Honeybee (*Apis mellifera* L.) Larval Toxicity Test, Single Exposure

Newsletter-10 | October 2021

Pollinators influence the production of fruits, vegetables, seeds, nuts, and berries. Thus, without pollinators, the plant and animal kingdom cannot be alive longer. Nearly 90% of flowering plants need a pollinator. Among different pollinators, *Apis mellifera* L. contributes one-third of pollination from honeybees. It goes foraging at 3 - 8 km from the hive, sometimes even longer (Beekman and Ratnieks, 2000). Bees are an essential regulator for terrestrial ecosystem conservation. It influences ecological relationships, maintaining genetic variation in the plant community and floral diversity through pollination. Agrochemicals are used to control harmful pests and to prevent crop yield losses. Extensive use of agrochemicals causes adverse effects on the honeybee. Effects of agrochemicals are varied on bees, such as acute and/or chronic toxicity. As a result of adult bee exposure, while foraging in diverse environments, a honeybee hive can act as a reservoir for many of the hazardous substances that occur in its environment.

Figure 1 : Spraying of pesticide in the field

Figure 2 : Frame containing a queen and worker honeybee

The survival of adult bees exposed to pesticides is the primary consideration in environmental risk assessments undertaken to determine the potential of a pesticide affecting honeybees (Desneux et al., 2007 and Medrzycki et al., 2013). However, due to contaminated nectar and pollen collected by foragers honeybee, larvae are also exposed to pesticides. Honeybee brood health is a critical component in colony survival. The larval diet exposes larvae to the environment directly (Babendreier et al., 2004). Pollen or nectar containing pesticides may be harmful to the brood of a colony; therefore, laboratory methods for assessing adverse effects on larvae development are required. Due to environmental variation, in *in vivo* testing, the effects of pesticides on honeybee brood are not easily possible. Thus, rearing of bee larvae as *in vitro* method was developed (Aupinel et al., 2007; Crailsheim et al., 2012 and Schmehl et al., 2016). It is recommended for regulatory trials assessing pesticide toxicity to larvae (Aupinel et al., 2007).

JRF GLOBAL
Pioneering Solutions since 1977 - Responsibly

Recently, JRF has validated the honeybee (*Apis mellifera* L.) larval toxicity test, single exposure (OECD TG 237). In this study, the organophosphate insecticide, dimethoate (DMT) was used. Below is the schematic representation of the important steps of the larval toxicity test.

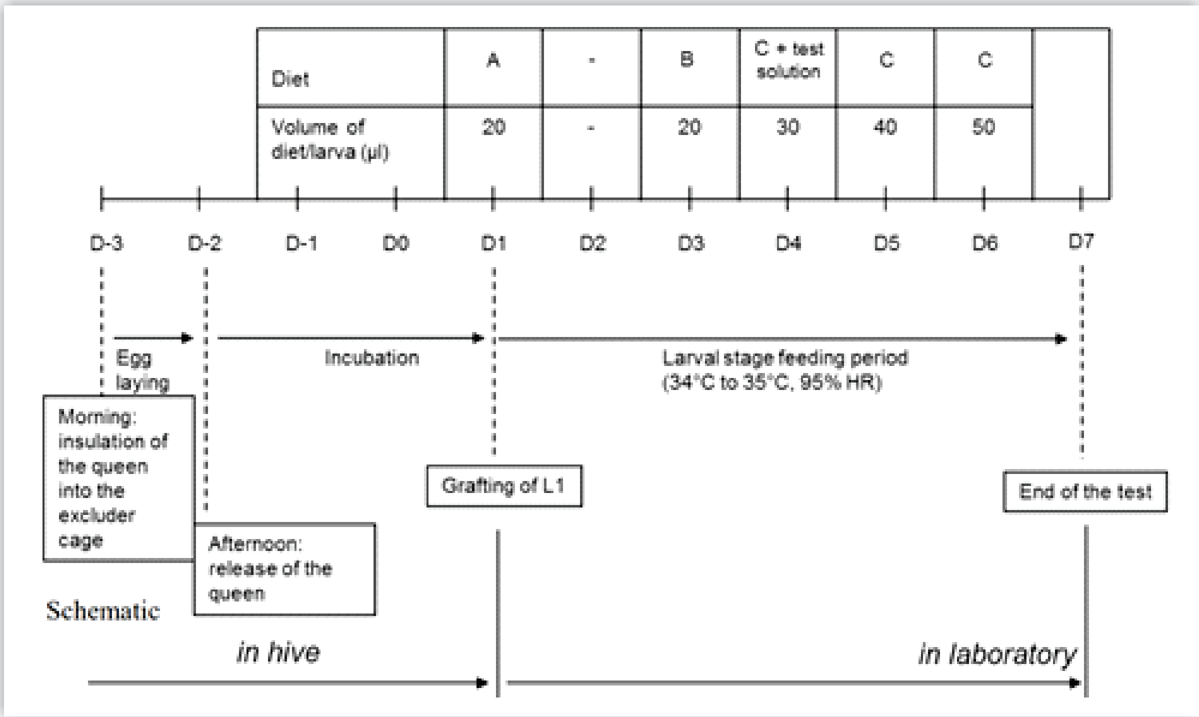

Figure 3 : Schematic representation of the important steps of the larval toxicity test (Source: OECD TG 237)

The honeybee larvae were obtained from the apiary, which is maintained at the Jai Research Foundation, Vapi, Gujarat. The experiment was conducted during the egg-laying period of the queen. Healthy honeybees in the same health condition were used. The larvae, which were not formed a "C" shape, were grafted. The larvae were selected from three healthy and adequately fed hives of the queen-right colony. Honeybee larvae were exposed to different concentrations of reference standard dimethoate along with appropriate control. After exposure, each group of the larva was observed for mortality, as well as for the behavioural symptoms at the interval of 24, 48, and 72 h.

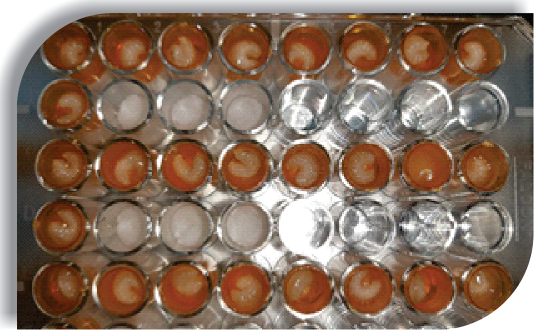

Figure 4 : 48-well plate containing honeybee larva

The median lethal dose (LD_{50}) of dimethoate at an interval of 48 h and 72 h by a single exposure to honeybee larva, *Apis mellifera* L., was 1.472 and 0.500 µg dimethoate/larva, respectively. The 48 h LD_{50} of dimethoate is in line with the published value (Aupinel et al., 2007). This result proves the efficiencies of the test system and the reliabilities of test conditions. Results of the present study indicate that dimethoate exposure caused a dose-related effects on larval mortality.

References

Aupinel P, Fortini D, Michaud B, Marolleau F, Tasei JN and Odoux JF, Toxicity of dimethoate and fenoxycarb to honeybee brood (*Apis mellifera*), using a new in vitro standardized feeding method. Pest Manag Sci 63: 1090–1094 (2007).

Babendreier D, Kalberer N, Romeis J, Fluri P and Bigler F, Pollen consumption in honeybee larvae: a step forward in the risk assessment of transgenic plants. Apidologie, 35, 293–300 (2004).

Beekman M and Ratnieks FLW, Long-range foraging by the honeybee, *Apis mellifera* L. Functional Ecology 14: 490–496 (2000).

Crailsheim K, Brodschneider R, Aupinel P, Behrens D, Genersch E, Vollmann J, et al., Standard methods for artificial rearing of *Apis mellifera* larvae. J Apic Res 52: 1–16 (2012).

Desneux N, Decourtye A and Delpuech JM, The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52: 81–106 (2007).

Gregorc A and Ellis JD, Cell death localization in situ in laboratory reared honeybee (*Apis mellifera* L.) larvae treated with pesticides. Pest Biochem Physiol 99: 200–207 (2011)

Gregorc A, Evans JD, Scharf M and Ellis JD, Gene expression in honeybee (*Apis mellifera*) larvae exposed to pesticides and Varroa mites (Varroa destructor). J Insect Physiol 58: 1042–1049 (2012).

Medrzycki P, Giffard H, Aupinel P, Belzunces LP, Chauzat MP, Claßen C, et al., Standard methods for toxicology research in *Apis mellifera*. J Apic Res 52: 1–60 (2013).

OECD, 2013: The Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, OECD 237, Honeybee (*Apis mellifera*) Larval Toxicity Test, adopted by the Council on July 26, 2013.

Schmehl DR, Tomé HVV, Mortensen AN, Martins GF and Ellis JD, Improved protocol for the in vitro rearing of *Apis mellifera* workers. J Apic Res 55:113–129 (2016).

Zhu W, Schmehl DR, Mullin CA and Frazier JL, four common pesticides, their mixtures and a formulation solvent in the hive environment have high oral toxicity to honeybee larvae. PLoS One 9: e77547 (2014).

About the Author:

Jigar R. Rana, Ph.D.
Group Leader – Ecotoxicology

Jigar is a senior officer, leading a team of Ecotoxicology. He has very good experience of conducting aquatic and terrestrial studies and has been actively involved in validation of Ecotoxicity studies. He is a member of Society of Toxicology, India. He has professional experience of more than 10 years in CRO industry.

About the Author:

Pritee D. Singh, M.Sc.
Jr. Research Officer – Ecotoxicology

She is a specialised Study Director for the aquatic and terrestrial studies. She has vibrant experience in handling difficult test items. She is research-oriented and actively involved in the validation of Ecotoxicity studies. At a very young age, she is the co-author of the paper, published in the peer-reviewed journal. She has an experience of more than three years in the CRO industry.