Acknowledgements

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References


LÜCKMANN, J., R. BECKER, 2016: Honeybee brood studies under field conditions: Is there a difference of the brood termination rate compared to semi-field studies? - Poster on the 26th Annual Meeting of SETAC Europe, 22-26 May 2015, Nantes, France.


2.2.P Toxicity of oxalic acid on in vitro reared honeybee larvae

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Abstract

Varroa destructor is considered as a serious pest of honeybees (Apis mellifera) and its resistance to acaricides has been reported since the early 1990s. Because large colony loses are yearly reported from over the world, new methods of treatment for Varroa mites are still in focus of many scientists. In our bioassay, we determined the lethal concentration 72 h LC50 of 2.425% oxalic acid solution following single spray exposure of honeybee larvae under laboratory conditions (Guideline OECD 237, 2013).

Keywords: honeybee larvae, oxalic acid, spray exposure, OECD 237

Introduction

Oxalic acid (OA) is a naturally occurring carboxylic acid used worldwide in apiculture to control Varroa destructor. It’s mode of action of OA is unknown, but the direct contact between them is required (Aliano et al. 2006). Some authors attributed its acaricidal action partly to a sensitivity of this species to acid pH (Maggi et al. 2016; Nanetti 2017). The instructions for administration of the authorised veterinary medicinal products with OA as an active ingredient recommend spraying,
trickling and evaporation as three main application methods (EMEA 2003). Results from several studies showed the efficacy greater than 90% in honeybee colonies when broodless or almost broodless colonies have been treated with the tricking method (Gregorc and Planinc 2001; Charrière and Imdorf 2002; Nanetti et al. 2003). Observed efficacy in broodright colonies was only around 60% (Hatjina and Haristos 2005; Gregorc and Planinc 2004). When the oxalic acid treatment occurs in broodright colonies, honeybee larvae may be exposed to OA via diet and, potentially, per cuticula (by evaporation and/or spraying). Rapid and consistent distribution of oxalic acid dihydrate within a colony was shown by macro-computed tomography (Rademacher et al. 2017). Two in vivo studies showed adverse effect of oxalic acid on bee brood following direct spray application (Higes et al. 1999; Gregorc et al. 2004), but so far, toxicological data on individual in vitro reared bee larvae have not been available.

Materials and Methods
The honeybee larvae were reared in vitro using the methodology described by Aupinel et al. (2007) and Guideline OECD 237 (2013). Authorised veterinary medicinal products containing oxalic acid dihydrate with the recommended dosage of 0.3 ml of 3% (w/v)/dm2 comb have been licenced in many countries worldwide over recent years (EMA 2017, 2018). In our bioassay, we tested nominal concentrations of 0% (control), 0.87%, 1.75%, 3.5% and 7.0% of oxalic acid (VWR BDH Prolabo* Chemicals) in spraying form (recommended dosage of 0.3 ml of 3% (w/v)/dm2 comb is covered). Respective tested doses are 0 (control) μg OA/larva, 16.1 μg OA/larva, 32.3 μg OA/larva, 64.6 μg OA/larva and 129.2 μg OA/larva. Twelve larvae from each of three colonies (12 larvae × 3 per tested group; n = 3), allocated on 48-well culture plate, were homogeneously sprayed with a manual sprayer (Lenz; NS 19/26) before feeding on day 4 with respective solution prewarmed on 37.5 °C from a 25 cm distance from the plate at right angle (90°). Control was sprayed with distilled water. Larval mortalities were checked and recorded at the time of feeding on days 5 and 6 and at the termination of the test on day 7 and are expressed in the number of dead larvae and in a corrected percentage according to Guideline OECD 237 (2013).

Results
The effects of spray exposure of oxalic acid on honeybee larvae reared in vitro were assessed according to Guideline OECD 237 (2013). The results (Tab. 1) showed the highest observed corrected mortality of 97% on day 6 in the tested nominal concentration of 7.0% and the lowest corrected mortality of 3.1% at the lowest tested concentration of 0.87% on day 7. Only the mortality observed in the lowest tested concentration of 0.87% showed no statistical significance compared to control. The established 72-h lethal concentration which kills 50% of tested individuals (LC50) following the single spray exposure of oxalic acid for A. mellifera larvae was 2.425% with a 95% confidence interval of 2.073–2.835 (χ² = 0.03753; df = 2; slope = 4.19590; intercept = − 1.61392; P(F) < 0.001). The no-observed-effect concentration (NOEC) was estimated to be 0.87%.
**Tab. 1** Mortality of honeybee larvae (*Apis mellifera carnica*) after single spray exposure of oxalic acid (dose-response test)

<table>
<thead>
<tr>
<th>Test concentration (nominal) [%]</th>
<th>Rearing day</th>
<th>n</th>
<th>P (exact)</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Oxalic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Mortality (larvae)</td>
<td>36</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>P (exact) Significance*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.87</td>
<td>Mortality (larvae)</td>
<td>36</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>Corrected mortality [%]**</td>
<td>36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.75</td>
<td>Mortality (larvae)</td>
<td>36</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>-</td>
<td>Corrected mortality [%]**</td>
<td>36</td>
<td>2.9</td>
<td>21.2</td>
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<tr>
<td>3.50</td>
<td>Mortality (larvae)</td>
<td>36</td>
<td>5</td>
<td>22</td>
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<tr>
<td>-</td>
<td>Corrected mortality [%]**</td>
<td>36</td>
<td>8.8</td>
<td>72.7</td>
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<tr>
<td>7.00</td>
<td>Mortality (larvae)</td>
<td>36</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>-</td>
<td>Corrected mortality [%]**</td>
<td>36</td>
<td>73.5</td>
<td>97.0</td>
</tr>
</tbody>
</table>

* Fisher’s Exact Binomial Test with mortality at 7 d: Two-sample comparisons between sample and control (Alpha is 0.050; one-sided greater); Ho (no effect) is accepted, if the probability p(exact) > Alpha; p(exact) is the probability that the increase in category "Dead" observed in the treatment(s) is due to chance.
+ significant; - non-significant
- - - not relevant
** treatment response compensated using Abbott’s formula
n number of tested larvae

**Conclusion**

Oxalic acid is the active ingredient of several authorised veterinary medicinal products and is becoming more prevalent as a Varroa control method in apiculture around the world. According to the instructions for administration, they can be used on colonies with and without brood. In our study performed according to Guideline OECD 237 with a spray way of exposure on Day 4, we demonstrated a dose-response adverse effect of oxalic acid on honeybee larvae under the laboratory conditions. Despite the recommended spray application with 2.1% OA solution is slightly lower than LC50 observed in our study, it may be harmful to bee brood when present during application.

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**References**


Abstracts: Poster


