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Using oxalic acid in water solution in control of Varroa mites and its influence on honey bees

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Abstract. We studied the toxicity of water solutions containing various concentrations of oxalic acid dihydrate to bees and Varroa mites (*Varroa destructor*) using by spraying honey bee (*Apis mellifera*) colonies with no brood or little brood in beehive conditions in West-Viru County, Estonia. A water solution of 0.5% OA gave effective control of the mite and was not toxic to bees whereas higher concentrations of OA (1.0 and 1.5%) were highly toxic to bees. In autumn, spraying test colonies that had little capped brood once or twice with a 0.5% OA solution gave effective mite control (92.94 ± 0.01% and 91.84 ± 0.02%, respectively) with no noticeable toxicity to bees.

Keywords: *Apis mellifera*, honey bee, *Varroa destructor*, oxalic acid.

INTRODUCTION

Some synthetic drugs such as **Apistan®** and **Bayvarol**®. are successfully used against honey bee parasitic mites *Varroa destructor*, but they leave the residues in honey and wax and may be dangerous for human health. Spraying honey bee colonies with 2 or 3% oxalic acid water solution has been used to control the Varroa mite since the 1980's. At first, 2% oxalic acid, at a dose of 100–150 ml per colony, was found to be very effective in mite control and, at the same time, harmless to bees (Takeuchi and Harada, 1983). More recently, spraying 5–8 ml of 3% OA solution per comb has been recommended (Radetzki et al., 1994). A single spray applied during a brood-free period, has given, on average, 94.5–98.8% (Imdorf et al., 1997) and 97.29% (Radetzki et al., 1994) effectiveness against the mite, whereas spraying twice has given up to 99.5% (Nanetti et al., 1995). Usually, no negative toxic effects have been observed in bees. Lately however, some negative after effects have been reported, namely a decrease in brood area after spraying four-times (Higes et al., 1999); others found that this concentration weakened colonies in the winter following treatment, particularly after two or three treatments during the summer or autumn (Rademacher & Harz, 2006).

The effect of solutions containing less than 2% OA has been little studied so far. Aqueous oxalic acid solutions in concentrations 1.7 and 1.4% have been reported to kill Varroa mites whereas a 0.7% aqueous solution had no effect (Takeuchi & Sakai,

1983). We found that even 0.5% OA solution guarantees the highly effective mite control in our pilot tests. Despite extensive recent research, optimum OA concentrations as well as OA amount per comb have not been established so far. The aim of our present study was to determine the concentration of oxalic acid solution that would guarantee sufficient control of the Varroa mite disease with low bee mortality.

MATERIALS AND METHODS

Two experiments were conducted from 2003–06 to test a range of different concentrations of oxalic acid for their toxicity to Varroa mites and honeybees. The studies were carried out at an apiary in West-Viru County, Estonia. The bees were housed in Estonian standard one-box long hives with a frame size of 414×277 mm, with a side area of 1000 cm^2. The ambient temperature during the experiments was 4–12°C. Hives were provided with Varroa screens. The solutions with different concentrations were sprayed using a hand-operated atomizer directly onto bees on combs removed from the hives and replaced after spraying. The strength of each colony was estimated according to the Liebefeld method (Gerig, 1983, Imdorf et al., 1987) before and after the spraying experiments. Seven days after the last spraying, dead mites were counted and colony strength estimated again. Apistan strips were then placed in each test and control colony to determine the number of mites that had survived the test. A strip was placed into each comb space, except for both side comb spaces and kept there for 6 days, after which mites had fallen onto the sheet of paper placed under the varroa screen on the bottom of the hive were counted. In determining the effectiveness of the control, the cumulative mite-fall after oxalic acid and subsequent control treatment was assumed to be 100%.

Experiment 1. Water solutions with 0.5, 1.0, 1.5% OA (April 2003).Twelve colonies were used with three test hives and a control colony in each experimental group. The brood was cut out of the brood comb and the queens were caged to prevent egg-laying prior to treatment. Test colonies were sprayed with different concentrations of OA aqueous solutions four times at 3–6 day intervals depending on the weather, control colonies were sprayed with same amount of water. The bees were sprayed until wet at dosage 25 ml per comb. The bees outside the combs were sprayed additionally with 25 ml OA solution. Thus for an 8-frame colony $8 \times 25 + 25 = 225$ ml solution of OA was applied.

Experiment 2. A water solution with 0.5% OA was applied once or twice (September 2005). Forty colonies were split into 2 groups, each having 15 test colonies and 5 control colonies. The area of capped brood was assessed in each colony. Group 1 test colonies were sprayed once and group 2, twice with a 6-day interval. Dosage per comb was the same. Group 1 control colonies were sprayed once and group 2 control colonies twice with the same amount of water. Dead mites were counted and colony strength assessed 7 days after the final spraying. The following spring (30 March 2006), colony strength was reassessed and mite survival assessed.

For statistical analyses the program Statistica 7.0 and one-way ANOVA was used. The means were compared by the LSD Test, and *t*-test was applied at a significance level of $p < 0.05$.

RESULTS

Experiment 1, in which bees in brood-free colonies were sprayed four times in early spring (April) with 0.5, 1.0 and 1.5% OA in aqueous solution confirmed the results of the earlier pilot research (Fig. 1).

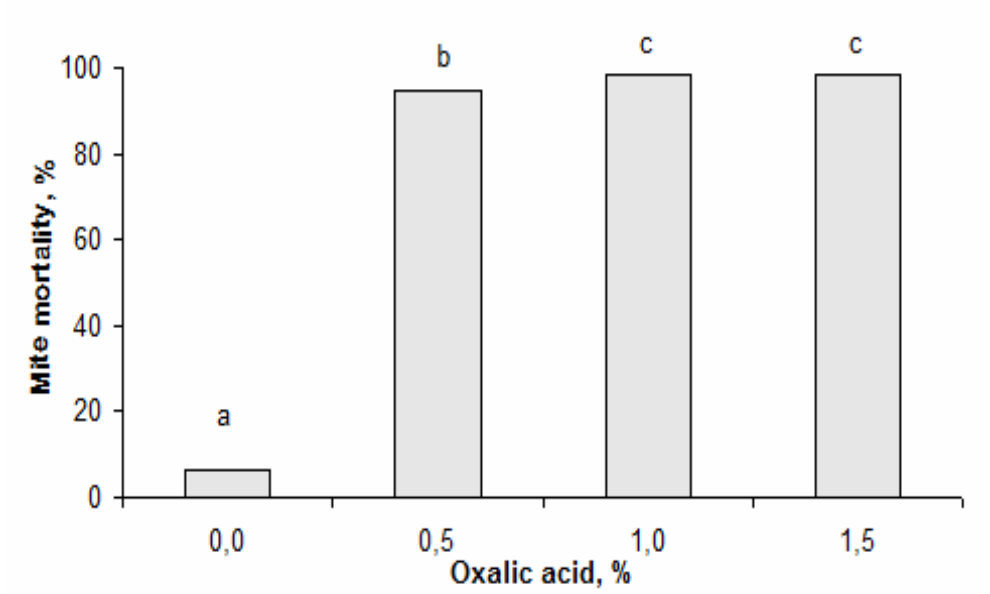

Figure 1. Effect of spraying bees in brood-free colonies in April 2003 with 0.5; 1.0 and 1.5% oxalic acid dihydrate in aqueous solution four times on the mortality of Varroa mites (dose 25 ml per comb, n = 3). Mean values marked with same letters do not differ significantly. The 0.5% concentration was significantly less effective than the 1.0 and 1.5% concentrations (ANOVA one-way, LSD test, $F_{3;8} = 4466.64$, $p = 0.000$).

Solutions of 1.0 and 1.5% OA gave similar mite control (98,39 ± 0,01% and 98,28 ± 0,01%); the 0.5% aqueous solution gave a weaker, but significant effect (94,92 ± 0,00%) (ANOVA, LSD test, $F_{3;8} = 4466.64$, $p = 0.00$) (Fig. 1). However, the 1.0 and 1.5% concentrations were highly toxic to bees: about twice the number of bees in the colony died according as estimated in the Liebefeld method (Gerig; 1983, Imdorf et al., 1987) before and after the spraying experiments (*t*-test, $p < 0.05$). The 1.0 and 1.5% concentrations weakened the colonies considerably, whereas the 0.5% solution had no noticeable toxic effect (Table 1). In both groups, two of the three colonies were severely affected; the weakening was noticeable after the third and fourth spraying. In cool weather, dead bees were found in the hive behind the partition board and a few bees were seen to leave the hive, stagger and die in front of the entrance.

Experiment 2 showed that spraying colonies with 0.5% OA in aqueous solution, in September when they had little sealed brood, was highly effective in killing mites

(Fig. 2). The average efficacy in colonies sprayed once was 92.94 ± 0.01% and in those sprayed twice, 91.84 ± 0.02%; in control groups only 5.41 ± 0.01% (ANOVA, $F_{3;36}$ = 683.96, p = 0.00) of mites died. Treatment effects were significantly lower in colonies with more capped brood during spraying (r = -0.806, n = 30). Spraying once or twice with a dose of 25 ml per comb proved non-toxic to bees: test colonies were no weaker than control colonies after the autumn spraying or the following spring (ANOVA, LSD test, $p > 0.05$). The number of dead mites ranged from 436 to 9164 per colony, on average 3329.74 ± 500.94 mites.

Table 1. Effect of spraying bees in brood-free colonies in April 2003 with 0.5; 1.0 and 1.5% oxalic acid dihydrate in aqueous solution (dose 25 ml per comb, n = 3) four times on colony size. The 1.0 and 1.5% concentrations weakened the colonies considerably (t = 2.3206, p = 0.05 and t = 9.3228, p = 0.0007, t-test) whereas the 0.5% solution had no noticeable toxic effect (t = 0.2579, p = 0.8092, t-test).

Concentration of OA in solution, %	Number of experiments (N)	Number of bees in colony		Decreasing no of bees, %
		Before treatment	After treatment	
0	1–3	12,026 ± 817	11,660 ± 1040	3.1 ± 2.4
0.5	4–6	12,613 ± 1505	12,027 ± 1706	4.7 ± 2.6
1.0	7–9	11,147 ± 264	3887 ± 3117	65.1 ± 26.6
1.5	10–12	13,273 ± 147	2860 ± 1107	78.5 ± 8.7

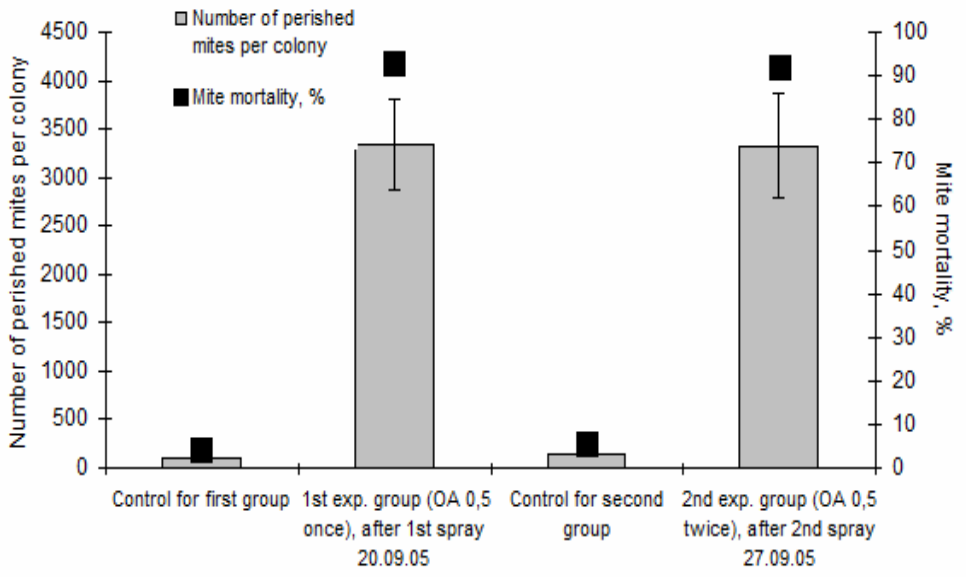

Figure 2. Effect of spraying bees in colonies with some capped brood with 0.5% oxalic acid dihydrate in aqueous solution (dose: 25 ml per comb, n = 15), either once (13.09.05) or twice (13.09.05 and 19.09.05) in autumn on Varroa mite mortality

Different letters indicate statistically significant differences (ANOVA one-way, $F_{3;36} = 683.96$, $p = 0.00$).

DISCUSSION

In the spraying experiments, 0.5% OA in aqueous solution was as effective as 1 and 1.5% solutions for mite control (Fig. 1) but less toxic to bees (Table 1). Spraying once or twice with 0.5% OA in aqueous solution, in September 2005, showed equally high efficacy on mite control (Fig. 2) and little toxicity to bees. We conclude that a single spray of this concentration and at a dose of ca 25 ml per comb should be used for effective Varroa control. Spraying later, mainly at the end of September for Estonian conditions, when there is less brood in the hive, can increase its effectiveness. In Central European conditions presumably it would be suitable to treat in November or even in December depending on the presence of the brood. The treatment effect can also be increased by spraying bees on combs from different directions, i.e. wetting them as much as possible with an OA solution. The spraying method is the more suitable in amateur apiaries. Use of low spray concentrations of OA harmless to bees could probably be successfully integrated in normal beekeeping practice with or without only small areas of capped brood. If the number of mites is high the colonies should be treated in early autumn, after the honey harvest, using the spraying with 0.5% OA or other natural substances like formic- and lactic-acid which are less harmful to the honey bees and to the person applying the treatment.

CONCLUSIONS

We conclude that 0.5% oxalic acid in a water solution at a dose of 25 ml per comb applied as a single spray in honeybee colonies with little or no brood achieves effective control of the Varroa mite. Spraying with a weak solution of OA is less harmful to the honey bees and to the person applying the treatment.

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