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STUDY OF TOXIC PROPERTIES AND EFFICIENCY OF ECOLOGICAL AND CHEMICAL PREPARATIONS IN *APIS MELLIFERA* BEE COLONIES

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The aim of this study was to determine the toxicity and efficiency of ecological (Bienen Wohl) and chemical (Gabon) preparations applied for bee treatment from varrosis under the climatic conditions of Northern Lithuania. In order to determine the level of bee contamination with *Varroa destructor* mites, bees from three places of a beehive were taken and narcotized with ethereal vapours. The degree of infestation was expressed in per cent and was equal to the number of mites divided by the number of studied bees and multiplied by 100. In order to determine the degree of contamination with mites, at least 200 insects were taken from each bee colony. In order to determine the efficiency of preparations during the autumn feeding, standard plastic underframes with Vaseline-anointed sheets of paper (a certain number of mites stay alive after treatment) were put under the combs. After the autumn treatment with Gabon and Bienen Wohl and evaluating toxicity of preparations on bee wintering, the number of dead bees and mites faund at the bottom of the hive after flying rout of bees was determined in spring. When using Gabon, 2 strips impregnated with the preparation were place in each of 5 beehives; when using Bienen Wohl 20 ml of the preparation were embedded between the frames of each of 5 beehives.

In the case of moderate contamination with mites equal to 10.9±0.6 %, during the autumn 1346 ± 281.3 mites dropped away after using Gabon, what made 123.4 mites for one contamination per cent. In the case of moderate contamination with mites equal to 12.1 ± 0.6 %, 472 ± 87.7 mites dropped away after treating with Bienen Wohl preparation, what made 39 mites for one contamination per cent. When cleaning the bee families in spring after the flying rout 97 ± 7.5 dead mites and 948 ± 116.8 dead bees ($P < 0.001$) were determined after treating in autumn with Gabon; 125 ± 27.0 mites and 538 ± 167.8 bees ($P< 0.001$) after using Bienen Wohl preparation. Therefore during autumn chemical preparation had stronger acaricidal effect than ecological preparation ($P<0.001$). Though Biennen Wohl had in 45.3 % less toxic action to bees ($P< 0.001$) and more effect to mites during winter period in comparisson with Gabon

Key words: *Apis mellifera*, *Varroa destructor*, Bienen Wohl, Gabon.

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INTRODUCTION

Considerably damage in apiculture is made by provocators of bee diseases. First sources of *Varroa jacobsoni* mites in Lithuania were discovered in 1979, and despite drastic measures were taken (the honeybee colonies were put to sleep or burned), varrosis spread wider (Balžekas & Kriščiūnas 1980). 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 & Trueman 2000). Bee deseases started to spread especially when pesticides came into wide use against deseases and pests of the plants and also during importation of bee queens and families from foreign republics (Balžekas & Petkevičienė 1977). Sick bee families can infect healthy families, they are weak and in disadvantageous natural conditions die quite fast (Harris et al. 2003). Apiarians often mix healthy and infected bee colonies, transfer whole colonies from one place to other. Mites spread when bees workers thieve other colonies as well. Healthy bees grab their remainders of food at the same time infesting themselves with disease spreaders. Contamination from weak and dead bee families comes together with honey, ambrosia, combs, tools and inventory of apiculture, unattended apiaries (Dubovičienė 2010).

During a few latter years most apiarians experienced some detriment when whole bee families died. That had resulted impairment of economical situation of apiarians, besides since plants are pollinated worse, danger to biological diversity increases. 84% of plants depends on pollination of the bees (http://agroeta.lt 2011).

Varroa destructor mites are pests devastating *Apis mellifera* bees the most, they bring huge damage both in Lithuania and elswhere in the world. In autumn contaminated bees store less food reserve, therefore in spring number of bee colonies decreases. Bees are dying if number of mites is not controled. Accordingly it is necessary to use some cure to protect bee colonies (Webster & Delaplane 2001, Bacandritsos et al. 2007). Widely using synthetic varroacides in many regions of Europe mites become resistant to these compounds (Lipinski & Szubstarski 2007). The largest number of bee colonies is in the end of spring or in the summer, and starts decreasing during second half of summer when population of mites grows bigger. The more mites access brood chamber, the more damaged is develompent of bees (Webster & Delaplane 2001). When infestation with mites is wider, bees queens of newly formated bee families often winter not insemination (Pileckas et al. 2012). By J. Ellis (2013), in these latter years massive ruination of bee colonies in the world is observed because of so called Colony Collapse Disorder (CCD). It is supposedly that there are several reasons causing CCD: *Varroa destructor* mites, pesticides, genetically modified crops, climatic changes, electromagnetic fields, but none of these reasons are finally proved (Caroline et al. 2001, Didvalis & Gaidamavičius 2011, Ellis 2013).

Area of bee varrosis grows in 10-15 km per year, but the speed of spreading depends on temperature of atmosphere (Stevenson et al. 2005). Bees contaminated by mites are less resistant to infective diseases, their behaviour changes (Shen et al. 2005). Synthetic chemical compounds, organic acids that might be found in nature, essential oils are used to treat bee varrosis (Bogdanov et al. 2002, Ruffinengo et al. 2005, Bacandritsos et al. 2007, Lipinski & Szubstarski 2007, Sammarato et al. 2008). Fight with *Varroa* mites is difficult because these pests are adjusted to the cycle of bees development. It is necessary to take some actions as during the first year after contamination with mites bees work as usual, during the second – families weaken and during the third – they die (Mutinelli et al. 1994).

Production of bees is used not only for food but also as treatment for various human diseases. Therefore to prepare ecologicaly clean production of bees is particularly important. Biological methods to fight with mites could be promising (Chandler et al. 2001).

The aim of this study was to determine the toxicity and efficiency of ecological (Bienen Wohl) and chemical (Gabon) preparations applied for bees treatment from varrosis under the climatic conditions in North Lithuania.

MATERIALS AND METHODS

Research was conducted in the 2011–2012 in the apiary of Vilgirdaičiai in Northern Lithuania. In order to determine the level of bee contamination with *Varroa destructor* mites, bees from three places of a beehive (the intensity of bee infestation differs depending on hive places) were taken and narcotized with ethereal vapours. Bees were taken from the central and two peripheral combs by shaking them down onto a plate at the bottom of which there was a gauze plug soaked with ether. All bees and mites on them were counted. The degree of infestation was expressed in percentage and was equal to the number of mites divided by the number of studied bees and multiplied by 100. In order to determine the degree of contamination with mites, at least 200 insects were taken from each bee colony. The residue level of *Apis mellifera* honeybee infection with mites was estimated after the treatment with Gabon and Bienen Wohl preparation in the same honeybee colonies. The number of mites fallen from bees has been chosen to be efficiency criterion of substances used against mites. 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.

When using Gabon, 2 strips impregnated with the preparation were placed in each of 5 beehives. One strip was hanged in second interframe, other strip was placed in the middle of the nest among frames. Gaps between combs where the strips were hanged were enlarged that the strips would not touch combs and bees could pass through. Long running strips of preparation Gabon PA-92 which is effective in defeating *Varroa* mites, were placed in bee families having covered hatches. There was 1.2–1.7mg of active material acrinathrin in each strip. Woodwork strips with Gabon were covered polymeric compound with acrinathrin, which in small amounts segregates on the surface of strip by diffusion process. In bee family, this preparation spreads while bees touch the strips or each other. Mites and bees when rolled out of the chambers get this preparation while touching. Gabon PA-92 can not be used during winter when bees are bunched together.

When using Bienen Wohl 20ml of the preparation were embedded between the frames of each of 5 beehives. Bienen Wohl is emulsion made from oxalic and lemon acids with essential oils and propolis. Bienen Wohl arouses instinct of cleansing to the bees. Drops of this preparation adhere to bee hairs and this way is spreaded over whole hive. Bees may scratch the mites and therefore family recovers. Sick, infected by parasites and weak bees leave the hive in a few days. Families should be prepared in late afternoon or about evening when the air temperature is 5-28°C. It is necessary to heat preparation to the body temperature (about 35°C) before using it, and to shake it properly just before driping. The preparation was kept in the hives for 14 days, then the subframes were taken out and the fallen mites were counted.

After autumn treatment of bees with Gabon and the ecological preparation Bienen Wohl and evaluating the degree of preparation toxicity on bees during winter, the number of dead bees and mites found at the bottom of the hive after flying rout of bees was determined in spring. Also the number of naturally fallen mites per 7 days in the family was estimated before the treatment with preparations. The number of fallen mites was examined.

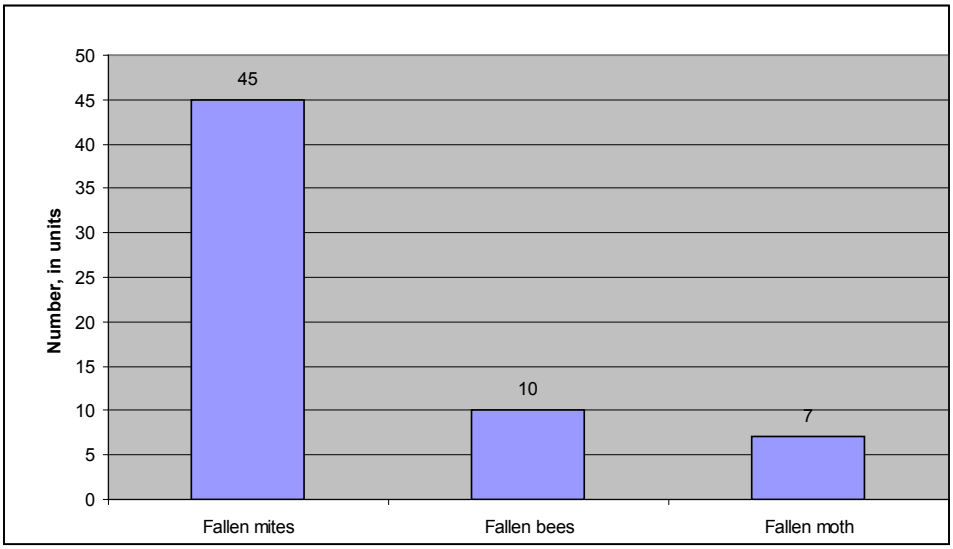

Fig. 1. Spontaneous depuration of bees.

Investigation data was 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 L. A. Tucker (2003). The difference was considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Amount of naturally fallen mites during 7 days period on subframes was estimated before processing with ecological and chemical preparations (Fig. 1). If during May more than 5 mites per day fall, and during June fall more than 10 mites per day, the spring treatment of bees is needed. If in the begining of August natural fall of mites reaches more than 33 units per day, treatment is needed just after removing honey (Uselis 2008). Though spontaneous depuration of bees progresses slowly and mites separation and accessing into subframes is rather random process. Approx. 45 ± 2.6 mites and approx. 10 ± 0.4 bees were found on the subframes to be naturally dead. Conditionally large amount of dead bees were found in underframes, this could be explained by the factor that smaller bees just rolled out of the combs could penerate through the net of underframe. This phenomenon is not observed when bees are in normal size.

Dynamics of mite distribution in the bee colonies was estimated. While investigating 1200 drone hatches in the magazine and nest chambers it was found that 33.3% of the drone hatches were infected with *Varroa destructor* mites. In 920 covered hatches of bee workers near the frame tempter, 200 mites were found: contamination of bee workers reached 21.7%, and it was 1.5 times smaller ($P < 0.001$) than contamination of drones. When examined 196 capped bee queens cells, *Varroa destructor* mites were not found (Fig. 2). One covered larvae of drone got approx. 0.3 of mite, one covered larvae of bee worker got 0.2 of mite, and one cradle of bee queen got 0 mites ($P < 0.001$). Therefore during the summer season amount of mites could be reduced by eliminating drone hatches, especially if bees are not treated from mites in autumn nor in spring. However eliminating drone hatches too often may demoralize bee colonies, stimulate them to snitch (Pileckas et al. 2012). Usually mites are found in the cells of hatches when there are some hatches of drones or other bees in the nest. Then it is porposeful to use slowly acting of treatment means, that affecting airway or nervous system of the mites. When there are no hatches in the nest, mites move onto bees, and then they can be

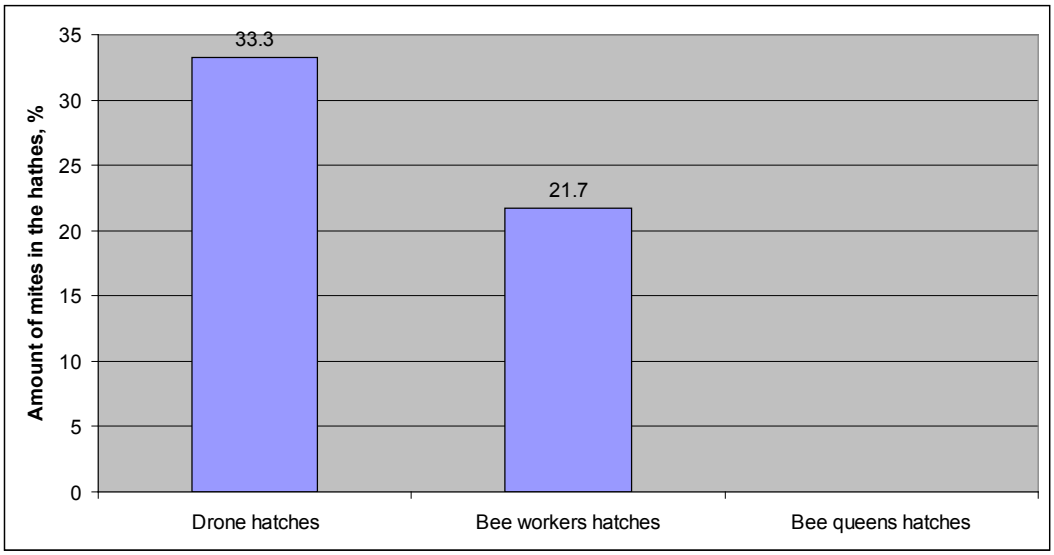

Fig. 2. Percentage of bee hatches contamination with mites during the period of main honey producing.

Table 1. Efficiency of anti-mite preparations during autumn feeding of the bees

Contamination of bees (%)	Preparation	Fallen mites	Amount of fallen mites to one percent of contamination
10.9 ± 0.6	Gabon	1346 ± 281.3*	123.4
12.1 ± 0.6	Bienen Wohl	472 ± 87.7	39.0

*P < 0.001

eliminated using contact medicaments (http://www.nmvrvi.lt). Wintering bees are much more infected than summer bees.

Two anti-mite preparations were used to fight with *Varroa destructor* during autumn feeding of the bees (Table 1): these were Bienen Wohl and Gabon, which were applied on 5 families each in 30th Sepember. When average infestation with mites is 10.9%, 1346 mites fallen after treatment with Gabon, and that made 123.4 mites for one percent of contamination. When average infestation was 12.1%, 472 mites fallen after treatment with Bienen Wohl, and that made 39 mites for one percent of contamination. 64.9% more mites fallen after using Gabon than after using Bienen Wohl ($P < 0.001$). Therefore during autumn Gabon had stronger toxic effect on mites than Bienen Wohl. Though strips of Gabon are not so effective as before (95–97%), but efficacy is still not smaller than other preparations of treatment made on the basis of acids or thymol (Dubovičienė 2010). It is estimated that mites resistant to acaricides are not so vital and do not reproduce fast, otherwise during couple of years they would spread over whole Lithuania as it happened in the beginning of varrosis invasion in 1979. It is more porposeful to use Gabon strips during autumn feeding of bees because onetime application of this preparation to bee colony is enough. Bienen Wohl preparation fissions during about 2 weeks, therefore it is recommended to apply it additionally. Besides, its use is influenced by temperature of atmosphere as well.

Toxicity of these preparations to bee colonies prepared to winter is also estimated. Strips of Gabon was not removed and left together with bees during the winter. After spring flying rout of the bees while cleaning bottom of the hive dead bees and mites were counted (Fig.

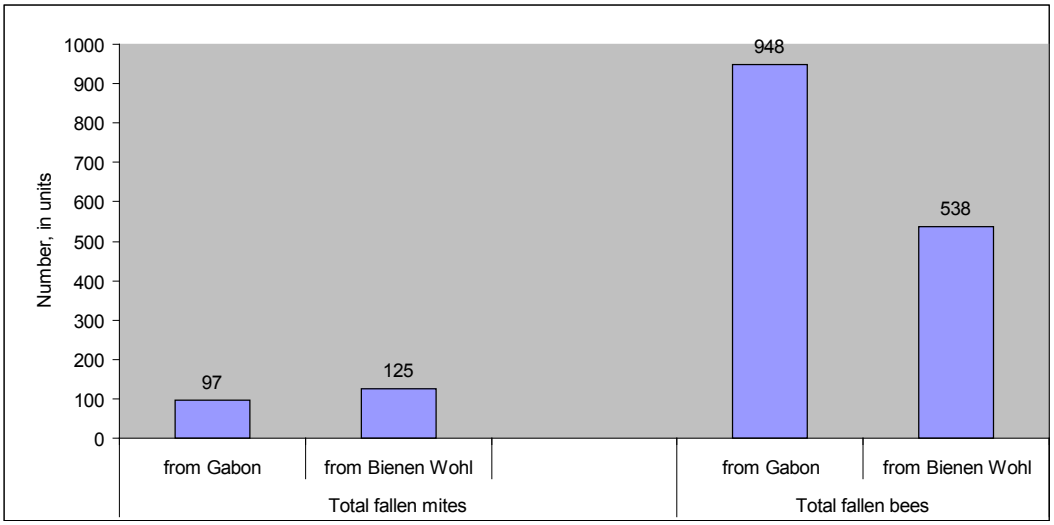

Fig. 3. Comparative estimation of toxicity of Gabon and Bienen Wohl during bee wintering.

3). Gabon was less effective to mites and had stronger toxic impact to bees compared with Bienen Wohl preparation ($P < 0.001$). After keeping strips of Gabon during winter, approx. 97 ± 7.5 fallen mites and 948 ± 116.8 fallen bees ($P < 0.001$) were found in spring, and bee infestation with mites reached 10.2% or 9.5 mites for one percent of contamination. Approx. 125 ± 27.0 fallen mites and 538 ± 167.8 fallen bees ($P < 0.001$) were found after winter when using ecological preparation Bienen Wohl: bee contamination with mites reached 23.2%. Late onetime treatment of bee colonies did not decrease invasion of mites, it may be because after treatment temperature of atmosphere fell down signally. Because of this reason bees bunched together and preparations could not spread onto seperate individuals of the colony suitably. Therefore not only preparations influence efficiency of treatment, but also time they where applied and climatic conditions (Bogdanov et al. 2002, Bacandritsos et al. 2007, Lipinski & Szubstarski 2007).

CONCLUSIONS

Spontaneous depuration of bees progresses slowly and do not protect their from *Varroa destructor* mites.

Time when bee colonies where affected with anti-mite preparations influences their efficiency. During autumn feeding chemical preparation Gabon had better anti-mite effect than ecological preparation Bienen Wohl ($P<0.001$). Though Bienen Wohl had less toxic effect to bees ($P<0.001$) and more effect to mites during winter period in comparisson with Gabon.

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