Bull Environ Contam Toxicol (2012) 89:921–924
DOI 10.1007/s00128-012-0773-x

Oral Toxicity of Fipronil Insecticide Against the Stingless Bee *Melipona scutellaris* (Latreille, 1811)

Clara Tavares Lourenço · Stephan Malfitano Carvalho · Osmar Malaspina · Roberta Cornélio Ferreira Nocelli

Received: 24 April 2012 / Accepted: 2 August 2012 / Published online: 12 August 2012
© Springer Science+Business Media, LLC 2012

Abstract For a better evaluation of the model using *Apis mellifera* in toxicology studies with insecticides, the oral acute toxicity of the insecticide fipronil against the stingless bee *Melipona scutellaris* was determined. The results showed that fipronil was highly toxic to *M. scutellaris*, with a calculated LC_{50} (48 h) value of 0.011 ng a.i./µL of sucrose solution and an estimated oral LD_{50} (48 h) of 0.6 ng a.i./bee. Our results showed that *M. scutellaris* bee is more sensitive to fipronil than the model specie *A. mellifera*.

Keywords Uruçu bee · Ecotoxicology · Pesticide · LC_{50}

Stingless bees are responsible for pollination of 30 %–90 % of the Brazilian native flora, depending on the ecosystem in which they are present, and for pollination of up to 33 % of the crops (Imperatriz-Fonseca and Nunes-Silva 2010). The stingless bee *Melipona scutellaris* (Hymenoptera, Apidae, Meliponini) is popularly known as Uruçu and is found mainly in the Brazilian Northeast, living in warm and wet forested areas. Such specie is well adapted to the weather and ecology of São Paulo State (Nogueira-Neto 1997). This stingless bee has size similar to the honey bee *Apis mellifera* L., 1758 and its colonies are maintained in "meliponaries", that are similar to apiaries. The hive of *M. scutellaris* is comprised of comb shaped overlapping disks surrounded by pots of food and its managed is similar to that of the honey bee. Besides its ecological importance as pollinators of native plants in Brazil, *M. scutellaris* is considered a promising pollinator species for rearing on a large scale, to use in protected or field crops, due its ease of maintaining strong hives, which can be easily transported and multiplied. It is considered an efficient pollinator of Solanaceae species, due its ability to perform buzz pollination (Imperatriz-Fonseca et al. 2006). However, due to the accessibility of crops or the proximity of crops to native forest areas, *M. scutellaris* is vulnerable to anthropic actions. One cause of increased mortality rates of these bees is insecticide poisoning (Johnson 2010).

Fipronil ($C_{12}H_4Cl_2F_6N_4OS$) acts on the insect nervous as a non-competitive inhibitor of the gamma-aminobutyric (GABA) and glutamate (GluCl) receptor. It acts by blocking the chloride channels, thus eliminating the normal inhibition of nerve impulses and resulting in hyperactivity, followed by paralysis and death (Barbara et al. 2005; Gunasekara et al. 2007; Stenersen 2004). The toxicity of fipronil was previously described for the honey bee *A. mellifera* (Decourtye et al. 2005; Mayer and Lunden 1999; Tingle et al. 2003), as well as *Megachile rotundata* Fabricius, 1787, *Nomia melanderi* Cockerell, 1906 (Mayer and Lunden 1999), and *Scaptotrigona postica* Latreille, 1807 (Jacob 2012). For *M. scutellaris*, only the topical toxicity of such insecticide was determined (Lourenço et al. 2012). As trace amounts of fipronil may be present in the pollen and nectar of the treated plants, the intoxication of bees through feeding is one possibility (Thompson 2010). Thus, the aims of this work were to determine the oral lethal concentration (LC_{50} at 48 h) of the insecticide fipronil for *M. scutellaris* foragers and to assess whether

C. T. Lourenço (✉) · R. C. F. Nocelli
Centro de Ciências Agrárias (CCA), Universidade Federal de São Carlos (UFSCar), Rod. Anhanguera, Km 174, Araras, SP 13600-970, Brazil
e-mail: claratavareslourenco@yahoo.com.br

S. M. Carvalho · O. Malaspina
Centro de Estudos de Insetos Sociais (CEIS), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Av. 24 A, 1515, Bela Vista, Rio Claro, SP 13506-900, Brazil

the *A. mellifera* honey bee is a good model for toxicological studies, considering the diversity of Brazilian stingless bees.

Materials and Methods

Three colonies of *M. scutellaris* from the Universidade Estadual Paulista (UNESP) Rio Claro campus were used in the experiment. The hives were maintained in a protected room and the bees were allowed free access to the external environment through a plastic tube connected to the nest entrance. Throughout the experiment, the colonies were monitored for overall health, the queen's laying capacities, and general foraging activity and food availability. Furthermore, a sucrose solution at 60 % was prepared with lemon juice and provide to colonies (Brighenti et al. 2011).

Assays were carried out at the UNESP Center for the Study of Social Insects (CEIS) according to the directives of the Organization for Economic Cooperation and Development number 213 (OECD 1998). To ensure genetic variability among the colonies and to obtain a more reliable value for LC_{50}, the forager bees for each treatment were collected from three different colonies. Thus, each treatment (defined by the concentration of fipronil used) included three distinct groups of forager bees, which originated from each one of three different colonies. So, each treatment consisted of three replicates, each of them with ten bees from one colony, with a total of thirty specimens per treatment.

To determine the oral LC_{50} of the fipronil (95 % of purity, Bayer CropScience, Brazil) to *M. scutellaris* foragers, a stock solution (1000 ng active ingredient/µL acetone) was prepared. This solution was diluted in a solution of 50 % sucrose to obtain concentrations of 0.005, 0.01, 0.03, 0.05 and 0.5 ng a.i./µL sucrose solution, which were chosen based on the oral LC_{50} value of fipronil for Africanized *A. mellifera* (Roat et al. 2010) and *S. postica* (Jacob 2012). The control group received a sucrose solution added of 2 % of acetone, which was the maximum concentration of this solvent in the fipronil-containing solutions.

During the assays, the forager bees were kept in cages of 250 mL of volume, fed through microtubes (1.5 mL) punched in extremities, filled in with a 50 % sucrose solution and kept in a chamber of biochemical oxygen demand (BOD) at 29 ± 1°C and relative humidity of 70 ± 5 %. For 72 h after initiating the bioassays, assessments were made every one, four and 24 h to record any signs of abnormal behavior as well as the number of dead bees. Additionally, the volume of sucrose solution consumed by the bees was quantified daily, weighing of the feeders on an analytical balance, immediately before the bioassays and after 24 h.

Statistical analyses to determine the LC_{50} values were performed using a log-logistic model from the "drc" package (Analysis of Dose–Response Curves) and compiled using the statistical software R® (2012).

Results and Discussion

The calculated value of LC_{50} (48 h) of fipronil to *M. scutellaris* bees was 0.011 ng a.i./µL sucrose solution ($C.I._{95\ \%}$ = 0.005–0.02 ng a.i./µL sucrose solution) (Fig. 1) showing that these bees are more sensitive to fipronil than the Africanized honey bees *A. mellifera* (1.27 ng/µL of diet by Roat et al. 2010) and *S. postica* (0.24 ng/µL of diet by Jacob 2012). Following Johansen and Mayer (1990) and Lourenço et al. (2012), this phenylpyrazole insecticide was considering highly toxic to *M. scutellaris*, independent of the administered route, showing a topic LD_{50} (48 h) of 0.41 ng a.i./bee (Lourenço et al. 2012).

Considering that the diet containing fipronil do not result in an antifeeding effect (Colin et al. 2004; Mayer and Lunden 1999) and that each *M. scutellaris* consumed an average volume of 55 µL of sucrose solution per day, the estimated oral LD_{50} (48 h) of this insecticide to *M. scutellaris* was 0.6 ng a.i./bee. This value is ten times smaller than the oral LD_{50} (48 h) of fipronil to *A. mellifera*, which was found by Decourtye et al. (2005) which was 6 ng a.i./bee.

This difference in the responses of various bee species to insecticide exposure was previously described by Desneux et al. (2007). Changes in pesticide susceptibility among bee species were also observed by several others (Nocelli et al. 2012), with most of the results indicating that the honey bee *A. mellifera* was more tolerant to insecticide in comparison with species of stingless bees. Furthermore, studies with the *A. mellifera* honey bee showed that sublethal doses of fipronil can also be of concern because changes in

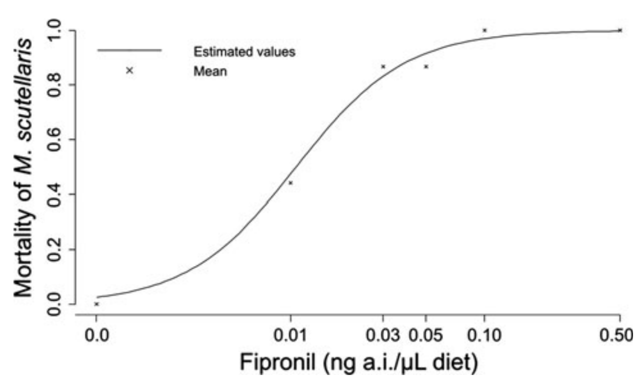

Fig. 1 Acute toxicity (48 h) by oral administration of the insecticide fipronil to foragers of *M. scutellaris*

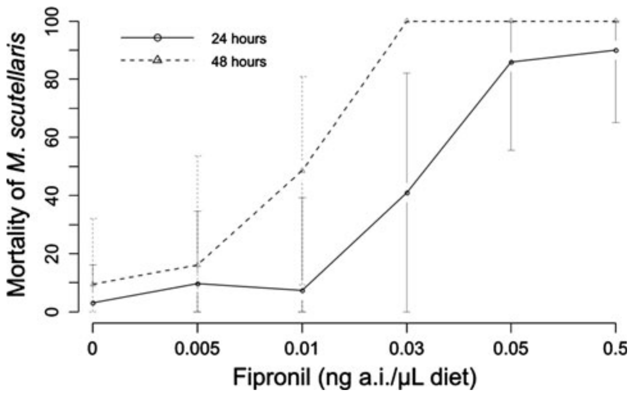

Fig. 2 Mortality evolution of foragers of *M. scutellaris* when exposed at different doses of fipronil. *Bars* show the confidence interval at 95 %

behavior, such as in feeding and foraging, can affect the entire colony (Colin et al. 2004). These findings reinforced the notion that wild bees are a pollinating group at high risk for pesticide exposure and toxicity (Brittain et al. 2010).

The highest concentrations of fipronil used in this work (0.05 and 0.5 ng a.i./μL sucrose solution) resulted in mortality rates of 85.9 % and 90 %, respectively, fallowing 24 h of exposure. After 48 h, the three highest concentrations of fipronil resulted in 100 % mortality (Fig. 2).

Before their death, the bees showed the expected signs of intoxicationby firponil, these being initial tremors followed by paralysis. After 48 h, the surviving bees in the group treated with 0.01 and 0.005 ng a.i./μL sucrose solution also displayed the same symptoms. The symptoms of intoxication that were observed after oral administration were the same described for these bees after topical administration of fipronil doses of 2.0, 2.5 and 5.0 ng a.i./bee (Lourenço et al. 2012) or for *A. mellifera* exposed to fipronil on contaminated diet at 2 g a.i./L of sucrose solution (Colin et al. 2004).

When the lethal or behavioral effects of insecticides are replicated in bees under laboratory conditions, the greater impact of the pesticide under natural conditions is highlighted. Thus, it is important to establish limits on pesticide use, considering the consequences on biodiversity, economic losses from beekeepers and crop producers, and the awareness of society concerning the pesticides in environmental (Pham-Delègue et al. 2002).

The use of diverse pollinator species in toxicological studies allows a better understanding of the spectrum of bee responses. This is especially important when compared the results of non-*Apis* bee with those of the current model *A. mellifera* (Brittain and Potts 2011).

In this way, new studies on *M. scutellaris* behavior after contamination with sublethal doses of fipronil are being conducted.

Acknowledgments This study was supported by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior, CAPES Brazil (http://www.capes.gov.br/) .

References

Barbara GS, Zoube C, Rybak J, Gauthier M, Grünewald B (2005) Acethylcoline, GABA and glutamate induce ionic currents in cultured antennal lobe neurons of the honeybee, *Apis mellifera*. J Comp Physiol A 191:823–836

Brighenti DM, Carvalho CF, Brighenti CRG, Carvalho SM (2011) Inversão da sacarose utilizando ácido cítrico e suco de limão para preparo de dieta energética de *Apis mellifera* Linnaeus, 1758. Ciênc Agrotec 35:297–304

Brittain C, Potts SG (2011) The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. Basic Appl Ecol 12:321–331

Brittain CA, Vighi M, Bommarco R, Settele J, Potts SG (2010) Impacts of a pesticide on pollinators species richness at different spatial scale. Basic Appl Ecol 11:106–115

Colin ME, Bonmatin JM, Moineau I, Gaimon C, Brun S, Vermandere JP (2004) A method to quatifify and analyse the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch Environ Contam Toxicol 47: 387–395

Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski K, Cluzeau S, Pham-Delègue MH (2005) Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Arch Environ Contam Toxicol 48:242–250

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

Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS (2007) Environmental fate and toxicology of fipronil. J Pestic Sci 32:189–199

Imperatriz-Fonseca VL, Nunes-Silva P (2010) As abelhas, os serviços ecossistêmicos e o código florestal brasileiro. Biota Neotrop 10:59–62

Imperatriz-Fonseca VL, Saraiva AM, De Jong D (2006) Bees as pollinators in Brazil: assessing the status and suggesting best practices. Holos, Ribeirão Preto

Jacob CRO (2012) Efeitos do inseticida fipronil sobre os corpos pedunculados de operárias *Scaptotrigona postica* (Latreille, 1807) (Hymenoptera, Apidae, Meliponini). Masters dissertation, Universidade Estadual Paulista, Rio Claro, SP

Johansen CA, Mayer DF (1990) Pollinator protection: a bee and pesticide. Wicwas, Cheshire

Johnson R (2010) Honey bee colony collapse disorder. CRS, Washington

Lourenço CT, Carvalho SM, Malaspina O, Nocelli RCF (2012) Determination of fipronil LD_{50} for the Brazilian bee *Melipona scutellaris*. Julius Kühn Archiv 426 (in press)

Mayer DF, Lunden JD (1999) Field and laboratory tests of the effects of fipronil on adults female bees of *Apis mellifera*, *Megachile rotundata* and *Nomia malanderi*. J Apicult Res 38:191–197

Nocelli RCF, Malaspina O, Carvalho SM, Lourenço CT, Roat TC, Pereira AM, Silva-Zacarin ECM (2012) As abelhas e os defensivos agrícolas. In: Imperatriz-Fonseca VL, Canhos DAL, Saraiva AM Polinizadores no Brasil–contribuição e perspectivas para a biodiversidade, uso sustentável, conservação e serviços ambientais. EDUSP, São Paulo

Nogueira-Neto P (1997) Vida e criação de abelhas indígenas sem ferrão. Nogueirapis, São Paulo

OECD Organization For Economic Cooperation And Development (1998) Honeybees, acute oral toxicity test. OECD guidelines for the testing of chemicals, 213. Available in http://www.oecdbookshop.org/oecd/display.asp?K=5LMQCR2K7R34&DS=Test-No.-213-Honeybees-Acute-Oral-Toxicity-Test. Accessed 18 Jun 2012

Pham-Delègue MH, Decourtye A, Kaiser L, Devillers J (2002) Behavioural methods to assess the effects of pesticides on honey bees. Apidologie 33:425–432

Roat TC, Carvalho SM, Nocelli RCF, Silva-Zacarin ECM, Malaspina O (2010) Acute toxicity of fipronil to newly-emerged honeybee *Apis mellifera* L., 1758 (Hymenoptera, Apidae) africanized. 9 Encontro sobre Abelhas Ribeirão Preto

Stenersen J (ed) (2004) Interference with signal transduction in the nerves. In: Chemical pesticides: mode of action and toxicology. CRC Press, New York

Thompson HM (2010) Risk assessment for honey bees and pesticides—recent development and "new issues". Pest Manag Sci 66:1157–1162

Tingle CC, Rother JA, Dewhurst CF, Lauer S, King WJ (2003) Fipronil environmental fate, ecotoxicology and human health concerns. Rev Environ Contam Toxicol 176:1–66