2.2 Progress on the Osmia acute oral test - findings of the ICPPR Non-Apis subgroup solitary bee laboratory testing

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DOI 10.5073/jka.2020.465.017

Abstract

The publication of the proposed EFSA risk assessment guidance document of plant protection products for pollinators highlighted that there are no study designs for non-*Apis* pollinators available. As a result the risk assessment of non-apis pollinators uses *Apis* pollinator data with so-called assessment factors to compensate for the lack of knowledge on other species. To fill part of this knowlegde gap an acute oral test for solitary bees was developed within the ICPPR non-apis group.

Ringtests have been conducted in 2018 to validate and improve the suggested protocol. And in 2019 a standardized protocol has been tested by all participants once more. The tests have been performed with *Osmia bicornis*, *Osmia cornuta*, *Osmia lignaria* and *Osmia cornifrons*. A summary of the ringtest results of both years will be given and further recommendations will be presented.

2.3 Stingless bee ring test: acute contact toxicity test

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DOI 10.5073/jka.2020.465.018

Abstract

There is much discussion about the representativeness of *Apis mellifera* specie in relation to stingless bees and how protective the schemes are. Thus, since 2016 Brazil has been investing in the development of a method that can be applied to different species of stingless bees. Since 2017 Brazil has a new pesticide registration procedure, which includes the risk assessment process for bees. However, all required studies are still performed with the species *Apis mellifera*, since there are no standardized protocols with native Brazilian species. In order to meet the growing demand for analysis and to ensure the availability of protocols that can answer the questions regarding the representativeness of *A. mellifera* in relation to the biodiversity of Brazilian bees, we have developed a stingless bees protocol for possible standardization and use in the risk assessment process. The protocol was developed from adaptations to OECD 214 protocol for *A. mellifera* and initially tested with the species *Scaptotrigona postica*. During its development, the best collection method, the most suitable experimental cage and anesthesia times were established. The proposed protocol was tested using the active ingredient dimethoate between October 2018 and March 2019. The contact LD_{50} were: 24h - 4.34 to 6.66 ng / µL; 48h - 3.08 to 5.39 ng / µL; 72h - 2.31 to 4.27 ng / µL; and 96h - 1.92 to 4.12 ng / µL. The method proved feasible and the protocol was presented during a workshop held in Rio Claro in January 2019 where a proposal for standardization throughout the national territory was presented. For the ring test the project has 13 laboratories: 7 universities, 3 research institutes and 3 private laboratories. Currently, the laboratories have been

equipped and all involved are being trained to begin the first round of testing from September 2019. The Brazilian experience will be presented during the 13th SETAC Latin America for the exchange of experiences and discussion of more species-oriented methods from the tropical and subtropical regions of the Americas, with the aim of creating a network aimed at protecting local species.

2.4 Standardization of an *in vitro* rearing method for the stingless bee species *Scaptotrigona postica* larvae and its application for determining the toxicity of dimethoate on the larval phase

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DOI 10.5073/jka.2020.465.019

Abstract

Currently, Brazil has a full framework for pesticide risk assessment established for *Apis mellifera*, based on North America's approach. However, the use of an exotic species as model-organism as a substitute for native species of Brazil (stingless bees) has been questioned. An *in vitro* larval rearing method has already been described for the Brazilian native *Melipona scutellaris* but, *Scaptotrigona postica* species has shown potential to be suitable for testing, mainly because its high numer of individuals per hive comparing to the other stingless bee species and for do not belongs to the list of endangedered species, like *M. scutellaris*. Thus, we aimed to establish an *in vitro* larval rearing method for *S. postica* and to apply it for determining the toxicity of dimethoate on larval phase. Larvae of 24 hours old were transferred to acrylic plates and five different procedures were carried out, considering the humidity control and the required fungus *Zygosaccharomyces* sp. as essential for the success of larval survivorship. Each replicate consisted of 100 larvae, totalng 4,800 larvae. Mortality and emergence parameters of the individuals, as well as the progress of the larval development were assessed, in order to check the efficiency of these methods. The intertegular distance, head width and wings asymmetry were assessed from the individuals emerged from the most efficient method. The same parameters were checked on individuals emerged from *in vivo* brood combs. The chosen method consisted of the deposition of the pure larval food followed by adding KCl and NaCl solutions 72 and 120 hours after the larval transference, respectively. This procedure was applied to determine the lethal concentration 50% (LC_{50}) of dimethoathe, the standard active ingredient for toxicological tests, established by OECD. The active ingredient, obtained from Sigma-Aldrich (Pestanal), was directly diluted in the larval food, and successive subsequent dilutions were performed in the food, in order to reach the following concentrations to be offered to the larvae (in ng a.i./ larva): 250, 200, 150, 100, 50 and 25. Each bioassay was carried out 4 times (20 larvae/concentration in triplicate). The negative control consisted of the pure larval food. The dose-response data were assessed with binomial generalized linear models, using the Cauchit function, for determining the LC_{50} for 24 and 48 hours. The analysis was performed in the R software (R Core Team). The best procedure indicated emergence/larvae, emergence/pupae and mortality/larvae of 93.44, 97.6 and 2.85%. The mean of intertegular distance for the *in vitro* method was 136.5 mm and for *in vivo* of 127.7 mm. For the head width, *in vitro* showed 92.58 mm and *in vivo* was 89.88 mm. The t test indicated no significative difference between the *in vivo* and *in vitro methods* ($p > 0.05$). Regarding the wings asymmetry, the ANOVA Procrustes indicated a significative difference in the centroid size only in the "individual effect", on individuals emerged from both *in vitro* ($F = 11.33$; $p < 0.0001$) and *in vivo* ($F = 38.35$; $p < 0.0001$) treatments, and in the wing venation pattern in the "individual effect" *in vitro* ($F = 12.03$; $p < 0.0001$) and *in vivo* ($F = 12.13$; $p < 0.0001$), and in the "size effect" on individuals emerged from the *in vivo* treatment ($F = 0.50$; $p < 0.0005$). The tests with dimethoathe indicated a LC_{50} (in ng a.i. /larva) of 172.48 and 156.33 for 24 and 48 hours, respectively. The mais points for the success of the *in vitro* rearing were the humidity control, the non-use of eggs for transference, and to the use of acrylic plates manufactured which the size simulates the real dimensions of brood cells. The differences showed in some patterns of the wings asymmetry on individuals emerged from *in vitro* treatment are considered normal, since we can observe also on *in vivo* emerged individuals. These little variations in morphology are common in nature, especially because of environmental stresses. Thus, our results obtained *in vitro* may be used for representing *in vivo* conditions. According to the OECD, to be possible carry out a toxicological comparison by LC and/or LD values, is necessary that the experimental method has been