Non-uniform distribution of treated sucrose solution via trophallaxis by honeybees, affects variability of homing success, gene expression and mortality

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Introduction

Based on our own observations and the recently published article[1] food sharing via trophallaxis (exchange of liquids between colony members) might lead to a non – uniform distribution of the tested sucrose solution between caged bees. This inhomogeneous food distribution could affect the outcome of ecotoxicological experiments, creating high variability among individuals within a replicate. Here we test this hypothesis, using small units either with ten or two bees on various well established parameters, such as homing success rate, gene expression and mortality of adult honeybees.

Method

Homing success: According to the RFID homing flight ring-test protocol, bees were orally exposed to different sub-lethal concentrations of thiamethoxam (0, 0.11, 0.33 or 1 ng/bee). For each treatment we tested various feeding schemes (two and ten bees per cage) and performed three replicates each. In all groups, homing flight success was assessed after 24h.

Gene expression: For analyses of *vittellogenin* gene expression patterns, returned bees of the homing test (including both feeding schemes) were collected and frozen for subsequent molecular analysis. Two brains were pooled to one RNA sample. RNA isolation, cDNA synthesis and qPCR was done as described before[2]. In total 8 samples for each treatment and feeding scheme were analyzed.

Mortality (LD_{50}): According to the TG OECD 213, bees were orally exposed to different concentrations of dimethoate (0, 0.033, 0.07, 0.1, 0.13, and 0.35 µg/bee). Otherwise, the same set up and number of replicates as for the homing success test was performed (see above). Mortality was always assessed after 24h.

Results

Homing success: Homing flight success rate, at 1 ng thiamethoxam per bee, was significantly lower in the group of ten bees compared to the two bees approach, as well as to the control. However, a very large variability of homing success rate was detected in the ten bees feeding group, but not in the two bees approach. For the other doses, similar trends were obtained (Fig. 1).

Gene expression analysis: *vitellogenin* is an important transcript regulating foraging activity (less expression in foragers compared to nurse bees) and *vitellogenin* expression is induced upon thiamethoxam exposure[2]. Strikingly, Run D (two bees feeding) shows less variation in the higher dose than 10 bees feeding (Run A, B and C) (Fig. 2).

Mortality (LD_{50}): Acute toxicity data with dimethoate showed that group feeding scheme with ten bees per cage resulted in higher mortality values when compared to the two bees feeding scheme (at same dosing levels). As a consequence the LD_{50} value is higher for the latter (Fig. 3).

Conclusion

Generally, our results revealed that for the various parameter's assessed (homing success, gene expression and mortality rate) a higher variability was observed in the ten bee feeding scheme, compared to the two bee feeding scheme. This high variability might be likely caused by inhomogeneous dose/food distribution, for example by over- or under dosing individual bees within a replicate. Interestingly, food intake within the two bees feeding scheme was generally faster and more homogenous (visual observations), since the chance to feed directly on the sugar solution increased manifold. Hence, a more accurate and uniform dosing distribution can be expected, under this feeding regime thus minimizing potential (false) variability among gathered data (between runs, replicates and treatments).

Therefore, we suggest that feeding (treatment of interest) in smaller groups or even single honeybees should be considered for future experiments via oral application, aiming to minimize the food-transfer based variability deriving from trophallaxis and to determine the exact dose consumption per bee.

The here suggested new feeding scheme would also be necessary to reliably compare endpoints of toxicological studies with single dosed wild bees i.e. for regulatory purposes.

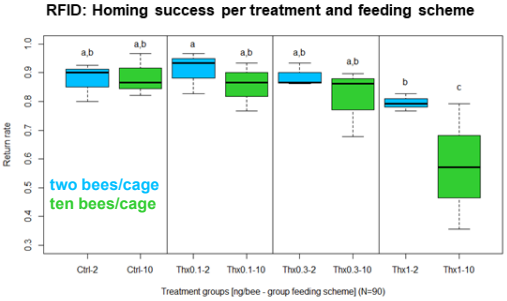

Fig. 1: Boxplot: Homing flight success per treatment and feeding scheme. Literals differentiate statistically significant ($p<0.05$) groups, validated by Chi-Square-Tests.

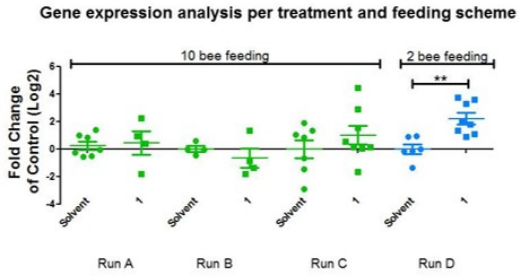

Fig. 2: Abundance of vitellogenin transcript in the brain of honey bees following exposure to 1 ng/bee thiamethoxam (Run A-C: ten bees feeding scheme, Run D: 2 bees feeding scheme. Significant differences with p-value of ≤ 0.05 are marked with asterisks, validated by ANOVA followed by a Bonferroni`s multiple comparison test.

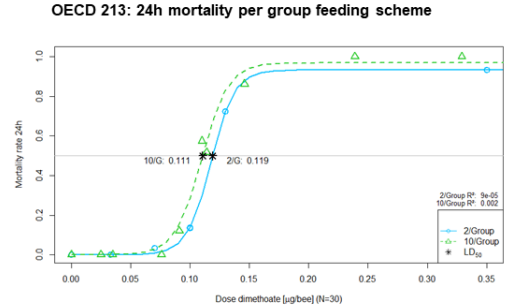

Fig. 3: LD_{50} dose-response model for dimethoate with two, resp. 10 group feeding schemes. 2 group feeding showed a more accurate and closer LD_{50} value compared to the reported LD_{50} value of 0.1257µg/bee by Baskar et al.[3]

REFERENCE [1] Brodschneider R, Libor A, Kupelwieser V, Crailsheim K, PloS ONE 12(3) (2017)
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[3] Baskar K, Sudha V, Tamilselvan C ScholeReps 1(1) (2016)

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