Control of varroa mites with formic acid and thymol in Africanized honey bee colonies

Dr. Rafael A. Calderón F., PhD.

Bee Pathology Program
Tropical Beekeeping Research Center (CINAT)
Universidad Nacional (UNA), Costa Rica

Co-authors: Marianyela Ramírez – Fernando Ramírez
I. Introduction

- *Varroa destructor* is an ectoparasite of serious economic importance for beekeeping worldwide.

- Severe colony mortality is reported in European Honey Bee (EHB) colonies in Europe, Asia and North America.

- At present, varroa is considered as a factor contributing to recent decline of honeybee colonies North America - Europe.
Varroa mites: dorsal and ventral view
- In order to reproduce varroa female mites enter worker or drone brood shortly before the cell is sealed.

- The reproduction of varroa mites is restricted to sealed brood.

- Reproduction is a significant factor determining its virulence and has a major impact persistence of both host and parasite.
- Because varroa populations increase when brood is present.

- It would be expected that in tropical climates, where brood rearing takes place year-round.

- The effect of varroa would be even more devastating than in temperate climates.
- However, reports for Africanized Honey Bees (AHB) in Brazil indicate tolerant / resistant to varroa.

- Colony losses are not reported for AHB in Brazil.

- The mechanisms for this apparent resistance or tolerance are not well understood.
Varroa situation in Costa Rica / Central America

- *V. destructor* was detected in Costa Rica = September 26 1997.

- It was confirmed as a Korean haplotype (pathogenic).

- As a consequence of varroa incidence in the apiaries.

- Beekeepers reported loss colonies and decrease honey production.
Treatment of the AHB colonies in Costa Rica

- Because beekeepers are afraid of reduced honey yields due to high infestation rates of varroa.
- A high percentage of beekeepers treat their colonies annually.
- Synthetic acaricides (fluvalinate-flumethrin) have been used for mite control.
- There are few reports of alternative products for varroa control.
High varroa infestation levels in AHB - Costa Rica

- Rates of 190 fallen mites per day.
- More than 8,000 mites per colony.
- Occurrence of Deformed wing virus and Kashmir virus in mite infested colonies (varroa is a vector of different virus).
- Revealed varroa is a serious problem on AHB in Costa Rica.
Main objective: evaluate formic acid and thymol for the treatment of *Varroa destructor* in Africanized bees.
II. Materials and Methods

Study site:

- The study was carried out Atenas, Alajuela (Central Valley) - Costa Rica.

- It was conducted from June to October 2012.

- November - April = dry months.

- May - October = rainy months.
Atenas-Alajuela is located in the Central Valley of Costa Rica.
- Experimental apiary was formed for 16 AHB colonies.
Bee species

- **AHB**: Africanized honey bees.
- Highly defensive bees = called “Killer bees”.
- The degree of Africanization of the colonies was analyzed using electrophoresis = enzyme hexokinase (Hk) on polyacrilamide gels.
Experimental design: Tested products

- Mite Away Quick Strips (MAQS®)
- Apiguard®
Mite Away Quick Strips (MAQS)

- Gel formulation of formic acid 65%.
- One strip of 150 g was applied per colony over the combs.
- We divided the strip.
- One application was made during 2 weeks.
Apiguard®

- Gel formulation of Thymol 25%
- We made 2 applications of thymol = total 50 g per colony.
- It was applied over the combs.
Control colonies

The natural mite mortality was evaluated in these colonies
After treatments

- Four strips of flumethrin were applied in all colonies
- During four weeks
- Eliminate remanent mites = shock treatment
Varroa Traps

- Varroa traps were put in the bottom board of each colony to collect mites

- The traps were replaced every week
Effectiveness

To calculate the percentage of effectiveness we applied this formula:

Effectiveness = \frac{\text{Fallen mites with formic acid or thymol}}{\text{Total mites of the colony}}

Total mites = mites with the product + mites with flumethrin
Mite mortality in capped brood cells

- A capped brood sample was analyzed 24 and 72 h after treatments.
- We evaluated the mother mite and offspring mortality.
We evaluated the mortality of varroa stages:

- Adult mite
- Male
- Protonymph
- Deutonymph
- Daughter
III. Results.
Table 1. Mite mortality with formic acid (average ± ES).

<table>
<thead>
<tr>
<th>Sampling period (d)</th>
<th>1</th>
<th>3</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>30</th>
<th>Total</th>
<th>Flum</th>
<th>Efect %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallen mites</td>
<td>621 ±83</td>
<td>181 ±65</td>
<td>128 ±41</td>
<td>127 ±52</td>
<td>5 ±1</td>
<td>4 ±1</td>
<td>1065 ±118</td>
<td>59 ±22</td>
<td>94.7</td>
</tr>
</tbody>
</table>

- A similar effectiveness was found in Argentina= 92% and 94% (Eguaras 2003, Eguaras et al. 2003).
Fallen mites with formic acid= 3 colonies

- Most of the mites were in the phoretic period.

- In colonies with few brood the mites will be in adult bees (Bailey and Ball 1991).

Figure 1. Colonies with a high mortality during the first 3 days.
Two peaks of fallen mites with formic acid = 4 colonies

- Phoretic mites and mites in capped brood cells.
- In colonies with brood most of the mites are in the capped cells for reproduction (Amrine et al. 2007).

Figure 2. Colonies with fallen mites at 24 h and day 15.
Effectiveness of thymol (Apiguard®)

Table 2. Varroa mortality with thymol (average ± ES).

<table>
<thead>
<tr>
<th>Sampling period (days)</th>
<th>1</th>
<th>3</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>30</th>
<th>Total</th>
<th>Flum</th>
<th>% Efect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallen mites</td>
<td>53 ± 15</td>
<td>191 ± 60</td>
<td>351 ± 88</td>
<td>143 ± 68</td>
<td>158 ± 45</td>
<td>11 ± 4</td>
<td>905 ± 210</td>
<td>29 ± 9</td>
<td>96,9</td>
</tr>
</tbody>
</table>

- Similar results were reported by Loucif-ayad *et al.* 2010 = Effectiveness of 95% with 12.5 g of Apiguard®.
Fallen varroa mites with the treatment of thymol

- Most of the mites fallen at day 8.
- Loucif-ayad *et al.* 2010 indicated that most of the mortality with thymol occurred at first week.
- We observed a second peak during the third week. It occurred after the second application.

Figure 3. Mite mortality distribution.
Varroa mortality in the control colonies

Table 3. Natural mortality of varroa (average ± ES).

<table>
<thead>
<tr>
<th>Sampling period (days)</th>
<th>1</th>
<th>3</th>
<th>8</th>
<th>15</th>
<th>22</th>
<th>30</th>
<th>Total</th>
<th>Flum</th>
<th>Natural mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallen mites</td>
<td>30 ± 13</td>
<td>55 ± 45</td>
<td>79 ± 46</td>
<td>67 ± 57</td>
<td>335 ± 285</td>
<td>149 ± 131</td>
<td>715 ± 531</td>
<td>2072 ± 1874</td>
<td>25.7</td>
</tr>
</tbody>
</table>

- It was similar to africanized bees in México = 22% (Espinosa and Guzmán 2007).
- Calderón 2011= reported more than 30% of fallen mites in africanized bees in Costa Rica.
**Comparison of the fallen mites**

**Figure 4. Varroa mortality at days one and eight.**

Day 1
Significant differences (p<0.0001) were found in the treatment with formic acid.

Day 8
Significant differences (p<0.0255) for the thymol.
Differences in the mortality period of the mites

Formic acid
- Eliminated most of the mites at the beginning.
- One application.

Thymol
- Most of the mites fallen one week after.
- Two applications

Figure 5. Varroa mortality with thymol and formic acid.
### Table 4. Percentage of mite mortality in capped brood

<table>
<thead>
<tr>
<th>Stages</th>
<th>Formic acid</th>
<th>Thymol</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother mites</td>
<td>95</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Protonymph</td>
<td>88</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Deutonymph</td>
<td>41</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Females</td>
<td>78</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>Males</td>
<td>84</td>
<td>11</td>
<td>16</td>
</tr>
</tbody>
</table>
Only formic acid killed the mites inside the cells.

Van Alten *et al.* 2009= found 66% of mite mortality with 150 g of MAQS®.

In CR was reported 56% of varroa mortality with FA liquid (Calderón *et al.* 2000).

There is no reports of mite mortality with thymol. In this study we found less than 10% of varroa mortality. It was similar to control colonies.
Negative effects of formic acid

- We found some brood mortality in the cells.

- In addition, mortality of emerging bees.

- Mitchell and Vanderdussen 2010 found larvae mortality in the first 3 days with MAQS®.

- In this study after 4 weeks colonies have a normal development.
IV. Conclusions

- Both formic acid and thymol showed a high effectiveness in the control of varroa mites in africanized bees in the tropical conditions of Costa Rica.

- Formic acid has a rapid effect in the control of varroa and require one application, while thymol needs two applications and a longer period.

- Both products could be considered as a good alternative in an integrated pest management program for varroa in africanized bees.

- In addition, a high mortality of the different stages of varroa were found in capped brood cells in colonies treated with formic acid.
VI. Acknowledgements

- The financial support to take part in APIMONDIA 2013 was provided by:
  – Scholarship Department, Universidad Nacional of Costa Rica.
  – Tropical Beekeeping Research Center (CINAT).
-Thank you very much for your attention…