RESIDUE DETERMINATION IN HONEY AFTER A SPRING TREATMENT WITH THYMOVAR AND FORMIC acid

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ABSTRACT

In the Netherlands Thymovar and formic acid are permitted to control the mite *Varroa destructor* in honeybees. Because the risk of residue in honey both Thymovar and formic acid are advised to be applied at the end of the summer after honey harvest. Due to a heavy *Varroa destructor* infestation, it can be necessary to conduct a treatment in spring.

In this study we have determined thymol and formic acid residues in honey when a honey super is placed on a hive immediately after termination of a treatment with Thymovar or formic acid in early spring.

The study was conducted with three groups of 10 honeybee colonies placed on a location where a good honey flow could be expected. One group was treated with Thymovar, one group was treated with formic acid and a third group was used as a control. The thymol was applied during 21 days. The formic acid treatment was applied during 14 days. Formic acid 60 % was evaporated with a Nassenheider evaporator. After the treatments honey supers were placed on all hives. As soon as two combs with honey were sealed completely, samples were taken. The honey samples were analysed on formic acid and thymol residue.

The thymol residue in the honey from the group treated with Thymovar (average 0.384 mg/kg) was significant higher than the thymol residue in the honey from the control group (average 0.036 mg/kg). The formic acid residue in honey from the group treated with formic acid (average 143.6 mg/kg) was also significant higher than the formic acid residue in the honey from the control group (average 48.6 mg/kg). Both Thymovar and formic acid treatments increased residue levels in the honey.

INTRODUCTION

In the Netherlands Varroa control with a combination of a biotechnical method plus organic acids or Thymovar is recommended. Thymovar and formic acid are advised to be applied at the end of the summer after honey harvest. In case of a heavy Varroa destructor infestation, a spring treatment is advised.

In this study thymol and formic acid residues in the honey are determined in case a honey super is placed on a hive, immediately after termination of a treatment with Thymovar or formic acid in early spring.

MATERIALS AND METHODS

Thirthy queen right honeybee colonies were placed in an area were a good honey flow could be expected. Each honeybee colony was equipped with a Varroa bottom board (Universalboden Theodor Martin). The 30 honeybee colonies were at random divided into three groups of 10 honeybee colonies. The 10 honeybee colonies of each group were placed next to each other. The distance between the three groups was 5 meters at least. One group was treated with formic acid and one group was treated with Thymovar. The third group was used as the control group. To ensure that the growth of the honeybee colonies was not impeded, an extra brood chamber was placed under the honeybee colonies.

Treatments

Thymovar was applied according to the instructions that are provided with the commercial product. The treatment with Thymovar was started on 19 March 2003 (day 0). On each honeybee colony one Thymovar plate was applied on top of the combs. On day 21 removal of the plates from the honeybee colonies ended the treatment with Thymovar. Thymol evaporation from the Thymovar plates was not determined.

The treatment with formic acid was started one week after the start of the Thymovar treatment (day 7). For this treatment a Nassenheider evaporator was used with 60 % formic acid. The formic acid treatment ended on day 21. The recommended evaporation rate is 6 – 10 ml per day. The amount of formic acid evaporated was recorded on day 11, day 14 and day 21.

Mite fall

The mite fall was counted on the bottom board on day 7, day 21, day 27, day 35 and day 40.

Honey harvesting

On day 21 directly after the treatments were ended, honey supers were placed on every honeybee colony. Three weeks later the honey supers were taken off. The honey was harvested directly after the removal of the honey supers. The sealed honey was sampled in glass sampling jars. After harvesting the samples were stored at 5° C for two weeks.

Analysis of the honey

The honey samples from the honeybee colonies that were treated with formic acid were analysed spectrophotometrically. The analyses were carried out with a Boehringer Mannheim testkit. The honey samples from the control honeybee colonies were analysed in the same way.

The honey samples from the honeybee colonies that were treated with Thymovar were analysed by de Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek, Zeist (TNO). This analysis was performed with GS-MS. The honey samples from the control honeybee colonies were analysed in the same way.

Statistical analysis

Formic acid residues and thymol residues were compared between the treatment and the control using ANOVA ($P < 0.05$).

RESULTS AND DISCUSSION

Formic acid

There was a significant difference between formic acid residue levels in the honey of the colonies that were treated with formic acid (average 143.6 mg/kg) and the formic acid residue levels in the honey from colonies that were used as controls (average 48.6 mg/kg, table 1). Due to the formic acid treatment the formic acid residue levels in the honey had increased. As there is no MRL (Maximum Residue Level) for formic acid, the taste threshold was used as a maximum. The average formic acid residue level in the honey from honeybee colonies that were treated with formic acid just remained below the taste threshold which is between 150 mg/kg and 600 mg/kg (Bogdanov et al, 1999). The highest formic acid residue that was measured was 205.3 mg/kg, which exceeds the lower limit of the taste threshold. This means that in some cases the formic acid in the honey can be tasted.

Table 1: Average formic acid residue in the honey of the control honeybee colonies and the honey of honeybee colonies treated with formic acid. (Average residues followed by a different letter are significantly different)

Treatment	Average formic acid residue (mg/kg)	Standard Deviation	Min - Max	n honeybee colonies
control	48.6 [a]	17.9	28.7 – 91.6	10
formic acid	143.6 [b]	43.5	71.8 – 205.3	10

There was a significant variation between the honeybee colonies that were treated with formic acid, in the amount of evaporated formic acid (minimum 145 ml, maximum 280 ml) in some cases the amount that evaporated exceeded the recommended amount. The formic acid residue found in the honey also showed a significant variation (minimum 71.8 mg/kg, maximum 205.3 mg/kg). A high evaporation resulted in both low formic acid residue levels as high formic acid residue levels. The same goes for a low evaporation with both high formic acid residue levels and low formic acid residue levels.

Variation in formic acid residue levels between the honeybee colonies that were used as controls was significant. This variation in natural formic acid residue was also determined by Bogdanov et al (2002). The variance in formic acid residue of the honey from honeybee colonies that were treated with formic acid had a larger range (71.8 mg/kg – 205.3 mg/kg) than the variance in formic acid residue of the honey from the honeybee colonies that were used as a control. This indicates that the variation in formic acid residue levels in the honey of honeybee colonies that were treated with formic acid was caused by the treatment with formic acid and not only by the natural variation in formic acid levels in honey.

Thymovar

There was a significant difference between the thymol residue levels in the honey of the honeybee colonies that were treated with Thymovar (average 0.384 mg/kg) and the thymol levels in the honey from honeybee colonies that were used as controls (average 0.036 mg/kg, table 2). Due to the Thymovar treatment the thymol residue levels in the honey had increased. As there is no MRL (Maximum Residue Level) for thymol, the taste threshold was used as a maximum. The average thymol residue level of the honey from honeybee colonies that were treated with Thymovar was below the taste threshold, which is between 1.1 mg/kg and 1.3 mg/kg (Bogdanov et al, 1999).

Table 2: Average thymol residue in the honey of the control honeybee colonies and the honey of honeybee colonies treated with Thymovar. (Average residue followed by a different letter are significantly different)

Treatment	Average thymol residue (mg/kg)	Standard Deviation	Min - Max	n honeybee colonies
control	0.036 [a]	0.011	0.020 – 0.060	10
Thymovar	0.384 [b]	0.120	0.270 – 0.600	10

The variation in thymol residue levels between the honeybee colonies treated with Thymovar was significant (minimum 0.27 mg/kg, maximum 0.60 mg/kg). The variation in thymol levels between the honeybee colonies used as controls was minimal (minimum 0.02 mg/kg, maximum 0.06 mg/kg).

Variation in formic acid and thymol residue

The variation in residue levels was probably influenced by colony size en the size of the brood nest. These two factors affect relative humidity, temperature and air circulation within the colony, which have an effect on the evaporation of formic acid and of thymol from the Thymovar plates.

CONCLUSION

Both the formic acid treatment and the Thymovar treatment increased the levels of these substances in the honey in case a honey super was placed immediately after treatment.

The average formic acid residue level is below the taste threshold. The maximum measured formic acid residue level however exceeds this taste threshold.

When formic acid treatment is used in spring, it should be taken in to account that formic acid residue could effect the taste of the honey.

The average thymol residue level in honey and the maximum measured thymol residue level in honey is below the taste threshold.

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