

## EFFICACY OF DIFFERENT ECOLOGICAL METHODS FOR HONEYBEE (*APIS MELLIFERA*) VARROA PREVENTION IN SPRING

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**Abstract.** The study was carried out with the aim to examine the efficacy of naturally occurring chemicals and mechanical methods for honeybee (*Apis mellifera*) varroa prevention in spring and infestation dynamics during the active season in the treated and untreated colonies under practical Lithuanian beekeeping conditions. Forty colonies were used for formic and oxalic acid, sugar syrup, drone comb trapping treatments and monitoring of the dynamics of varroa infestation. The effect of the oxalic acid treatment was higher than that of the formic acid. However, the formic acid treatment showed higher negative effect on honeybee mortality than the oxalic acid treatment. The honeybee drop after the first and second applications was, respectively, 15 times and 16.8 times higher compared with the oxalic acid treatment. Honeybee sprinkling with sugar syrup increased mite drop 2.5-2.7 times compared with the natural mite drop. However, it also increased the attraction of honeybees to rob foragers. Drone comb trapping significantly reduced infestation of honeybee colonies and it also increased aggressiveness and disorientation of honeybees.

Monitoring of infestation dynamics in the treated and untreated colonies showed that during the summer the infestation of untreated honeybees increased 1.7 times. Oxalic acid treatment in spring did not protect from varroa spread. Although after a week in the oxalic acid treated colonies the infestation of honeybees decreased 5.6 times, afterwards the infestation of monitored honeybee colonies had been constantly increasing and in autumn exceeded the initial level of infestation.

**Keywords:** *Varroa destructor*, *Apis mellifera*, oxalic acid, formic acid, sugar syrup, drone comb trapping.

## EKOLOGINIŲ PRIEMONIŲ, TAIKOMŲ BIČIŲ VARROOZĖS PREVENCIJAI PO PAVASARINIO BIČIŲ *APIS MELLIFERA* APSISKRAIDYMO, EFEKTYVUMAS

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**Santrauka.** Tyrimų tikslas buvo įvertinti natūralių cheminių bei mechaninių priemonių, taikomų bičių varroozės prevencijai, efektyvumą po pavasarinio bičių apsiskraidymo ir bičių užkrėstumo dinamiką gydytose ir negydytose šeimose. Oksalo rūgšties, skruzdžių rūgšties, cukraus vandeninio tirpalo ir tranų perų pašalinimo poveikio tyrimai atlikti 40 šeimų. Gydymui panaudojus oksalo rūgštį, žuvo daugiau erkių ir mažiau bičių, negu panaudojus skruzdžių rūgštį. Po pirmo ir antro bičių šeimų apdoravimo skruzdžių rūgštimi žuvo atitinkamai 15 ir 16,8 kartus bičių daugiau, negu panaudojus oksalo rūgštį. Bičių apipurškimas cukraus vandeniniu tirpalu erkių kritimą padidino 2,5–2,7 karto palyginti su kritimu netaikant gydymo. Tačiau, bites apipurškus cukraus vandeniniu tirpalu, padidėjo bičių plėšikavimo atvejų. Traninių perų šalinimas ženkliai sumažino erkių kiekį vasaros sezono metu, bet dažnas traninių perų šalinimas demoralizavo bičių kolonijas – padidėjo bičių agresyvumas, polinkis spiesti ir plėšikauti.

Erkių plitimo dinamikos tyrimai parodė, kad negydytų kolonijų erkėtumas per vasarą padidėjo 1,7 karto. Bičių kolonijų gydymas oksalo rūgštimi pavasarį vėliau neapsaugojo nuo erkių plitimo. Nors apdorojus oksalo rūgštimi per savaitę kolonijose erkių sumažėjo 5,6 karto, vėliau erkėtumas nuolat gausėjo, o rudenį pasiekė ir netgi viršijo pavasarinį lygį.

**Raktažodžiai:** varroozė, bitės, oksalo rūgštis, skruzdžių rūgštis, cukraus tirpalas, traninių perų šalinimas.

**Introduction.** The *Varroa* bee mite *Varroa destructor* (formerly *Varroa jacobsoni*) has been described as the most serious pest of the European honeybee (*Apis mellifera*) following introduction into bee hives, *Varroa* mites invade brood cells, where offspring produced by female mites feed on the developing host bee causing

debility and/or death (Anderson and Trueman, 2000; Stevenson et al., 2005). Targeted investigations indicated (Stevenson et al., 2005) that the maximum rate of local spread of *Varroa* could be in the order of 12 km/year (interquartile range 10-15 km/year). Honeybees infected by *varroa* mites have low vigour, are completely

undeveloped, when they leave the comb cells, they have lower weight and reduced life length (Pileckas et al. 2009). Some bees have deformed wings, although the latter is not a typical feature of *varroa* infestations, but monitored in bees with viral diseases (Nordstrom 2003). The mites could be the carriers of virus diseases in honeybees (Chantawannakul et al. 2006, Sanpa et al. 2009). Some honeybees from colonies with small and large parasite populations show aggressive behaviour (Martin et al. 2001). Significant differences were found between the stocks in *varroa* mite intensity (mites per adult bee) and in *varroa* mite load (Tarpy et al., 2007), and this diversity offers rich potential of genetic resources for selection on mite resistance (Büchler et al., 2010).

Different control measures have been developed for *varroa*. Most of these measures are based on the use of synthetic acaricides called also miticides or varroacides. The commonly used varroacides belong to three different chemical groups: pyrethroids, formamidine, and organophosphate. The mite can be controlled by the pyrethroids flumethrin and tau-fluvalinate (Williams, 2000) and natural, non-toxic substances, such as different organic acids and essential oils (Charrière and Imdorf, 2002; Gregorc and Planinc, 2002; Ruffinengo et al., 2005; Basandritsos et al., 2007; Akyol and Yeninar, 2008; Pileckas et al., 2008). Acaricide resistant mites have appeared in many countries of Europe and the world (Charrière and Imdorf, 2002; Lipinski et al., 2007; Akyol and Yeninar, 2008; Elzen et al., 2001). Chemical control of *varroa* with acaricides has the disadvantage of variable efficacy mostly due to increasing resistance to these acaricides (Floris et al., 2001; Charrière and Imdorf, 2002). Moreover, these control methods are problematic for a few reasons: some methods are effective (acaricides) but leave residues in the hive products, thereby jeopardizing product quality. Second, highly effective acaricides often lead to the mites evolving resistance to the applied drugs. Third, some control methods are less problematic from residue point of view but are often less effective, more laborious, or damage the bees to varying degrees (Fries and Bommarco, 2007). Although the long-term strategy of chemical control of the *varroa* in honeybee colonies is usually based on delay of appearance of discussed resistance to the given acaricide and thus replacing the ineffective ones, it is advisable to integrate chemical methods with wider use of nonchemical control techniques (Lipinski et al., 2008). Due to lipophilic and persistent nature of synthetic acaricides, they accumulate in wax, and to a smaller extent in honey, therefore, natural, non-toxic substances are used increasingly throughout Europe (Bogdanov et al., 1998; 2002; Korta 2003).

The emergence of *Varroa* infestation of Lithuanian honeybee (*Apis mellifera*) was identified in the colonies (apiaries), using Caucasian Queens and honeybee transportation to melliferous plants (Lisas, 1980).

The objective of the present study was to examine the efficacy of natural occurring chemicals and mechanical methods for honeybee (*Apis mellifera*) *varroa* prevention in spring and infestation dynamics during the active

season in the treated and untreated colonies under practical Lithuanian beekeeping conditions.

**Materials and Methods.** The study was conducted at the apiaries of Radviliškis district: the latitude of 55° 35' to 55° 40' N and the longitude 23° 40' to 23° 45' E. Forty colonies were used in the study. The colonies were divided into five groups. Five colonies were monitored without treatment (Group 1), ten colonies in Group 2 received oxalic acid, and ten colonies in Group 3 received formic acid. With the aim to establish the ability of bees to clean themselves from mites, ten colonies (Group 4) were sprinkled with sugar syrup prepared by the ratio of 1:1. Honeybees were sprayed until the surface of the body covered with sugar syrup aerosol. Comb trapping method (Group 5) that removes capped drone brood from the hives was used in five colonies.

Natural mite drop was monitored in all the groups before treatments. Each hive was equipped with standard plastic sub-frames and vaseline daubed sheets (for alive mites after treatment). The bees were taken from three parts of the hives and sent to sleep using ether vapour. The mites were collected and counted. The level of infestation was expressed as the percentage and was counted using the following formula: Number of Mites / Number of tested Honeybees x 100.

Oxalic acid treatment was applied by Varro Verdampfer evaporator (Switzerland). Oxalic acid treatment was performed on the 1st and 12st May. Nine grams of oxalic acid hydrate was evaporated for 3 min and then the plates with oxalic acid were removed, and the hives were heat sealed for 10 min. The evaporation plates were placed at the comb with brood. Ambient air temperature was 10.8 ÷ 12.3°C.

Formic acid treatment was applied by Nassenheider dispenser (Germany). Application with 40 ml chemically clean 60% formic acid per colony was applied by evaporation using an evaporation plate of 18 cm<sup>2</sup> for 5 days.

For management of *varroa* reproduction in Group 5 special 420 mm x 100 mm frames were used for drone comb trapping. Combs in the upper and lower parts of these frames were designed for worker and drone brood, respectively. Once the cells were capped, comb parts with drone brood were removed and combs without brood were inserted again. The cells were open, removed brood and mites were examined and killed. Empty drone combs were soaked in the solution of acetic acid for 12 hours and then after washing and drying were used for another cycle.

Additionally the dynamics of *varroa* infestation in untreated and oxalic acid treated colonies were monitored monthly beginning with the 1st of May and ending, respectively, on the 20<sup>th</sup> of August and the 1<sup>st</sup> of September before autumn bee feeding.

**Statistical analyses.** The infestation of honeybees used in the study was subjected to descriptive analysis. The data of the mite and honeybee drop before and after the treatments were subjected to one-way analysis of variance (ANOVA) with Tukey's tests to determine the significance of differences of means before and after

applications within treatments using MINITAB 15 software package. The differences were regarded as significant when  $P < 0.05$ . The results were expressed as means and standard deviations.

**Results.** Before the oxalic and formic acid treatments, the average infestation of honeybees used in the study was, respectively, 7.09 and 7.76 % ( $P > 0.234$ ) with a similar natural daily mite drop. The mite drop in the oxalic acid treated colonies on the second day and after 12 days was, respectively 8.2 and 21.6 times higher than the natural mite drop (Table 1). However, repeated oxalic acid application increased honeybee drop. After repeated oxalic acid treatment the honeybee drop was 2.6 times higher ( $P < 0.05$ ) than after the first application. The effect of the formic acid treatment was lower than that of the

oxalic acid. The mite drop in the formic acid treated colonies after the first and second applications was, respectively, 1.4 and 7.4 times higher than the mite drop before the treatment (Table 1). However, the formic acid treatment showed higher negative effect on honeybee mortality than the oxalic acid treatment. The honeybee drop after the first and second applications was, respectively, 15 times and 16.8 times higher compared with the oxalic acid treatment. Moreover, six colonies died after spring formic acid treatments, in the rest four colonies the quantity of drone brood increased and accounted for approximately 85% of the total brood. The introduction of new queens into these colonies caused swarming and collapse of colonies.

Table 1. **Efficacy of former acid, oxalic acid and sugar treatments**

Variables	Treatment		
	Oxalic acid	Formic acid	Sugar syrup
	(n=10)	(n=10)	(n=10)
Infestation level before treatment, %	7.1 ± 1,20	7.8 ± 1,22	13.0 ± 2.31
Effect of treatment on mite drop (number of insects):			
Natural mite drop	18.9 ± 3,78	17,6 ± 1.90	25.6 ± 6.47
After I application	155.1 ± 68.34	23.8 ± 4.96	69.5 ± 12.04
After II application	409.1 ± 53.15	130.8 ± 11.02	63.9 ± 18.17
P value	<0.001	<0.001	<0.001
Effect of treatment on honeybee drop (number of insects):			
After I application	0.8 ± 0.01 1.03	12.0 ± 2.36	
After II application	2.1 ± 1.45	35.4 ± 5.40	
P value	0.033	<0.001	

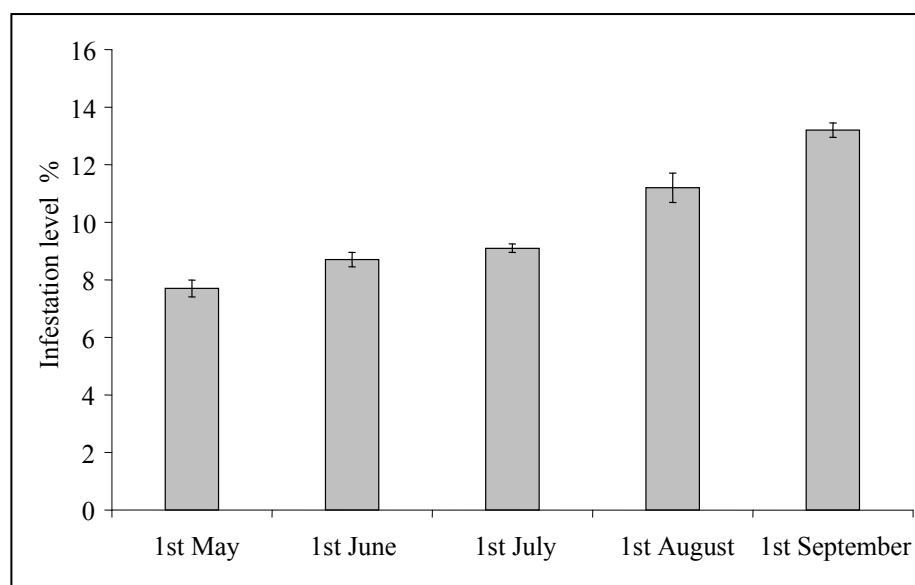


Figure 1. **Dynamics of *Varroa destructor* infestation in the untreated honey bee colonies**

The mite drop in the sugar syrup treated colonies after the first and second sprinkling was, respectively, 2.5 and 2.7 times ( $P < 0.01$ ) higher than the natural mite drop (Table 1). Although honeybee sprinkling with sugar syrup increased grooming behaviour of honey bees, it was insufficient for complete honeybee cleaning. Wet bees

were observed crawling and some colonies attracted robbing foragers.

Drone comb trapping during the summer period highly decreased the honeybee infestation level. There were 764 (70.9%) *Varroa destructor* mites in the 1078 capped cells of the drone brood. In 279 cells of the same

combs on the third and fourth days of larva development period there were 59 mites (21.2% infested larva). This showed that the infestation of larva at 3–4 days of age was treble lower ( $P < 0.001$ ) than the infestation in the capped brood. Nearby the drone combs in 297 capped worker cells 37 *varroa* mites were found. The average number of *varroa* mites per capped drone and worker cell was, respectively, 0.71 and 0.13 ( $P < 0.01$ ). The infestation of the worker brood was 12.5%, id est the infestation of the worker brood was 5.7 times lower ( $P < 0.001$ ) than the infestation of the trapped drone brood. However, frequent drone trapping has increased aggressiveness and the amount of disoriented bees which attracted robbing foragers. The drone-trapped colonies

also showed higher swarming.

Monitoring of infestation dynamics in the treated and untreated colonies showed that during the summer the infestation of untreated honeybees increased 1.7 times. The highest increase of infestation was detected during August (Figure 1). Higher increase of *varroa* infestation was detected in the colonies with a lower initial infestation rate. Oxalic acid treatment in spring did not protect from *varroa* spread but after a week the infestation of honeybees in the oxalic acid treated colonies decreased 5.6 times (Figure 2). However, afterwards the infestation of monitored honeybee colonies had been constantly increasing and in autumn exceeded the initial level of the infestation.

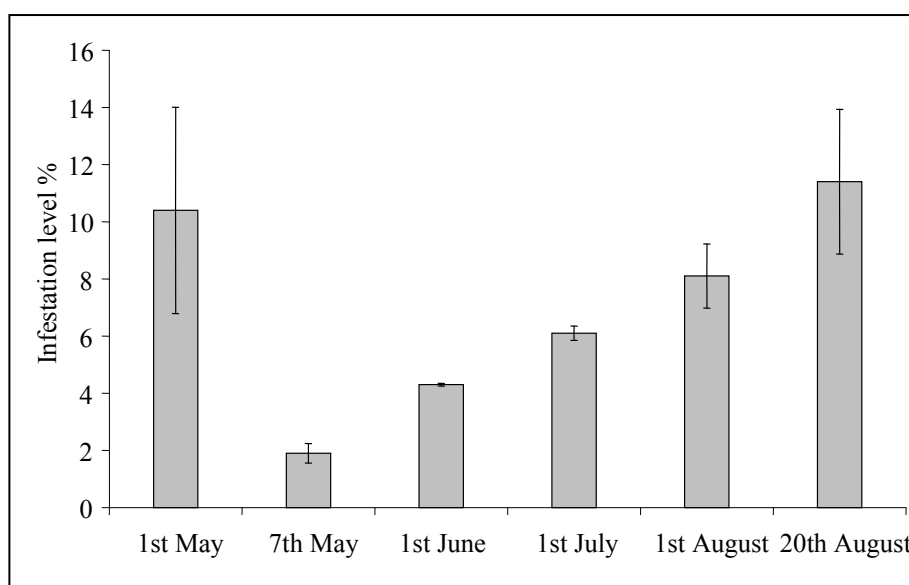


Figure 2. Dynamics of *Varroa destructor* infestation in the honey bee colonies after oxalic acid treatment in spring

**Discussion and Conclusions.** Oxalic and formic acids are natural constituents of honey and they are allowed for use in biological beekeeping (Bogdanov et al., 2002; Rosenkranz et al., 2010). These products have been studied in different countries. However, the climatic environment and the time of year highly influence the efficacy of applications (Sammarato et al., 2008). In this study, formic acid and oxalic acid treatments in honeybee colonies during the spring season resulted in an increase in mite drop and mortality after each application. However, after some time the mite drop reduced and at the end of summer the infestation level of the treated colonies increased to the level determined before their treatment. Moreover, formic acid treatments were associated with an increase in mortality of worker and queen bees (Elzen et al., 2004; Satta et al. 2005; Underwood wet al., 2005). The combination of the concentration of formic acid and the duration of fumigation during the treatment of honeybee colonies determine the efficacy of the treatment. Formic acid given as a low concentration over a long exposure time rather than as a high concentration over a short exposure time would allow effective control of *varroa* mites without

detrimental effects (Satta et al. 2005; Underwood wet al., 2005). Although the amount of formic acid used in this study was relatively not high, formic acid treatment showed detrimental effects too. Some colonies are unable to ventilate their hives (Mutinelli et al., 1994; Мельник, 1991). Oxalic acid offers a good opportunity for the treatment against *Varroa destructor* without side effects on the honeybees (Charrière and Imdorf, 2002; Bogdanov et al., 2002). However, there are some disadvantages of these natural compounds. The efficacy of organic acids depends on the evaporation pressure within the colony, the climatic and within-hive conditions and often is more variable, compared to registered acaricides (Sammarato et al., 2008; Rosenkranz et al., 2010). Results obtained in this study are in agreement with the stated disadvantages. The research demonstrated that dusting with powdered sugar could effectively increase the mite drop from a colony (Ellis et al., 2009). Two behaviours of honey bees, hygienic and grooming, are mechanisms of defence against brood diseases and parasitic mites, including *Varroa destructor* (Stanimirivici et al., 2005; Rosenkranz et al., 2010). In this study, the usage of sugar syrup increased the grooming. However, it was insufficient for

complete bee clean. Observed crawling wet bees, probably as a result of the large amount of liquid that was twice administered to each colony and their attraction to rob foragers, also, correspond to the results of Sammarato et al. (2008), who reported similar problems of liquid sucroside applications. Biological methods which include peculiarities of the biology of host and parasite are the real, sustainable approaches for *Varroa* treatment (Rosenkranz and Ziegelmann, 2010). *Varroa* reproduction is closely synchronized with the development of its host. Female mites deprived of feeding activated oogenesis when perceiving larval volatiles (Rosenkranz and Garrido, 2004). Female mites prefer drone brood. Since nurse bees spend much more time feeding drone larvae than worker larvae (Calderone and Kuenen 2003), the mites have ample opportunity to come into contact with drone larva. Thus, drone comb trapping is used for further treatment as a regulative mechanism for the host and parasite relationship. In our study drone comb trapping significantly reduced the infestation of colonies. However, it increased aggressiveness and the numbers of disoriented honeybees. Wilde et al. (2005) reported that swarming seems to be a promising strategy for the host to divide the population of the parasite and reduce mite reproduction through a broodless phase. Villa et al. (2008) reported that in years immediately after the arrival of *Varroa destructor*, swarming rates in Hymenoptera Apidae decreased, however, after five years in the presence of *Varroa destructor* the rates recovered to levels at least as high and higher as those seen before *varroa* arrived. In Lithuania, the mite infestation in *Apis mellifera* is under surveillance from 1976. Although the infested colonies were decimated and applied methods, such as introduction of the new Queen and quarantine of hives, were not effective, the transmission of *varroa* proliferated with annual rate of 3-4 km (Valentanavičius, 1981). It was determined that long presence of *varroa* infestation of Lithuanian bees could explain the increased swarming. Swarming seems to have resulted the increased infestation after treatments but was not a result of applied treatments alone.

It can be concluded that the effect of the oxalic acid treatment was higher than that of the formic acid. However, the formic acid treatment showed higher negative effect on the honeybee mortality than the oxalic acid treatment. Honeybee sprinkling with sugar syrup increased the mite drop 2.5–2.7 times compared with the natural mite drop but it also increased attraction of honeybees to rob foragers. Drone comb trapping significantly reduced infestation of the colonies. However, it increased aggressiveness and disorientation of honeybees.

Monitoring of infestation dynamics in the treated and untreated colonies showed that during the summer the infestation of untreated honeybees increased 1.7 times. However, after the oxalic acid treatment the infestation of monitored honeybee colonies had been constantly increasing and in autumn exceeded the initial level of the infestation.

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