Susceptibility of *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) to *Beauveria bassiana* (Bals.) Vuill.

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**Abstract**

Entomopathogenic fungi are frequently used as an alternative method for insect pest control. However, only a few studies have focused on the effect of these fungi on bees and on the selectivity of fungi to beneficial organisms in agroecosystems. The objective of the present study was to assess the susceptibility of worker bees of the species *Melipona scutellaris* (locally known as "uruçu") to the isolate (Biofungi 1) of the entomopathogenic fungus *Beauveria bassiana*. The experiment was carried through indirect contact between the fungal suspension and newly-emerged bees and topical application of the fungal suspension on the back of newly-emerged bees. The sampling design was completely randomized and comprised five treatments, which included four different concentrations of the fungus: $1 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$, $1 \times 10^8$ conidia/ml, and a control composed of distilled water. Each treatment had five replicates. The mortality data were subjected to an analysis of variance and a probit regression analysis, which provided an estimate of the lethal dose to 50% of the population (LD$_{50}$). The adjustment of the curves to the model was tested with a chi-squared test and differences between curves were tested with a test for parallelism. *Beauveria bassiana* was virulent to uruçu bees, killing the bees at the lowest dose used. These findings may help minimize the impact of this entomopathogen and, therefore, contribute to the maintenance of natural populations of these insects.
In the exposure by indirect contact, the bees were placed on the dorsum of each bee with a sterile 10μl micro syringe (BD Plastipak). Application, 1μl of each treatment was applied to the dorsum and indirect contact. In the topical application, filter paper sheets slightly moistened with 1 ml of each treatment for 5 min (Rother et al. 2009). After each treatment, the worker bees were placed in plastic containers (6.0 cm x 8.0 cm) and transferred to an acclimatized chamber.

Five worker bees were placed in each plastic container, totaling 125 individuals. The bees were fed with honey at 10% (100g/1000ml distilled water), placed on a sterilized wad of cotton to prevent contamination (Rother et al. 2009).

Data analysis

The experimental design was completely randomized. It was composed of five treatments and five replicates, totaling 25 plots. The mortality of worker bees was monitored at 24h intervals for 10 days. The results were corrected considering natural mortality in accordance with the Abbott formula (Alves 1998).

The corrected mortality data were subjected to an analysis of variance. For this analysis, the data were transformed using the formula X’ = arcsin (X/N), where X’ is the datum after the transformation, X is the mortality observed in the replicate i and N is the total number of insects in the experimental plot. Data normality was assessed with a Kolmogorov-Smirnov test and variance homogeneity was assessed with a Levene test.

Mortality data from different treatments were subjected to a probit regression analysis (Sokal 1958) in Statistica (Stat-Sof Inc.). This analysis provided an estimate of the lethal dose to 50% of the population (LD50). The adjustment of the curves to the model was assessed with a chi-squared test and differences between curves for the exposure methods were assessed with a test of parallelism (Alves 1998).

The mortality data at highest dose were used to build survival curves for the two exposure methods following the Kaplan-Meier method (Blanford et al. 2005). Based on these curves the time to mortality (or survival) of 50% of the insects (S50) was estimated. The curves were compared using a logrank test (P = 0.95) and a Gehan-Breslow-Wilcoxon test (GBW) in GraphPad Prism 5.0 (Motulsky 1995).

Results and Discussion

Under the experimental conditions, there was no significant effect (p > 0.05) of the exposure methods on the mortality of uruçú bees (M. scutellaris). However, there was a significant interaction between exposure methods and the doses applied (DF = 4.396; F = 17.68; P < 0.001). The corrected mortality caused by different doses of the fungal suspension was higher when applied on the dorsum of the bees than when bees got in indirect contact with the fungal suspension (Figure 1). Even at the lowest dose (10^2 conidia ml^-1), the worker bees were affected: they lost mobility and had an average mortality of 56%.

These results differ from those of Butt et al. (1994), who tested the pathogenicity of Metarhizium anisopliae to adult bees of A. mellifera and observed significant mortal-
A dose-response model ($\chi^2 = 2.897; \text{DF} = 2; P = 0.235$). The isolate of *B. bassiana* used in the experiments was pathogenic to uruçú bees and caused high mortality even at low doses. Based on the results obtained, a LD$_{50}$ of $2.04 \times 10^5$ conidia ml$^{-1}$ was estimated ($7.95.10^3; 3.70.10^5$) (Figure 2).

The data obtained from the exposure method of indirect contact with the fungal suspension at different doses showed high variability among doses, and did not fit the probit regression model ($\chi^2 = 26.811; \text{DF} = 2; P < 0.001$). With this result, it was not possible to estimate the LD$_{50}$ or make a comparison between exposure methods through the assessment of the parallelism of curves. For the comparison between different exposure methods, the survival curve at the two highest doses was compared with Mantel-Cox and GBW tests.

Data analysis through the construction of survival curves using the Kaplan-Meier method made it possible to assess mortality details over time, and allowed the identification of differences in the survival curve between exposure methods. We observed that the indirect contact method resulted in a lower mortality rate at the lowest doses in the beginning of the observation period, but in the end of the observation period the total mortality was also high.

Figure 3 shows that the topical application of the fungal suspension of *B. bassiana* ($10^8$ ml$^{-1}$) resulted in high mortality in the end of the experiment, with a survival of only 20.4% ($\pm 5.1$) of the bees; whereas the exposure method through indirect contact resulted in higher survival (69.1% $\pm 4.2$). The survival curves obtained for different methods of exposure to the fungus were significantly different from the control and from one another when compared by Mantel-Cox (Log Rank Test) and GBW tests (Table 1).

The mean survival value ($S_{50}$) estimated for the topical application method was 216.0 days (Table 1). For the con-
trol treatment or application by indirect contact, the amount of $S_{50}$ could not be estimated. For the control treatment or exposure method by indirect contact, $S_{50}$ could not be estimated because bee mortality did not exceed 50%, and the use of the Kaplan-Meier model to calculate $S_{50}$ is limited by the increased survival time of the individuals studied. The estimated value of hazard ratio (HR) between the two exposure methods was 0.35, between the topical application and the control it was 8.48, and between the indirect contact and the control it was 5.96 (Table 1). The hazard ratio estimates the difference in mortality between treatments based on the slope of the respective survival curves. In this particular case, the average mortality estimated for the topical application exposure method was consistently 35% higher throughout the experiment in comparison with the indirect contact exposure method.

**Table 1 – Comparison of survival curves estimated for uruçu bees (M. scutellaris) exposed to topical application or indirect contact with the fungal suspension of B. bassiana conidia (10⁶ ml⁻¹).**

<table>
<thead>
<tr>
<th>Method Combination</th>
<th>Median Survival ($S_{50}$)</th>
<th>Hazard Ratio (HR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical application x Control</td>
<td>216</td>
<td>-</td>
</tr>
<tr>
<td>Indirect contact x Control</td>
<td>192</td>
<td>-</td>
</tr>
<tr>
<td>Topical application x Indirect contact</td>
<td>26.17**</td>
<td>5.64</td>
</tr>
<tr>
<td>Topical application x Control</td>
<td>5.86 (2.76 to 12.45)</td>
<td>0.35 (0.22 to 0.61)</td>
</tr>
</tbody>
</table>

1 Significant at $P < 0.001$; 2 Confidence interval estimated (CI)

Delaplane & Mayer (2005) reported that methods used to apply the compounds may interfere with the results of toxicity assessment of pesticides on non-target insects in the laboratory; there may be an interaction between the active ingredients and exposure methods. Carvalho et al. (2009) tested four methods to assess the toxicity of pesticides to A. mellifera and found different responses according to the active ingredient used. Thiamethoxam and methidathion were highly toxic, with low median lethal time ($LT_{50}$) for topical application, supply of contaminated food, and indirect contact of bees to previously sprayed surfaces. Abamectin showed lowest $LT_{50}$ when provided in contaminated food, whereas deltamethrin showed highest toxicity when the insects were exposed to previously sprayed surfaces (Carvalho et al. 2009).

Pest control with entomopathogenic agents has the advantage of leaving no toxic residues, and therefore can be used for long periods with low environmental impact (Alves 1998). However, the susceptibility of stingless bees to other commercial isolates of entomopathogenic fungi should be tested in future studies, with special attention to the concentration. Only by knowing the effects of entomopathogenic agents on bees, it will be possible to achieve greater efficiency in pest control with minimal impact on these beneficial insects.

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**References**


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