100 adult bees, the percentage of cells with worker bee or drone brood affected by the mite and natural mite mortality.

The following five methods were tested in the same four experimental colonies selected from the colonies of the apiary. In every colony, all five methods were tested to get a reliable average value. Experimental colonies were selected of approximately equal average strength of bees and brood.

Vivo evaluation

Method 1. About 100 adult bees from the middle comb frame of a hive were collected using the exhauster and placed in the diagnostic device (a box 150×150 mm made of transparent plastic with a hinged lid and side walls having the interior height of 4 mm). In a closed box the bees were pressed by the lid and fixed in a stationary position. Then the bees were inspected and the counts of the number of the bees themselves as well as the mites on their body were made. The number of detected mites was divided by the number of bees in the box and multiplied by 100 to get the number of mites per 100 bees. After the counts, the bees were released back into their colony. This procedure was replicated four times to get a reliable average value.

Method 2. To do *Vivo* diagnosis of the brood, the newly constructed comb with a bee or drone (when available) brood before capping was taken and the square of 50 × 50 mm (containing approximately 100 worker bee or 80 drone cells) was looked through a strong light source or the sun. Counts of the number of mites were made, and calculated per 100 cells. The procedure was replicated four times to get a reliable average value.

Table 1.
The average rate of *Varroa* infestation (%) of adult bees in experimental colonies, evaluated by method 1 (April 25, 2012)

Number of the colony	Average number of bees per device	Average number of mites per device	Average rate of infestation, %
1	102.5	0.9	0.9
2	99.5	0.7	0.7
3	108.8	1.6	1.5
4	100.0	1.2	1.2
Average	102.7	1.1	1.1

Evaluation with the killing of bees or brood

Method 3. Approximately 100 bees were taken from a mid comb of the colony and shaken off in a bright tray filled with hot (+70 ... 90 ° C) 1% water solution of soda, vigorously stirred for 3–5 minutes. The mites fallen from the bees were counted to get the number of mites per 100 bees.

Method 4. To detect mites in brood, 100 cells with a bee or drone brood from a mid combs on the border of the upper and the middle third part located closer to the hive entrance were opened. The cell and larva/pupa (taken by tweezers) were examined for the presence of mites and the calculation of a percentage of cell infestation was made. Both procedures were replicated four times to get a reliable average value.

Evaluation of natural mortality of mites

Method 5. Hives with a sticky sheet, protected with a net having mesh size of 3 × 4 mm, which prevented the fallen mites to get back to the bees were used. The distance between the sheet and the net was 1 cm. The net covered the whole bottom of the hive. It was ensured that the net was not obstructed by propolis.

The number of mites fallen onto the bottom in each experimental hive was counted on the 7th day after cleaning of the bottom and placement of a new sticky sheet and respectively on the same day of testing of four previous methods. The collected material was dried and placed in a Petri dish. Counts of mites were conducted under the magnifying glass with 10–12-fold increase. The number of fallen mites was then calculated on the prorated 24-hour basis. The data of natural mite mortality were compared with the data of mites counted directly on bees by above indicated methods on the same day to correlate natural mortality and the total number of mites present in colonies (actual level of infestation).

Counts were made on entire sticky sheet. Only mature female mites were taken into counts. Females were recognized by the color of any shade of brown from light to dark and fully black. Females were separated from males or immature mites (not to cause future damage) by a smaller size, white, pearly white or yellow color of the latter two.

All data obtained in the experiments were processed mathematically by statistical analysis using Student's *t*-test, correlation and regression.

RESULTS

Early in the season experiments

The treatment of colonies in the experimental apiary against *Varroa mite* in September 2011 with Bipin (a.i. Amitraz) made it possible to reduce the rate of infestation of bees by the mite to 0.3–0.7 %. In the following months after the treatment until the formation of the wintering cluster, the mite infestation could slightly increase. It is well known that during the winter the mite does not reproduce in the bee brood until establishing of a comfortable temperature in the hive, so early in the season (March–April) the infestation of adult bees increased insignificantly since the end of the previous season.

Vivo evaluation

Method 1.

The testing of adult bees placed in the diagnostic device counts made in the beginning of the season (April 25, 2012) showed a slight increase in infestation of bees since the end of the autumn treatment (Table 1).

As seen from the Table 1, the average rate of *Varroa* infestation in experimental colonies evaluated by method 1 was 1.1 %.

Method 2.

Vivo diagnosis of the brood was made too, although the brood was of a little amount in the be-

Table 2.
The average rate of *Varroa* infestation (%) of the brood in experimental colonies, evaluated by method 2 (April 25, 2012)

Number of the colony	Average number of immature bees (mostly workers) per 50 × 50 mm square of comb	Average number of mites per 50 × 50 mm square of comb	Average rate of infestation, %
1	97.0	0.9	0.9
2	101.5	0.6	0.6
3	88.8	0.7	0.8
4	84.5	0.3	0.4
Average	92.9	0.6	0.7

Table 3.
The average rate of *Varroa* infestation (%) of adult bees in experimental colonies, evaluated by method 3 (April 25, 2012).

Number of the colony	Average number of bees per tray	Average number of mites per tray	Average rate of infestation, %
1	99.3	1.2	1.2
2	108.5	1.6	1.5
3	104.8	0.9	0.9
4	111.0	2.0	1.8
Average	105.9	1.4	1.3

ginning of the season. The results are presented in Table 2.

As seen from the Table 2, the average rate of *Varroa* infestation of brood in experimental colonies evaluated by method 2 was 0.7 %.

Taking into consideration that average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 1 was 1.1 %, the total infestation rate of the colonies was 1.8% (1.1+0.7) and the percentage of mites in the brood constituted 39% of all *Varroa* mites presented in the nest.

So, it is evident that appearance of the first spring brood, occupying a small square of combs, does not provide favorable conditions for breeding of the parasite.

Evaluation with the killing of bees or brood

Method 3.

The testing of adult bees placed in plastic trays filled with hot 1% water solution of soda was carried out on the same specimens, which were used for testing by method 1. The results are presented in Table 3.

As it is seen in Table 3, the average rate of *Varroa* infestation in experimental colonies evaluated by method 3 was 1.3 %.

The comparison of the average rate of infestation of bees evaluated by method 1 (1.1%) with the average rate of infestation of the same specimens of bees evaluated by method 3 (1.3%) showed that method 3 was 1.2 times more precise than method 1. It makes obvious that not all the mites in the samples can be seen when bees are tested by method 1 and significant amount of mites escapes from counts when testing bees by this method.

Thus, method 3 was more precise than method 1, because it lets distinguish higher values of *Varroa* infestation.

Method 4.

The results of the examination of the taken brood and cells for the presence of mites are presented in Table 4.

As seen from Table 4, the average rate of *Varroa* infestation of the brood in experimental colonies evaluated by method 4 was 1.0.

Taking into consideration that the average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 3 was 1.3 %, the total infestation rate of the colonies was 2.3% (1.3+1.0) and the percentage of mites in the brood constituted 43% of all *Varroa* mites present in the nest.

The value 2.3% should be considered as a true rate of *Varroa* infestation in experimental colonies, because these data were obtained by direct counting of mites on adult bees and on the brood.

Table 4.
The average rate of *Varroa* infestation (%) of the brood in experimental colonies, evaluated by method 4 (April 25, 2012)

Number of the colony	Number of immature bees (mostly workers) per sample	Average number of mites per sample	Average rate of infestation, %
1	100.0	1.3	1.3
2	100.0	0.8	0.8
3	100.0	1.1	1.1
4	100.0	0.9	0.9
Average	100.0	1.0	1.0

Table 5.
The average natural mortality of *Varroa* mite in experimental colonies, evaluated by method 5 (April 25, 2012)

Number of the colony	Number of mites fallen for 7-days period	Prorated 24-hour natural mite drop
1	9	1.3
2	21	3.0
3	17	2.4
4	12	1.7
Average	14.8	2.1

Table 6.
The average rate of *Varroa* infestation (%) of adult bees in experimental colonies, evaluated by method 1 (July 8, 2012)

Number of the colony	Average number of bees per device	Average number of mites per device	Average rate of infestation, %
1	106.8	3.7	3.5
2	97.0	2.1	2.2
3	100.3	2.6	2.6
4	103.5	3.1	3.0
Average	101.9	2.9	2.8

The comparison of the average rate of infestation of bees evaluated by method 4 (1.0%) with the average rate of infestation of bees evaluated by method 2 (0.7%) showed that method 4 was 1.4 times more precise than method 2. It makes obvious that not all of the mites in the samples can be seen when bees are tested by method 2 and significant amount of mites escapes from counts when testing bees by this method.

Thus, method 4 was more precise than method 2, because it lets distinguish higher values of *Varroa* infestation.

Evaluation of natural mortality of mites

Method 5.

The results of the examination of natural mortality of *Varroa* mites are presented in Table 5.

The data of natural mortality of *Varroa* mites presented in Table 5 show a low infestation level (2.1 mites per colony) in overwintered colonies. The data also indicate that autumn acaricides treatment have worked satisfactorily.

Mid-season experiments

In July, during the period of maximum development of bee colonies and their preparation for the main honey flow the increase of the number of bees and the corresponding increase of the infestation by mites was observed.

Vivo evaluation

Method 1.

The testing of adult bees placed in the diagnostic device showed a significant increase in infestation of bees since the beginning of the season (Table 6).

As seen from Table 6, the average rate of *Varroa* infestation in experimental colonies evaluated by method 1 was 2.8%, which was 2.5 times more

Table 7.
The average rate of *Varroa* infestation (%) of the brood in experimental colonies, evaluated by method 2 (July 8, 2012)

Number of the colony	Average number of immature bees (mostly drones) per 50 × 50 mm square of comb	Average number of mites per 50 × 50 mm square of comb	Average rate of infestation, %
1	89.0	5.2	5.8
2	99.8	4.7	4.7
3	87.5	4.5	5.1
4	103.3	5.7	5.5
Average	94.9	5.0	5.3

Table 8.
The average rate of *Varroa* infestation (%) of adult bees in experimental colonies, evaluated by method 3 (July 8, 2012)

Number of the colony	Average number of bees per tray	Average number of mites per tray	Average rate of infestation, %
1	107.8	3.8	3.5
2	103.3	3.2	3.1
3	101.8	4.5	4.4
4	99.5	3.2	3.2
Average	103.1	3.7	3.6

than the average rate of infestation evaluated on the 25th of April.

Method 2.

Vivo diagnosis of the brood showed the results presented in Table 7.

As seen from Table 7, the average rate of *Varroa* infestation of the brood in experimental colonies was 5.3 %, which was 7.6 times more than the average rate of brood infestation on April 25 (0.7%).

Taking into consideration that the average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 1 was 2.8 %, the total infestation rate of the colonies was 8.1% (2.8 + 5.3) and the percentage of mites in the brood constituted 65% of all *Varroa* mites present in the nest.

Evaluation with the killing of bees or brood

Method 3.

The results of he testing of adult bees by method 3 are presented in Table 8.

As it is seen from Table 8, the average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 3 was 3.6 %, which was 2.8 times more than the average rate of infestation evaluated on the 25th of April (1.3%).

The comparison of the average rate of infestation of bees evaluated by method 1 (2.8%) with the average rate of infestation of the same specimens of bees evaluated by method 3 (3.6%) showed that method 3 was 1.3 times more precise than method 1.

Thus, method 3 is more precise than method 1, because it lets distinguish higher values of *Varroa* infestation.

Method 4.

The results of the examination of the taken brood and cells for the presence of mites are presented in Table 9.

As seen from Table 9, the average rate of *Varroa* infestation of the brood in experimental colonies evaluated by method 4 was 6.2 %, which was 5.6 times more than the average rate of brood infestation on April 25 (1.0%).

Thus, 5.6–7.6 fold increase of the number of the parasite in the brood in July, compared to the beginning of the season, made evident that it took an "explosive" character, which is obviously associated with a sharp increase of the amount of brood in the nest before the main honey flow.

Taking into consideration that the average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 3 was 3.6 %, the total infestation rate of the colonies was 9.8% (3.6 + 6.2), which is 4.3 times more than in April (2.3%). Correspondingly, the percentage of mites

Table 9.
The average rate of *Varroa* infestation (%) of the brood in experimental colonies, evaluated by method 4 (July 8, 2012)

Number of the colony	Number of immature bees (mostly drones) per sample	Average number of mites per sample	Average rate of infestation, %
1	100.0	5.2	5.2
2	100.0	6.7	6.7
3	100.0	7.1	7.1
4	100.0	5.9	5.9
Average	100.0	6.2	6.2

Table 10.
The average natural mortality of *Varroa* mite in experimental colonies, evaluated by method 5 (July 8, 2012)

Number of the colony	Number of mites fallen for 7-days period	Prorated 24-hour natural mite drop
1	43	6.1
2	31	4.4
3	23	3.3
4	51	7.3
Average	37.0	5.3

Table 11.
The average rate of *Varroa* infestation (%) of adult bees in experimental colonies, evaluated by method 1 (September 6, 2012)

Number of the colony	Average number of bees per device	Average number of mites per device	Average rate of infestation, %
1	98.5	4.6	4.7
2	109.3	5.8	5.3
3	106.3	6.2	5.8
4	101.8	6.1	6.0
Average	103.9	5.7	5.5

in the brood constituted 63% of all *Varroa* mites present in the nest.

The value 9.8% should be considered as a true rate of *Varroa* infestation in experimental colonies, because these data were obtained by the direct counting of mites on adult bees and on the brood.

The comparison of the average rate of infestation of bees evaluated by method 4 (6.2%) with the average rate of infestation of bees evaluated by method 2 (5.3%) showed that method 4 was 1.2 times more precise than method 2.

Thus, method 4 is more precise than method 2, because it lets distinguish higher values of varroa infestation.

Evaluation of natural mortality of mites

Method 5.

The results of examination of natural mortality of *Varroa* mites are presented in Table 10.

The data presented in the table 10 show that the average natural mortality of *Varroa* mites has increased more than 2.5 times since last counts on April 25 (5.3 against 2.1 mites per colony) which reflects the corresponding increase of infestation level demonstrated in tables 6–9.

End of the season experiments

Vivo evaluation

Method 1.

The testing of adult bees in the diagnostic device made in the end of the season before acaricide treatment showed a further increase in infestation of adults since the last counts in the middle of the season though not so significant (Table 11). This increase is obviously associated with the regular reduction of the amount of brood in a nest to the end of a season due to which the majority of mites concentrates on adult bees.

Table 12.
The average rate of *Varroa* infestation (%) of the brood in experimental colonies, evaluated by method 2 (September 6, 2012)

Number of the colony	Average number of immature bees (mostly workers) per 50 × 50 mm square of comb	Average number of mites per 50 × 50 mm square of comb	Average rate of infestation, %
1	103.3	3.9	3.8
2	86.5	3.6	4.2
3	99.5	3.1	3.1
4	101.8	2.6	2.6
Average	97.8	3.3	3.4

Table 13.
The average rate of *Varroa* infestation (%) of adult bees in experimental colonies, evaluated by method 3 (September 6, 2012)

Number of the colony	Average number of bees per tray	Average number of mites per tray	Average rate of infestation, %
1	97.5	4.9	5.0
2	105.8	5.9	5.6
3	109.8	7.1	6.5
4	104.0	7.5	7.2
Average	104.2	6.3	6.1

As seen from Table 11, the average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 1 was 5.5%, or 1.9 times more than the average rate of infestation evaluated on the 8th of July (2.8%).

Method 2.

Carried out at the same time the *Vivo* diagnosis of the brood showed the results presented in Table 12.

As seen from Table 12, the average rate of *Varroa* infestation of the brood in the experimental colonies was 3.4 %, which was 1.6 times less than the average rate of the brood infestation on July 8 (5.3%). As it was noted above this decrease was associated with the reduction of the amount of the brood in the nest in this period of the colony development.

Taking into consideration that the average rate of *Varroa* infestation of adult bees in experimental colonies evaluated by method 1 on September 6 was 3.8 %, the total infestation rate of the colonies was 8.9% (3.4 + 5.5), which was 1.1 times more than the analogical index on July 8 (8.1%). The percentage of mites in the brood constituted 38% of all *Varroa* mites present in the nest.

Evaluation with killing of bees or brood

Method 3.

The results of the testing of adult bees by method 3 are presented in Table 13.

As it is seen in Table 13, the average rate of *Varroa* infestation of adult bees in the experimental colonies evaluated by method 3 was 6.1 %, which was 1.7 times more than the average rate of adult bees infestation on July 8 (3.6%).

The comparison of the average rate of infestation of bees evaluated by method 1 (5.5%) with the average rate of infestation of the same specimens of bees evaluated by method 3 (6.1%) showed that method 3 was 1.1 times more precise than method 1.

Thus, method 3 was more precise than method 1, because it lets distinguish higher values of *Varroa* infestation.

Method 4.

The results of the examination of the taken brood and cells for the presence of mites are presented in Table 14.

As seen from Table 14, the average rate of *Varroa* infestation of the brood in experimental colonies evaluated by method 4 was 5.2 %, which was 1.2 times less than the average rate of the

Table 14.
The average rate of *Varroa* infestation (%) of the brood in experimental colonies, evaluated by method 4 (September 6, 2012)

Number of the colony	Number of immature bees (mostly workers) per sample	Average number of mites per sample	Average rate of infestation, %
1	100.0	4.6	4.6
2	100.0	6.1	6.1
3	100.0	4.8	4.8
4	100.0	5.4	5.4
Average	100.0	5.2	5.2

Table 15.
The average natural mortality of *Varroa* in experimental colonies, evaluated by method 5 (September 6, 2012)

Number of the colony	Number of mites fallen for 7-days period	Prorated 24-hour natural mite drop
1	38	5.4
2	65	9.3
3	76	10.9
4	47	6.7
Average	56.5	8.1

brood infestation on July 8 (6.2%) also associated with the reduction of the amount of the brood in the nest.

Taking into consideration that the average rate of *Varroa* infestation of adult bees in the experimental colonies evaluated by method 3 was 6.1%, the total infestation rate of the colonies was 11.3% (5.2 + 6.1), which is 1.2 times more than in July (9.8). The percentage of mites in the brood constituted 46% of all *Varroa* mites present in the nest.

The value 11.3% should be considered a true rate of *Varroa* infestation in the experimental colonies, because these data were obtained by direct counting of mites on adult bees and the brood.

The comparison of the average rate of infestation of bees evaluated by method 4 (5.2%) with the average rate of infestation of bees evaluated by method 2 (3.4%) showed that method 4 was 1.5 times more precise than method 2.

Thus, method 4 was more precise than method 2, because it lets distinguish higher values of varroa infestation.

Evaluation of natural mortality of mites

Method 5.

The results of examination of natural mortality of *Varroa* mites are presented in Table 15.

The data presented in Table 15 show that the average natural mortality of *Varroa* mites has increased more than 1.5 times since last counts on July 8 (8.1 against 5.3 mites per colony) which reflects the corresponding increase of infestation level demonstrated in Tables 11–14.

Statistical analysis of the data obtained

The comparison of significance of averages

The comparison of significance of average rates of *Varroa* infestation in experimental colonies, obtained by different methods in all experiments, using Student's *t*-test is presented in Table 16. As seen from the table the average rate of *Varroa* infestation (%) in experimental colonies, obtained by method 1 in all experiments was 3.0 and by method 3–3.7. The difference between the averages obtained by methods 1 and 3 was significant at $P<0.05$ by Student's *t*-test.

The average rate of *Varroa* infestation (%) in experimental colonies, obtained by method 2 in all experiments was 3.2 and by method 4–4.1. The difference between the averages obtained by methods 2 and 4 was significant at $P<0.01$ by Student's *t*-test.

Correlation and regression analyses

To determine the dependence of natural mortality of the mite on the rate of infestation of adult bees we calculated coefficients of correlation, regression and the equation of regression. The results of correlation and regression analyses of the data of natural mortality of *Varroa* mite and the

Table 16.
The comparison of the significance of the average rates of *Varroa* infestation (%) in experimental colonies, evaluated by different methods

The comparison of the significance of differences between the averages obtained by different methods in all experiments using Student's *t*-test				
Average by method 1	Average by method 3	Average by method 2	Average by method 4	Values of Student's *t*-test at 11 degrees of freedom
3.0	3.7	3.2	4.1	
$t = 3{,}07$		$t = 3{,}55$		$t_{05} = 2{,}20$ $t_{01} = 3{,}11$
The difference significant at P<0.05		The difference significant at P<0.01		

Table 17.
The dependence of natural mortality of *Varroa* mite (number/24-hours) on the rate of infestation of adult bees (%) in the experimental colonies

The values of coefficients of correlation, regression and equation of regression of natural mortality of *Varroa* mite (numbers) on the rate of infestation of adult bees (%) and their significance by Student's *t*-test		
Coefficient of correlation	Coefficient of regression (mites/24-hours)	Values of Student's *t*-test at 10 degrees of freedom
$r = 0.74$	$b_{yx} = 1.15$	
$t_r = 3.52$	$t_b = 3.58$	$t_{05} = 2.23$ $t_{01} = 3.17$
The value significant at P<0.01	The value significant at P<0.01	
The equation of regression	Y = 1.15 X — 0.96	

rate of infestation of adult bees in experimental colonies are presented in Table 17.

As seen from the table 17, the correlative dependence between the rate of infestation of adult bees and the natural mortality of the mite was strong, which is proved by the value of coefficient of correlation (r) equal to 0.74 (significant at P<0.01 by Student's *t*-test).

The regression analysis showed that coefficient of regression (b_{yx}) of natural mortality of the mite on the rate of infestation of adult bees is expressed by the value 1.15. Coefficient of regression shows how the attribute Y (function) changes quantitatively when changing the attribute X (argument). Accordingly, the increase of the rate of adult bee infestation by 1% corresponds to the increase of natural mortality by 1.15 mites/24-hours.

The equation of regression of natural mortality of the mite in dependence on the rate of infestation of adult bees is expressed by the formula: Y = 1.15 X — 0.96.

Calculation of the ratio of *Varroa* mite drop and the total number of mites

Comparison of the average seasonal natural mortality of *Varroa* mites with the average seasonal rate of infestation of adult bees allowed us to calculate the ratio of the mite drop to the total number of mites on bees in the colony.

Considering that an average colony in our experiments contained 1.9 frames of bees early in the season, 12.5 frames — in the middle (at the main honey flow collection) and 7.2 — in the end of the season, the average seasonal strength of colonies was 7.5 frames of bees. It is assumed that an average frame of bees contains about 3000 individuals. Therefore, the average seasonal strength of experimental colonies was about 22500 bees.

As seen from Table 16, the average seasonal rate of *Varroa* infestation of adult bees determined by the most precise method 3 was 3.7 %. It means that from 22500 bees there were infested about 824 bees. If we compare this figure with the average seasonal 24-hour natural mite drop, equal to 5.2 mites, the natural mortality of 1 mite per 24-hours indicates the presence of about 160 mites on bees per colony.

DISCUSSION

Discussion on advantages and disadvantages of the tested methods is presented below.

Method 1.

Advantages of the method

The diagnosis is carried out on adult bees and therefore can be implemented at any time of a season. The bees fixed in the device are able to move their legs, but cannot move. It contributed to careful counting of bees and mites, and more precise evaluation of the degree of infestation. After counts, the bees can be returned alive back into their colony.

Disadvantages of the method

Diagnosis of varroosis is complicated by the fact that a mite is afraid of daylight, tries to hide

between the segments of bees. In addition, a mite has a gray-yellow color, which not always lets to notice it.

Method 2.

Advantages of the method

The method does not demand special devices. The diagnosis is carried out on brood before capping, thus keeping bees alive.

Disadvantages of the method

The diagnosis is carried out on bee brood and therefore it cannot be implemented at any time of a season. The method demands the acute eyesight of a viewer. The method is not too accurate.

Thus, method 2 can be used for the evaluation of rate of *Varroa* infestation of bee colonies only to a limited extent.

Method 3.

Advantages of the method

The diagnosis is carried out on adult bees and therefore can be implemented at any time of a season. The method does not demand complicated devices and can be carried out in field conditions with any suitable trays. The method does not demand expensive chemicals. The method is the most precise for adult bees evaluation.

Disadvantages of the method

The main disadvantage of the method is that it is a destructive one because of killing of captured bees during the evaluation process. The using of plastic trays does not let use boiling water which kills bees quickly and make mites to fall on bottom immediately. Therefore, it is better to use glass containers for this method.

Method 4.

Advantages of the method

The method is the most precise for brood evaluation because it lets the direct counting of mites on individuals. All mites are confined in cells, what contributes to more careful counting of mites, and therefore more precise evaluation of the degree of infestation. The method does not demand special devices and can be carried out in field conditions.

Disadvantages of the method

The shortcomings of the method are the continuation of its merits. The main disadvantage of the method is that it is a destructive one because of killing of opened immature bees during the evaluation process. The method is labor consuming. The diagnosis is carried out on a bee brood and therefore it cannot be implemented at any time of a season.

Method 5.

Advantages of the method

The method is easy applicable to practice and can be implemented at any time of a season. The method does not demand complicated devices. The net screen with a sticky sheet serves at the same time as a means of a passive control of a mite. Unlike the previous methods which require taking samples of bees and therefore depend on where the sample was taken from and can therefore be inaccurate, method 5 is an indirect one. It also does not disturb a colony by removing frames while sampling the entire bee population.

Disadvantages of the method

As for method 4, the shortcomings of the method 5 are the continuation of its merits. The method is indirect unlike the previous ones and therefore cannot be as precise. The method is time-consuming, since it takes two trips to the apiary to obtain the results.

CONCLUSIONS

1. All tested methods of the evaluation of *Varroa* infestation rate of bee colonies have their advantages and disadvantages. The methods using *vivo* evaluation are more sparing for bees but less precise. The methods using evaluation with the killing of bees or brood are the most precise but less sparing for bees. The indirect method is the most sparing but not as precise as the previous ones. It can serve as a supplemental to the direct ones. In total, method 3 was the most precise for evaluation of *Varroa* infestation of adult bees, method 4 — for immature bees.

2. The difference between the average rates of *Varroa* infestation, obtained in all experiments by method 1 and by method 3 was significant at $P<0.05$; by methods 2 and 4 — at $P<0.01$ by Student's *t*-test.

3. The correlative dependence between the rate of infestation of adult bees and the natural mortality of the mite is strong. The increase of the rate of adult bee infestation by 1% corresponds to the increase of natural mortality by 1.15 mites/24-hours. The equation of regression of natural mortality of the mite in dependence on the rate of infestation of adult bees is expressed by the formula: $Y = 1.15\ X — 0.96$.

4. Natural mortality expressed by one mite drop per 24-hours corresponds to the presence of about 160 mites on bees per colony.

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