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Original research paper

USING OXALIC ACID FOR VARROA MITE CONTROL IN HONEYBEE COLONIES DURING THE BEEKEEPING SEASON

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Summary: Twenty-one *Apis mellifera carnica* honeybee colonies were used to record the levels of Varroa mite mortality in the periods before and after oxalic acid (OA) treatments, which were conducted after the honey harvesting. The colonies each received from four to seven OA treatments. During the pre-treatment period, the daily natural mite mortality was estimated at 0.56 (±0.74). A high correlation was established between the daily pre-treatment mite mortality and the cumulative total of dead mites after each of the consecutive OA treatments (R = 0.92387). In the colony with the lowest daily mite mortality (0.08 ±0.05) there was no correlation with the number of mites that fell after the initial OA treatments conducted on August 1. The relative mite mortality ranged from 7.78 % (±1.68) during the brood period to 88.87 % (±8.41) in colonies without brood. Reducing a colony's mite population by employing OA treatments and a mite-control programme are discussed in this study.

Key words: honeybee; *Apis mellifera;* varroa control; oxalic acid

Introduction

Varroa destructor, a parasite of *Apis mellifera,* has to be controlled by the regular use of acaricides in order to maintain honeybee colonies. These are usually synthetic and their lipophilic and persistent characteristics result in a build-up of their residues in the wax and honey (1). Acaricide resistant mites have appeared in several European countries (2, 3). Natural, non-toxic substances to control varroa mites, such as organic acids (4) and essential oils (5, 6), have been developed and are increasingly being used by beekeepers (7).

Oxalic acid is a natural constituent of honey and EU regulations permit its use in biological beekeeping (EU Council Regulation, No. 1804/1999). Because of its high efficacy, OA is widely used in most Western European countries (7). Research has been conducted into the efficacy of oxalic acid applications (OA) as a method for controlling the mite in colonies, both with and without brood (8, 9, 10, 11).

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Experiments have been conducted in honeybee colonies by spraying (9), evaporating (12) and trickling an OA-water solution into the hive (13, 14, 15). During broodless periods, Radetzki (16), Nanetti et al. (17) and Imdorf et al. (18) found it to be highly effective in killing the mite and they estimated the elimination level at 97.3 %, 98.3 % and 99.5 %, respectively. When a capped brood was present, Mutinelli et al. (15) achieved 95 % efficacy after three treatments of a 5-%-OA solution and Brřdsgaard et al. (10) reported a 24 % efficacy of one spring treatment administered by trickling.

In our previous experiments 50 millilitres of an OA solution was used to treat one normally developed colony. Three OA treatments had an efficacy of 39.2 % when a brood was present and 99.4 % when there was no brood (13). This paper presents data from the periodic checking of the number of mites that had fallen onto the bottom of hives to determine the natural mite-fall. The aim was to establish the effectiveness of OA as a single substance for controlling varroa in honeybee colonies by using a sucrose-in-water solution (14). We also aimed to establish the optimal strategy for using oxalic acid applications to control mites during the

2002 season in colonies with capped broods and for winter treatments of broodless colonies.

Materials and methods

Twenty-one *Apis mellifera carnica* honeybee colonies, populated in national standard AŽ "back load" hives (19) with nine combs (41 x 26 cm) in each brood and honey compartment, were located at one site near Vipava. In the spring of 2002, metal sheets (38 x 29.8 cm) were placed on the floor of each of the hives in order to record the hives' natural mite mortality. On the sampling dates, the numbers of mites were recorded. The pre-treatment natural mite fall of each of the colonies was recorded on 6 different occasions for those colonies whose initial OA treatment was on August 1 and on 9 different occasions for those colonies whose treatment began on August 8. The mite mortality after each of the consecutive OA treatments was also recorded.

The number of OA treatments each colony received was determined after establishing the mite mortality before and after each treatment. The treatments were performed as follows:

- Group A, which consisted of five colonies (Nos. 1, 2, 8, 14, 19), received 7 OA treatments; on August 1, 8 and 20, September 7, October 10, November 14 and December 24;
- Group B – four colonies (6, 9, 17, 31) – received 6 OA treatments; on August 1, 8 and 20, September 7, November 14, and December 24;
- Group C – four colonies (11, 20, 21, 24) – received five OA treatments; on August 1, 8 and 20, November 14, and December 24;
- Group D – three colonies (4, 23, 29) – received five OA treatments; on August 8 and 20, September 7, November 14, and December 24;
- Group E – five colonies (3, 5, 7, 27, 28) – received four OA treatments; on August 8 and 20, November 14, and December 24.

The treatments were applied to each colony by trickling the OA solution over the combs, *in situ*, and squirting the bees in the brood compartment using a syringe. Respiration masks, protective glasses and rubber gloves were worn while applying the solution. The mite fall during the treatment period was recorded after each OA application.

The outside temperatures during the August OA treatments ranged from 30 to 33 ^{0}C. On September 7 the outside temperature was 24 ^{0}C, on October 10 it was 22 ^{0}C, on November 14 it was between 10 and 12 ^{0}C and on December 24 it was 6 ^{0}C.

The colonies received 50 millilitres of a 2.9 % OA and 31.9 % sucrose-in-water solution (w/w), using oxalic acid dihydrate (Riedel-de Haën), sucrose (sugar) and de-mineralised water (Gregorc and Planinc, 2001).

The percentages of mites killed by the experimental treatments (FTB) were estimated using the formula: FTB = FOA1 / (FOA1 + FOA2) x 100 (Gregorc and Planinc, 2001). FOA1 is the total number of mites that dropped during the consecutive treatments of colonies with capped broods and FOA2 is the number of mites that fell during the December treatment of the broodless colonies.

The efficacy of the treatments was also estimated by comparing the numbers of mites that fell before and after the treatments and the mite mortality between the consecutive OA treatments. The data analyses were performed by ANOVA (analysis of variance) with the use of the Statgraphic (20) programme.

Results

During the pre-treatment observation periods, a total of 39 days performed in intervals between May 2 and August 1, 2002, the average daily natural mite-death was estimated at 0.56 (±0.74). The average mortality per colony during the total observation period was estimated at 21.71 (±29.01) mites. In this period 1.45 % (±0.83 %) of the total varroa mite population died naturally. The average numbers of mites that dropped onto the bottom boards of each group are shown in Table 1.

Mite mortality after the first OA treatment was significantly higher ($P<0.01$) in the colonies of group A than it was in groups B, C, D and E. Statistically significant differences were also found between these groups. The number of mites that fell after the first OA treatment of the highly-infested colonies of group A correlated with the daily mite mortality prior to treatment (R = 0.81398) (Fig. 1). A high correlation was found when the total number of dead mites (R = 0.8851387) and the daily mite mortality (R = 0.92387) during pre-treatment period were compared to the cumulative total of dead mites observed after each of the consecutive OA treatments.

Table 1: The average (±SD) mite mortality after consecutive OA treatments. The number of mites that fell naturally prior to treatment and the % of natural mite mortality prior to the treatments compared to the total number of mites that fell during the experiment. The data relate to the five groups of colonies, each of which were exposed to a different number of OA treatments

Group	Mite mortality after the OA treatments (±SD)	Mite mortality prior to the OA treatments (±SD)	Mite mortality (%) prior to the OA treatments (±SD)
A	3107.80 (±1622.57)	60.00 (±40.84)	1.94 (±0.81)
B	1248.00 (±553.12)	1.00 (±1.15)	0.06 (±0.08)
C	754.25 (±331.82)	13.25 (±5.56)	1.77 (±0.66)
D	831.00 (±176.55)	14.33 (±4.04)	1.68 (±0.13)
E	520.40 (±271.14)	0.00 (±0.00)	0.00 (±0.00)

The cumulative mite mortality after the OA treatments of the different groups and the natural daily mite mortality prior to the OA treatments are shown in Figure 2. In the colony that had the lowest average daily mite mortality (0.08 ±0.05) there was no statistically significant correlation with the number of mites that fell after the initial OA treatment conducted on August 1.

In the colonies of group A, the average mite mortality after the first OA treatment was 204.4 (±81.60). The mite mortality after each OA treatment ranged from 114.4 (±150.80) mites after the seventh treatment conducted on December 24 to 1065 (±605.38) mites after the fifth treatment on October 5 (Figure 3). The number of mites that fell after the first OA treatment on August 1 was, on average, 341.48 (±352.17) times higher than the average daily natural mite mortality (0.55 ±0.78) monitored in the pre-treatment period.

The relative mite mortality during the brood period ranged from 7.78 % (±1.68) recorded after the first OA treatment conducted on August 1 to 88.87 % (±8.41), which was recorded after the November 11 OA treatment when the colonies were without brood. The relative values of mites that fell during the OA treatments are shown in Figure 4.

All the colonies had queens throughout the experiment and the normal death rate of the worker bees was not altered by the treatments. The development of the colonies over winter and the spring of 2003 were normal and comparable to the development of other colonies in the region.

Discussion

Counting the mites that drop onto the bottom board is a reliable diagnostic method (21). The correlation between the high natural mite mortality and the number of mites that fell after the OA treatments indicates the importance of measuring the natural mite mortality prior to treatments in order to accurately establish the degree of a colony's infestation.

The natural mite-mortality per day, which until the August OA treatments was estimated at an average of 0.56, increased up to a maximum of 1.54 mites per day. The colonies of the experimental apiary showed variations in the levels of mite infestation. The high daily natural mite-mortality correlates with the numbers of mites that fell after the first and subsequent OA treatments. The total mite mortality after several OA treatments also correlates with the pre-treatment natural mite mortality. A relationship between the mites in the hive debris and the mite population (22) is evident in colonies with approximately one mite "drop down" per day. In colonies with a low mite mortality (approximately 0.5 mite per day), the correlation is not as evident and considerable differences in mite mortality after the OA treatments are found. In these colonies the cumulative mite mortality during the OA treatments averaged 701 (±161) (Fig. 2).

It is important to continuously monitor the number of mites dropping in bee colonies in order to establish the appropriate timing and sequence of the summertime OA treatments. It seems that for colonies with approximately 1.5 natural mite-deaths per day a suitable programme of OA treatments should be established. In our experiment the reduction of the mite population by 7.78 % after the first, and 9.2 % after the second OA treatment ensured that the colonies remained viable. The efficacy of further OA applications increased up to 53.4 % and 88.9 % after the October and November OA treatments, respectively. The results of our experiment confirmed that using OA to treat

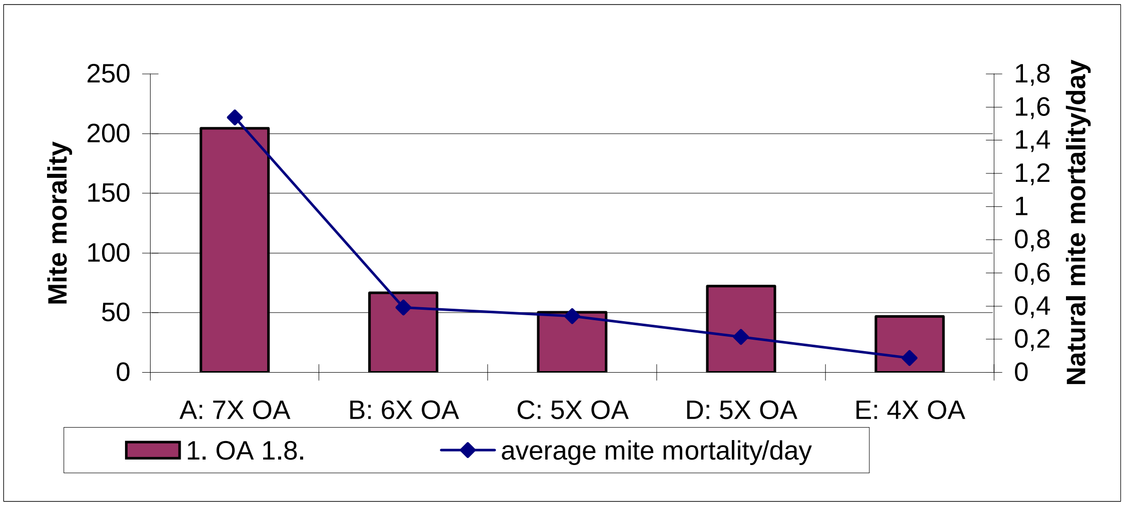

Figure 1: Diagram showing treatments groups (A-E) and the number of mites that fell after the first OA treatment conducted on August 1. The average natural mite mortality/day during the pre-treatment observation period is also shown to illustrate the high correlation between the two sets of figures (R = 0.81398)

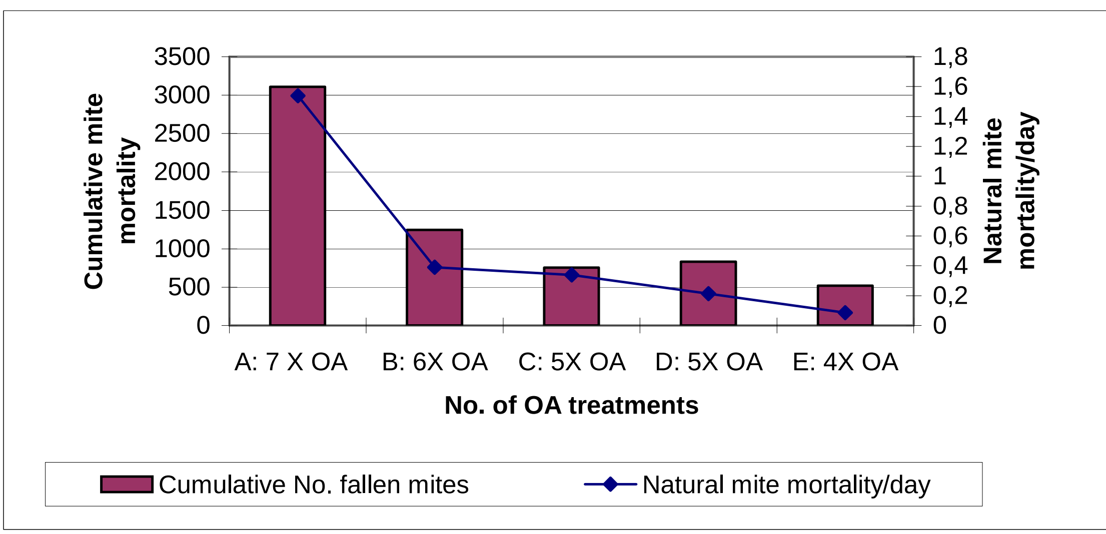

Figure 2: The cumulative mite mortality following the oxalic acid applications during the experiment is shown separately for each group. The average daily natural mite mortality during the pre-treatment observation period is also shown. As indicated, the colonies in each group received between four and seven treatments

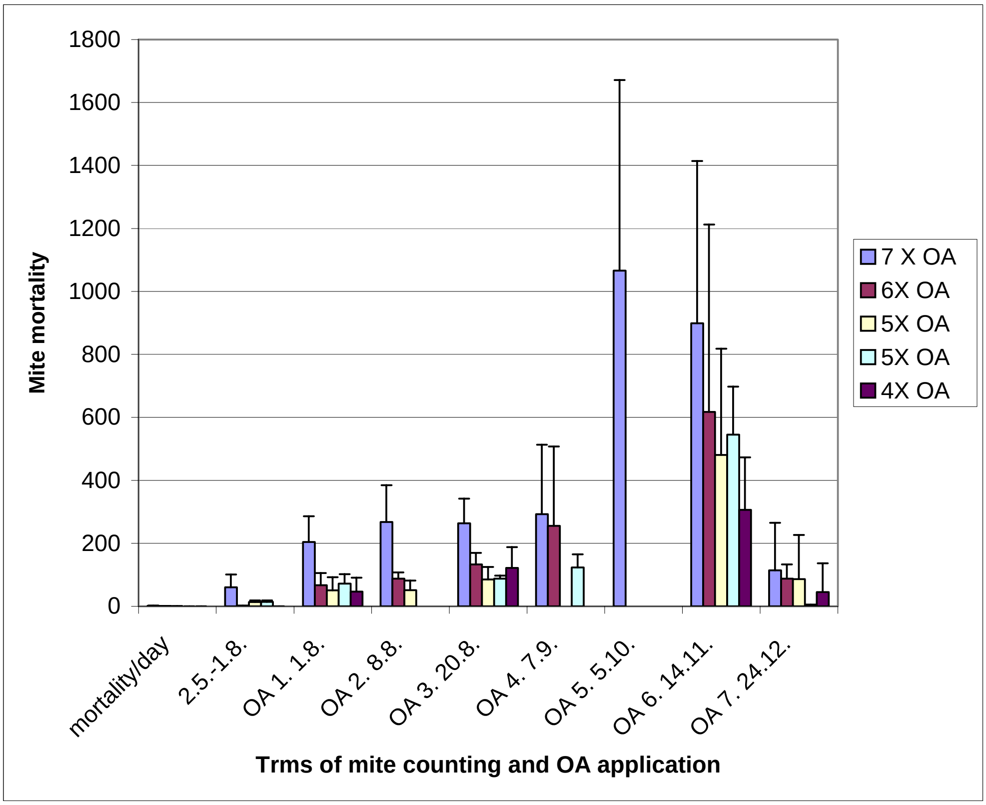

Figure 3: Mite mortality after each of the OA applications. All colonies received OA treatments on August 8, August 20, November 14 and December 24. The colonies in group A, which had a higher mite mortality during the experiment, were additionally treated during the brood period on August 1, September 7 and October 5. The results achieved by the final OA treatment, conducted on December 24, indicate that there were very few mites remaining in the colonies that were by then broodless and preparing for overwintering. Bars indicate standard deviation

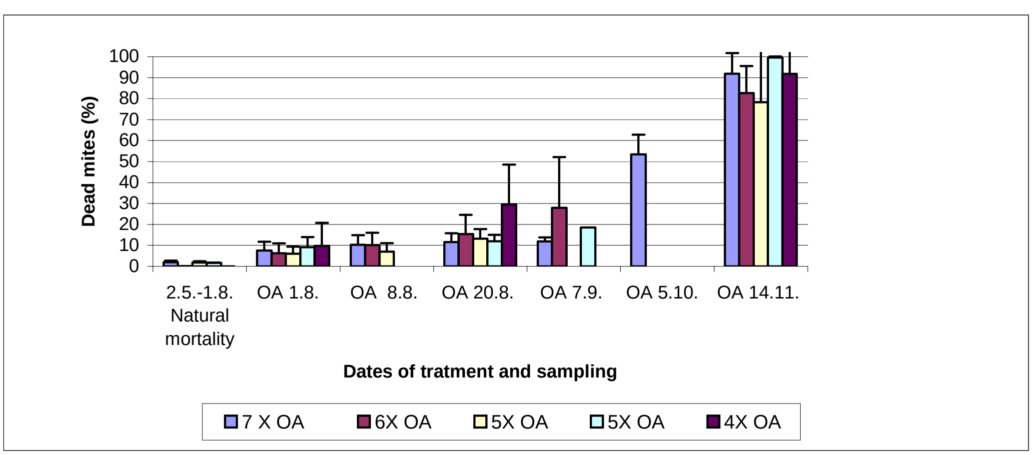

Figure 4: Relative OA efficacy expressed as a percentage of mites killed. The first set of columns labelled "2.5. - 1.8. Natural mortality" represents the percentage of mites that died naturally during the pre-treatment observation period. The other sets of columns represent the cumulative mite mortality after each OA treatment relative to the total mite mortality observed throughout the experiment. Bars indicate standard deviation

colonies without a capped brood is highly effective (13, 17). The consecutive OA treatments of colonies with brood initially had an efficacy of 12 % and 23 % (August), which rose to 51 % after the November treatment (13). Brødsgaard et al. (1999) established similar levels of OA efficacy against the mites and Gregorc and Poklukar (23) achieved a level of approximately 21 %.

The efficacy of the OA applications that we applied during the season are understated because mite reproduction within the colonies and mite re-invasion from both control and neighbouring colonies were not taken into account. Reducing a colony's mite population by employing OA treatments after the honey extraction is essential to ensure its normal development and wintertime survival. The use of an OA-only mite-control programme in a honeybee colony is an effective and useful method of reducing the mite population and helping the colony develop in specific climatic and geographical conditions.

Further experiments must be conducted in order to establish how to manage separate and specific treatments of highly-infested colonies within an apiary and to establish a method to evaluate the degree of a mite reinvasion.

Acknowledgements

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UPORABA OKSALNE KISLINE ZA ZATIRANJE VAROJ V ČEBELJIH DRUŽINAH V ČEBELARSKI SEZONI

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Povzetek: V enaindvajsetih čebeljih družinah smo ugotavljali naravni odpad varoj pred dajanjem oksalne kisline (OA) in po njem. V poskusu so bile čebelje družine v devetsatnih AŽ panjih. Kovinski testni vložki (38 x 29,8 cm) so bili vstavljeni v podnico panja spomladi 2002. Oksalna kislina je bila uporabljena po končanem pašnem obdobju in po iztočenju medu iz panjev. Družine so bile zdravljene od štiri- do sedemkrat. Vsaka družina je dobila 50 ml vodne raztopine oksalne kisline (2,9 % oksalne kisline in 32,9 % sladkorja v vodi). Odstotek mrtvih varoj na testnem vložku smo izračunali na osnovi števila odpadlih varoj po posameznem zdravljenju in ga primerjali s skupnim številom odpadlih varoj v posamezni družini v celotnem poskusu. Učinkovitost posameznega dajanja oksalne kisline smo ugotavljali s primerjavo odmiranja varoj po dodajanjih in analizirali varianco posameznih parametrov. Pred zdravljenjem je naravno odpadlo na dan 0,56 (±0,74) varoj. Korelacija je bila ugotovljena med dnevnim naravnim odpadom pred uporabo oksalne kisline in kumulativno vrednostjo odpadlih varoj po zaporednem dodajanju oksalne kisline (R = 0,92387). V družini z najnižjim naravnim odpadom varoj (0,08 ± 0,05) ni bilo korelacije s številom odpadlih varoj po prvem dodajanju oksalne kisline 1. avgusta. V času, ko je bila v družinah zalega, je bil relativni odpad varoj po uporabi oksalne kisline 7,78 % (±1,68), odpad v času brez zalege pa je bil 88,87 % (±8,41). V prispevku obravnavamo možnosti zmanjševanja populacije varoj v čebelji družini z uporabo oksalne kisline.

Ključne besede: medonosna čebela; *Apis mellifera;* zatiranje varoj; oksalna kislina