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Analysis of a dose-response assay in *Scaptotrigona bipunctata* bees, Lepeletier, 1836 (Hymenoptera: Apidae) using the logistic regression model under the Bayesian approach

Breno Gabriel da Silva[1*], Paula Ribeiro Santos[1], Cristian Marcelo Villegas Lobos[1], Tamiris de Oliveira Diniz[2], Naiara Climas Pereira[2] and Maria Claudia Colla Ruvolo-Takasusuki[2]

[1]Departamento de Ciências Exatas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Avenida Pádua Dias, 11, 13418-900, Piracicaba, São Paulo, Brazil. [2]Departamento de Biotecnologia, Genética e Biologia Celular, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: brenogsilva@usp.br

ABSTRACT. This paper shows the results of a dose-response study in *Scaptotrigona bipunctata* bees, Lepeletier, 1836 (Hymenoptera: Apidae) exposed to the insecticide Fastac Duo. The aim was to evaluate the lethal concentration that causes the death of 50% of bees (LC_{50}) and investigate the odd of mortality after exposure to different concentrations, using the logistic regression model under the Bayesian approach. In this approach, it is possible to incorporate a prior information and gives more accurate inferential results. Three independent dose-response experiments were analyzed, dissimilar in their lead time according to guidelines from the Organisation for Economic Co-operation and Development (OECD), in which each assay contained four replicates at the concentration levels investigated, including control. Observing exposure to the agrochemical, it was identified that the higher the concentration, the greater the odd of mortality. Regarding the estimated lethal concentrations for each experiment, the following values were found, 0.03 g a.i. L^{-1}, for 24 hours, 0.04 g a.i. L^{-1}, for 48 hours and 0.06 g a.i. L^{-1} for 72 hours, showing that in experiments with longer exposure times there was an increase in LC_{50}. Concluding, the study showed an alternative approach to classical methods for dose-response studies in *Scaptotrigona bipunctata* bees exposed to the insecticide Fastac Duo.

Keywords: lethal concentration; hamiltonian Monte Carlo; statistical modeling; binary regression; completely randomized design.

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Introduction

Bees, belonging to the class of pollinating insects, play a crucial role in the stability and conservation of biosystems. The authors Barbosa, Crupinski, Silveira, and Limberger (2017) and Ramírez, Ayala, and González (2018) mention that these insects are associated with pollination of crops, reaching on average in Brazil an approximate contribution of US$3,000 ha^{-1} in soybean production.

There are several species of bees classified as native Brazilian bees (Chuttonh, Chanbang, Sringarm, & Burget, 2016). Among these species is the *Scaptotrigona bipunctata,* Lepeletier, 1836 (Hymenoptera: Apidae), a stingless bee, found in South America (Bolivia, Brazil, Paraguay and Peru) and the Brazilian states of Acre, Ceará, Maranhão, Minas Gerais, Paraná, Pará, Rio Grande do Sul, Rio de Janeiro and Santa Catarina (Camargo & Pedro, 2013). Its colonies are quite populated and, despite presenting great potential as pollinators, research on their vulnerability to agrochemicals is limited (Diniz et al., 2020a).

The use of agrochemicals is one of the principal causes of contamination, harming the environment, as well as the insects that visit these crops, impacting biodiversity, deforestation and loss of native ecosystems, thus becoming one of the main disadvantages for the beekeeping sector (Dively & Kamel, 2012). The authors Sanchez-Bayo and Goka (2014), indicate that plants contaminated by specific agrochemicals, in full bloom, play a fundamental role in the mortality of pollinating insects, and may impair the behavior of bees affecting the colony.

In recent years, there has been a wide use of agrochemicals for pest management, the most common type of combination are pyrethroids and neonicotinoids because this arrangement increases their efficiency and decrease the probability of resistance (Sosa-Gómez & Omoto, 2012). In this context, the insecticide Fastac Duo stands out, a systemic and contact insecticide composed of two chemical groups: neonicotinoid and pyrethroid.

Neonicotinoids have become the most widely used class of insecticides, with large-scale applications ranging from plant protection, veterinary products and biocides in fish farming. They act as agonists of the post-synaptic nicotinic acetylcholine receptor of the insect central nervous system, causing a signalling blockage (Bridi, Larena, Pizarro, Giordano, & Montenegro, 2018). Pyrethroid insecticides also present low-dose efficiency, it acts on the sodium channels of the nerve cell membrane, altering depolarization and nerve impulse conduction (Santos, Areas, & Reyes, 2007).

As stingless bees are more sensitive to insecticides, the use of these compounds is threatening its survival, and it is critical to evaluate the toxicity of these components in these bees to ensure their protection (Arena & Sgolastra, 2014). Dose-response studies stand out as a methodology in different fields, such as agricultural and biological areas and more specifically in the plant, soil, entomological, animal sciences, microbiology, clinical and pharmaceutical. Among the potentialities observed in these studies, we highlight the possibility of evaluating the effects of controlled experimental factors (dose) on the measured responses.

In the literature, there are few dose-response studies for stingless bees (LC50), (Dorneles, Souza Rosa, & Blochtein, 2017; Moreira et al., 2018; de Jacob, Zanardi, Malaquias, Silva, & Yamamoto, 2019; Diniz et al., 2020b; Rosa-Fontana, Dorigo, Galaschi-Teixeira, Nocelli, & Malaspina, 2020), however, none of these studies analyzed the exposure of *Scaptotrigona bipunctata* bees to the insecticide Fastac Duo. Also, considering statistical methods, the studies mentioned used classical approaches in their analyses, such as logistic regression, probit regression, parametric and non-parametric tests. On the other hand, Jacob et al. (2019) used Bayesian inference as an alternative to classical inference, associated with cluster analysis using the Jaccard index.

Differently to classical methods, Bayesian methods coherently manage uncertainty through probability calculation, quantify several sources of simultaneous variation, incorporate a prior knowledge in modeling when possible and also allow a more accurate inference, taking advantage of all available information, which is ideal in decision making (Hennessey, Rosner, Bast Jr, & Chen, 2013). Therefore, this study aims to show the statistical results obtained by Bayesian methods, using logistic regression, inherent to the dose-response assay (LC50), in *Scaptotrigona bipunctata* bees exposed to the insecticide Fastac Duo.

Material and methods

Experiment description

Adult *Scaptotrigona bipunctata* forages were collected at the entrance of the colony when they returned from foraging, at the *Fazenda Experimental de Iguatemi* (FEI) (23°25'S and 51°57'W) of *Universidade Estadual de Maringá* (UEM) and taken to the Laboratory of Animal Genetics of the Department of Biotechnology, Genetics and Cell Biology at UEM. The insecticide used was Fastac Duo, a ready-made mixture of systemic and contact insecticides, composed of two different chemical classes: Neonicotinoid (Acetamipride) and Pyrethroid (Alpha-Cypermethrin). The main solution was diluted in water, based on the recommended dose for the whitefly pest, *Bemisia tabaci* (0.5332 g a.i. L^{-1}), and preliminary tests were performed to determine concentrations (0.0265 g a.i. L^{-1}; 0.053 g a.i. L-1; 0.0795 g a.i. L^{-1} and 0.106 g a.i. L^{-1}). The agrochemical was added to the syrup (water and sugar) and analyzed after the ingestion test. The control group received only syrup (0.001 g a.i. L^{-1}).

The bioassays were designed according to the Organisation for Economic Co-operation and Development [OECD] (1998) guidelines to evaluate the acute oral toxicity of agrochemicals in adult workers. Three bioassays were performed from March to May 2019, containing four replications, with 15 *Scaptotrigona bipunctata* adult workers per concentration, totalling 300 bees in each bioassay, with a duration of 24, 48 and 72 hours. Glass bottles containing filter paper at the bottom, a water-soaked cotton swab and a container with syrup + Fastac Duo and only syrup for the control group, were used. Bioassays were stored in a BOD incubator, maintained in 28 ± 2°C and UR 70 ± 10%, for 24, 48 and 72 hours, and evaluated. For each experiment, the LC50 was determined based on mortality.

Logistic regression model

The logistic regression model is widely used to model the dependency relationship between a binary variable (success/failure, killed/alive, yes/no) and a set of explanatory variables (independent variables).

In general, explanatory variables are a mixture of categorical and continuous variables (Giolo, 2017). The binary regression model is defined by (Equations 1 and 2):

$$\mathbf{Y}_i|\mathbf{x}_i \overset{i.i.d.}{\sim} Ber(\mathbf{p}_i),\ i=1,\ldots,n, \tag{1}$$

$$\mathbf{p}_i = E[Y_i|x_i], \tag{2}$$

where: $\mathbf{Y}_i = (Y_1, Y_2, \ldots, Y_n)^T$ expresses a vector n×1 independent binary random variables, with Bernoulli probability distribution, $\mathbf{x}_i = (x_{i1}, \ldots, x_{ik})^T$, where $i = 1, \ldots, n$ contains the vectors associated with the explanatory variables implying the construction of the matrix of the model described by $\mathbf{X} = [\mathbf{1}, \mathbf{x}_i]$, where vector $\mathbf{1}$ with dimension n×1, $\boldsymbol{\beta} = (\beta_0, \beta_1, \ldots, \beta_k)^T$ represents the vector of unknown parameters associated with the k explanatory variables. The aim of this model is to estimate the probability of success of a binary variable given a set of explanatory variables.

According to Giolo (2017), in binomial regression models it is necessary to consider some characteristics, where $E[\mathbf{Y}_i|\mathbf{x}_i]$ belongs to the interval [0,1] and not to the interval (-∞,∞) as in linear regression, in addition to the relationship between $\mathbf{x_i}$ and $E[\mathbf{Y}_i|\mathbf{x}_i]$ it has the form of an S, similar to the accumulated distribution of a random variable, this format motivated the use of logistic distribution to model $E[\mathbf{Y}_i|\mathbf{x}_i]$, and its distribution function is expressed by Equation 3:

$$F(\mathbf{Y}_i|\mathbf{x}_i) = \frac{\exp(\mathbf{x_i})}{1 + \exp(\mathbf{x_i})} \tag{3}$$

where (3) is a nonlinear function in a linear set of parameters, which if x_i tends to -∞ where $F(-\infty) = 0$ and if $\mathbf{x}_i$ tends to ∞ imply $F(\infty) = 1$. Shipe, Deppen, Farjah, and Grogan (2019) explain that due to the flexibility from the mathematical point of view and simple interpretation because it is based on odds, the logistic regression model has become the most used model with these characteristics. The probability of an individual with observed values x_i present a given success, is defined by Equation 4:

$$p_i = p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i) = \frac{\exp(\boldsymbol{\beta}'\mathbf{x}_i)}{1+\exp(\boldsymbol{\beta}'\mathbf{x}_i)} = \frac{\exp(\beta_0+\sum_{l=1}^{k}\beta_l x_l)}{1+\exp(\beta_0+\sum_{l=1}^{k}\beta_l x_l)} \tag{4}$$

where β_0 represents a constant, β_l are the unknown parameters of the regression and $\mathbf{x} = (1, x_1, \ldots, x_k)^T$ a vector containing the constant 1 and the observed values of independent variables. As a result of the Equation in (4), the probability of the individual not presenting the failure is expressed by Equation 5:

$$1\text{-}\mathbf{p_i} = 1 - p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i) = \frac{1}{1+\exp(\beta_0+\sum_{l=1}^{k}\beta_l x_l)} \tag{5}$$

McCullagh and Nelder (1989) highlight that in dose-response assays the aim is to find a sigmoidal curve that is a good fit to the data since this curve admits mathematical properties of a continuous distribution function. Such search can be performed with nonlinear models in the parameters, so the idea consists of doing a transformation in the sigmoidal curve in a way that it becomes a straight line, making it possible to use common regression procedures to estimate the parameters.

Therefore, performing a transformation, expressed by the natural log of the ratio between Equations (4) and (5) the result is a function called logit, a symmetric function, that is, it is the logarithm of an odds and can be described by Equation 6:

$$odds = \frac{p(\mathbf{Y_i}=\mathbf{1}|\mathbf{x_i})}{1-p(\mathbf{Y_i}=\mathbf{1}|\mathbf{x_i})} = \exp(\boldsymbol{\beta}'\mathbf{x}_i) \tag{6}$$

In the literature, there are other distributions commonly used in dose-response assays, which are the probit and complementary log-log, that according to McCullagh and Nelder (1989), because of the lack of fit, there is a need to verify a suitable model for this situation.

Bayesian estimation in dose-response trials

In statistics, the inference is the science of obtaining outcomes about a 'population' through a 'sample' of this population (Lee, 2013). In classical inference, the parameter θ is unknown and fixed, but in Bayesian inference, the parameter θ is unknown and random. So, in the Bayesian inference the uncertainty of the parameter θ is measured by a probability distribution. For the description of dead bees in 24, 48 and 72 hours (three independent experiments), the parameters to be estimated are the coefficients of regression, so $\beta = (\beta_0, \beta_1, \dots, \beta_k)^T$. Thus, information related to the vector of parameters β can be obtained through the likelihood function, which is defined as (Equation 7):

$$L(\boldsymbol{\beta}) = \prod_{i=1}^{n} [p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i)]^{y_i} [1 - p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i)]^{1-y_i} \tag{7}$$

where $p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i)$ is defined in Equation (4) and is related with β (the unknown regression coefficients) and x (independent variables). In this way, the structure of the Bayesian model is given by (Equations 8, 9 and 10):

$$\mathbf{Y}_i|\mathbf{x}_i \overset{i.i.d.}{\sim} \text{Ber}(p_i), \quad i = 1, \dots, n, \tag{8}$$

$$\mathbf{p}_i = p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i), \tag{9}$$

$$\beta_l \sim N(\mu, \sigma^2), \quad l = 0,1, \dots, k \tag{10}$$

A non-informative prior distribution will be used for β_l, since there are no reports of work on *Scaptotrigona bipunctata* bees in the applied or theoretical literature. Other prior distribution could be studied in practice (for instance, Jeffreys prior or Student t prior among others) and compare the results. However, is not the goal of this paper to compare the results of the posterior distribution by using different prior distribution. For our data set, the parameters β_0 and β_1 are defined as a normal distribution of mean 0 and variance 10^6, in which β_0 is associated with the intercept and β_1 refers to the parameter corresponding to the logarithmic variable of the concentration of the insecticide used, that is (Equations 11 and 12):

$$\beta_0 \sim N(0, 10^6) \tag{11}$$

$$\beta_1 \sim N(0, 10^6) \tag{12}$$

With these specifications established, the joint posterior distribution, according to the Bayes's theorem, showed in the structure of the Bayesian model, is (Equations 13 and 14):

$$\pi(\boldsymbol{\beta}|\mathbf{Y}, \mathbf{x}) = \frac{L(\boldsymbol{\beta}).\pi(\boldsymbol{\beta})}{\int L(\boldsymbol{\beta}).\pi(\boldsymbol{\beta})d\beta} \tag{13}$$

$$\pi(\boldsymbol{\beta}|\mathbf{Y}, \mathbf{x}) \propto \prod_{i=1}^{n} [p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i)]^{y_i} [1 - p(\mathbf{Y}_i = \mathbf{1}|\mathbf{x}_i)]^{1-y_i} \prod_{l=0}^{1} \exp(\beta_l^2), \tag{14}$$

Where $\propto$ represents proportionality. The joint posterior distribution in Equation (14) does not have a close form, therefore we use in our paper a variation of the Hamiltonian Monte Carlo (Betancourt, 2017) because produces samples for the joint posterior distribution in a more efficient way compare with the traditional Gibbs Sampling and Metropolis-Hastings algorithm (Betancourt, 2017). One of the sampling algorithms that is implemented in Stan and used in this work is the 'NUTS' (No-U-Turn sampler), which is a variation of the Hamiltonian Monte Carlo (Hoffman & Gelman 2011; Betancourt, 2017). It is well know, that the samples produces by the Monte Carlo sampling are highly correlated, so we need to do something for trying to avoid this problem. It is necessary to discard an initial sample (burnin) and perform jumps in the generated values (thin, which is the dilution interval used in the simulation) so that convergence occurs for a stationary distribution, resulting in the joint posterior distribution. Thus, until it reaches convergence, the number of iterations, burnin values and dilution interval, can change. In this paper, three chains were generated, with 11000 iterations for each of them, discarding a sample of 1000 initial values and considering dilution intervals equal to ten. In our paper, we use some traditional measures (not presented in the paper for simplicity) for assessing convergence (Stan Development Team, 2020) such as: traceplot that evaluates the mixing rate of the chain, autocorrelation plot that assess the dependent sampling, the effective sample size (ESS) that measure the amount by which autocorrelation in samples increases uncertainty (standard errors) relative to an independent sample, the Monte Carlo standard error that is the uncertainty about a statistic in the sample due to sampling error. In our paper, we just present the Potential Scale Reduction (R-hat) and the rule of thumb is that R-hat values for all less than 1.1 (Stan Development Team, 2020). For more details about how to assess convergence, please review the Stan Development Team (2020).

Statistical analysis and computational aspects

All data were analyzed in the Software R version 4.0.2 (R Core Team, 2020), the graphs of the statistical analysis were performed with the function ggplot of the package ggplot2 (Wickham, 2016). The function stan of the package rstan was used to fit the logistic regression model, under the Bayesian approach (Stan Development Team, 2020). Odds were retrieved to investigate whether insects exposed to higher doses of the insecticide reveal higher mortality odds. Intervals of Highest Posterior Density (HPD) were obtained through the boa.hpd function of the package boa from Smith (2007), to calculate the posterior mean of the distribution. More tails about the HPD interval could be found for instance in Chen and Shao (1999). Pearson's residuals from the fitted models were used as a diagnostic measure.

Results

First, a descriptive analysis was performed (300 bees in each bioassay), in which it was observed that for the first period (24 hours), the proportion of dead bees were 50.67%. For the second period (48 hours), the proportion of dead bees was 46.33%. Finally, for the 72 hours period, the proportion of dead bees decreased to 39.67%. Thus, it was observed that the percentage of deaths decreases over time. Regarding the scatter plot of logarithm of concentrations vs. the proportions of deaths, it was noticed a behavior close to a sigmoid (Figure 1), thus there was evidence to fit binary regression models, more precisely the logistic model.

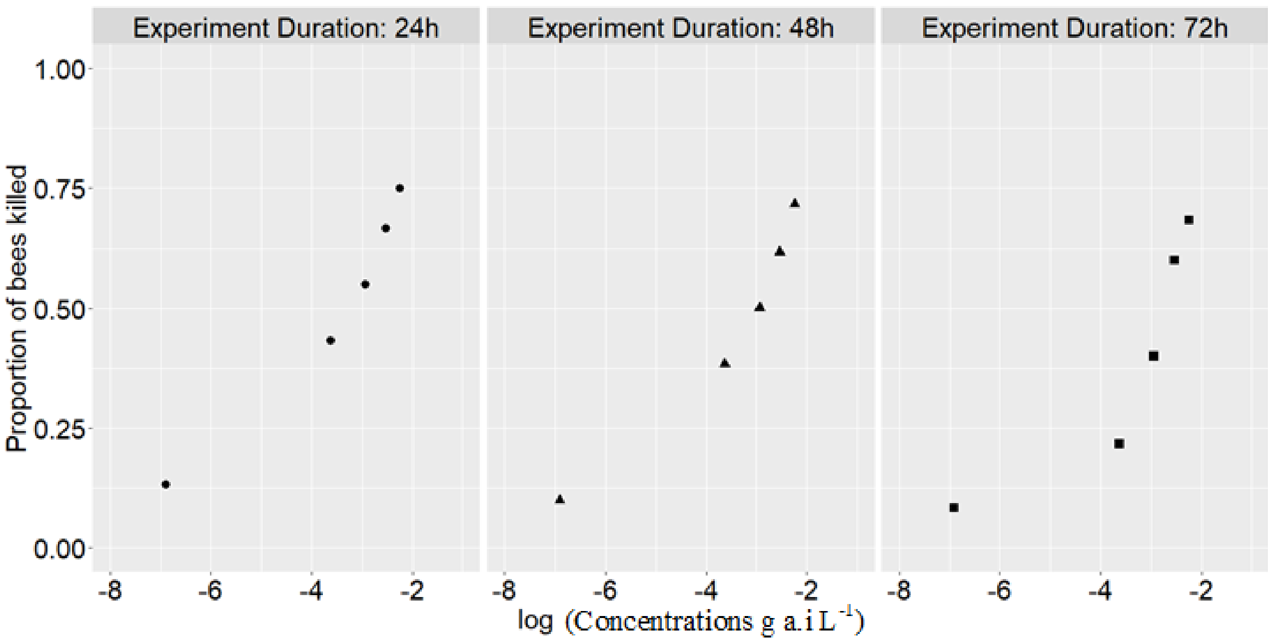


Figure 1. Scatter plot of the logarithm of concentrations depending on the sample proportions.

Table 1 presents the posterior mean estimates, the posterior Highest Posterior Density (HPD) and standard deviation for the intercept and logarithm of the concentration, showing that the logarithm of concentration is significant in all periods. Since the zero is not contained in 95% HPD intervals and the values of that the generated value chain showed a stationary behavior, where they are close to or equal to 1.

Table 1. Posterior mean, 95% HPD intervals and posterior standard deviation of logistic regression model.

Period	Parameters	Mean (95% HPD intervals)	Standard Deviation	$\hat{R}$ (R hat)
24 hours	Intercept	2.21 (1.64,2.77)	0.34	0.99
	log(Concentration)	0.62 (0.47,0.79)	0.10	0.99
48 hours	Intercept	2.15 (1.54,2.76)	0.37	1.00
	log(Concentration)	0.67 (0.50,0.85)	0.11	1.00
72 hours	Intercept	2.16 (1.42,2.84)	0.43	1.00
	log(Concentration)	0.78 (0.55,1.01)	0.14	1.00

In general, it was observed (Table 2) that the higher the concentration, the greater the odd of a bee dying after contact with the agrochemical, in all periods. However, regarding the odds, considering the concentration of log(0.0795) = -2.53, after 72 hours, it was observed that to increase one unit in the concentration of the insecticide Fastac Duo, it is expected about 20% increase in the mortality odds of *Scaptotrigona bipunctata* exposed to the agrochemical. Similarly, concerning the concentration of log(0.1060) = -2.24, after 72 hours, it is noted that to increase one unit in the concentration of the insecticide, it is expected about 50% increase in the mortality odds.

Table 2. Odd of mortality of *Scaptotrigona bipunctata* workers exposed to different concentrations of the insecticide Fastac Duo.

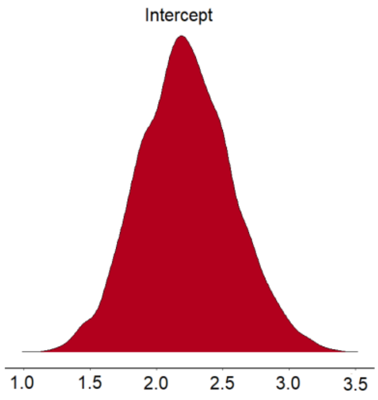
log(Concentrations)	Odds-24 hours	Odds-48 hours	Odds-72 hours
-6.90	0.12	0.08	0.04
-3.63	0.96	0.75	0.51
-2.93	1.47	1.19	0.89
-2.53	1.89	1.56	1.20
-2.24	2.27	1.89	1.50

With regard to the graphs of a posteriori densities, considering each of the experiments, it can be seen in the (Figures 2(a) and 2(b)), (Figures 2(c) and 2(d)), (Figures 2(e) and 2(f)), that the marginal distribution admits behavior close to a normal distribution, and furthermore, the most probable value of the parameters β_0 and β_1 considering the period of 24 hours is 2.21 and 0.62 respectively (Figures 2(a) and 2(b)). As for the period of 48 hours, the most probable value of the parameters β_0 and β_1 are 2.15 and 0.67 respectively (Figures 2(c) and 2(d)), and finally, regarding the period of 72 hours, it is observed that the most probable value of the parameters β_0 and β_1 are 2.16 and 0.78 respectively (Figures 2(e) and 2(f)).

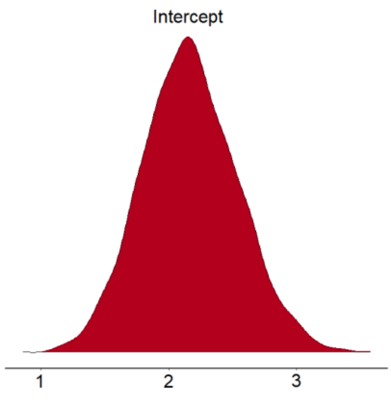
On (Figure 3), considering the logarithmic scale in the concentrations, the following LC_{50} results were obtained: -3.53 g a.i. L^{-1}, for 24 hours, -3.19 g a.i. L^{-1}, for 48 hours and -2.75 g a.i. L^{-1} for 72 hours. The fitted values are close to the actual values, showing a satisfactory adjustment of the model.

However, to obtain the original values of the concentrations it is necessary to calculate the exponential in the results thus the estimated values were 0.03 g a.i. L^{-1}, for 24 hours, 0.04 g a.i. L^{-1}, for 48 hours and 0.06 g a.i. L^{-1} for 72 hours. Therefore, the experiments with longer exposure times caused an increase in the LC_{50} of *Scaptotrigona bipunctata* workers.

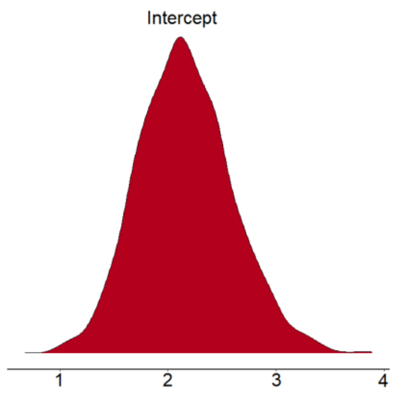
Figures 4(a), 4(c) and 4(e) show Pearson's residuals ($\mathbf{r_i^p}$), as defined by McCullagh and Nelder (1989), considering the Bayesian logistic model, in which it is expressed by the quotient of ordinary residuals $\mathbf{r_i^p} = \hat{\boldsymbol{\varepsilon}}_\mathbf{i} = \mathbf{y_i} - \hat{\boldsymbol{\mu}}_\mathbf{i}$ by its standard deviation $\sqrt{\mathbf{Var}(\hat{\boldsymbol{\mu}}_\mathbf{i})}$, $\hat{\boldsymbol{\varepsilon}}_\mathbf{i}$ being the estimated error, $\hat{\boldsymbol{\mu}}_\mathbf{i}$ the estimated values and $\mathbf{Var}(\hat{\boldsymbol{\mu}}_\mathbf{i})$ the variance function of the model, observing that it is contained in the borderline of -3 to 3, indicating no violated assumptions. Figures 4(b), 4(d) and 4(f) contain the Q-Q plot graphics, verifying that they approach the adjusted line, that is, there are indications that the residuals admit an appropriate behavior for all experimental periods considered.



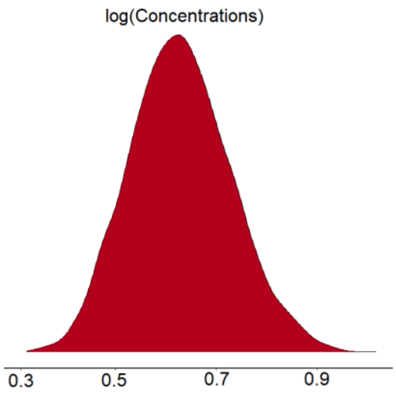
(a) Posteriori density of the intercept β_0 considering the 24h experiment



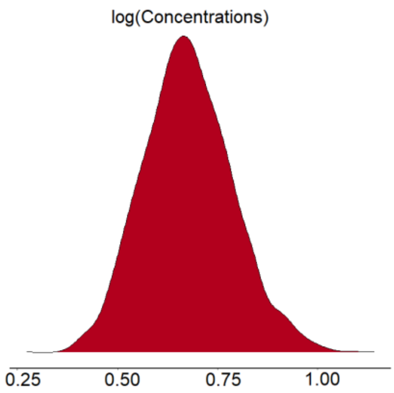
(c) Posteriori density of the intercept β_0 considering the 48h experiment



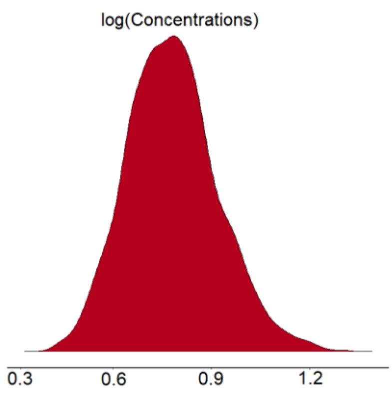
(e) Posteriori density of the intercept β_0 considering the 72h experiment



(b) Posteriori density of the log(Concentrations) β_1 considering the 24h experiment



(d) Posteriori density of the log(Concentrations) β_1 considering the 48h experiment



(f) Posteriori density of the log(Concentrations) β_1 considering the 72h experiment

Figure 2. Posteriori marginal distribution for the coefficients of the logistic regression model.

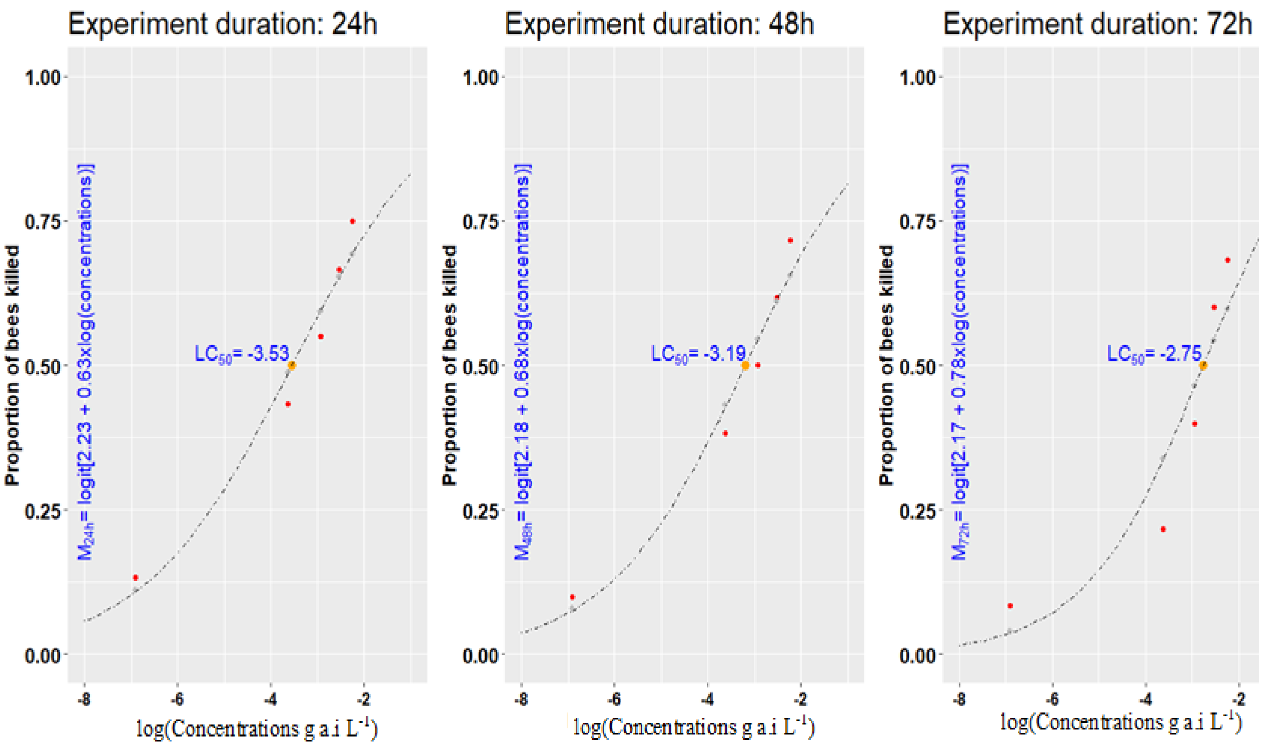
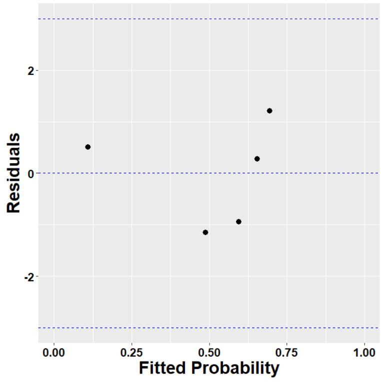
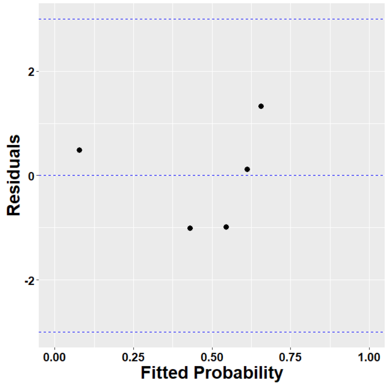


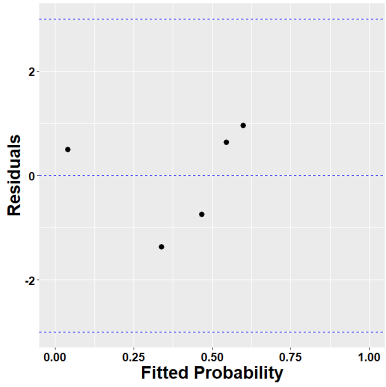
Figure 3. Lethal concentration curve of 50% Mortality of *Scaptotrigona bipunctata* after oral contamination with the insecticide Fastac Duo.



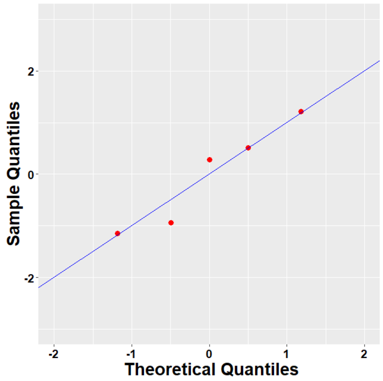
(a) Estimated probabilities vs. residuals (r_i^P) of the fitted model considering the 24h experiment



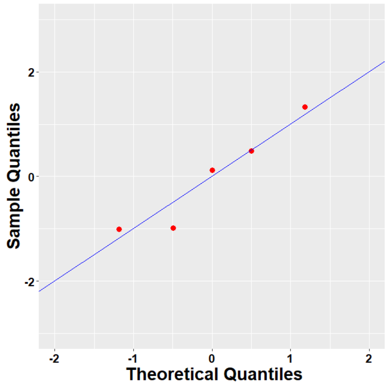
(c) Estimated probabilities vs. residuals (r_i^P) of the fitted model considering the 48h experiment



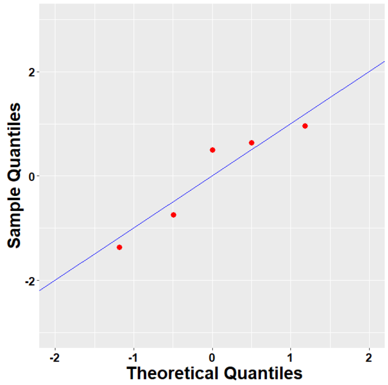
(e) Estimated probabilities vs. residuals (r_i^P) of the fitted model considering the 72h experiment



(b) Q-Q Plot (r_i^P) of the fitted model considering the 24h experiment



(d) Q-Q Plot (r_i^P) of the fitted model considering the 48h experiment



(f) Q-Q Plot (r_i^P) of the fitted model considering the 72h experiment

Figure 4. Diagnostic analysis of the logistic regression model for each of the experiments.

Discussion

Bayesian methods provide intuitive and meaningful inferences. Highlights that this method allows the researcher to answer complex questions more accurately and use all available data set information. The main difference between frequentist and Bayesian methods is that frequentist methods are based only on probability, while Bayesian methods use probability and information previously acquired through previous research or dialogue with the researcher (O'Hagan, 2004).

However, as this is a pioneer study, it was not possible to include informative priors in Bayesian analyses, so further research is necessary to include extra information in future analyses to avoid any evidence of subjectivity. Regarding the agrochemical used in this study, its composition is a combination of products of different chemical classes, considered highly toxic (Agência de Defesa Agropecuária do Paraná [Adapar], 2018). The values of estimated LC_{50} (0.03 g a.i. L^{-1}, for 24 hours, 0.04 g a.i. L^{-1}, for 48 hours and 0.06 g a.i. L^{-1} for 72 hours) were lower when compared to those reported in the literature for this compound.

In a study conducted by Diniz et al. (2020b) the estimated LC_{50} for *Scaptotrigona bipunctata* bees orally contaminated with the insecticide Fastac Duo were 0.07 g a.i. L^{-1} to 24 hours, 0.10 g a.i. L^{-1}, for 48 hours and 0.0665 g a.i. L^{-1} for 72 hours. However, our results were higher when compared to studies that estimated LC_{50} for only one chemical class of agrochemical, which is the most common pesticide reported in the literature. The LC_{50} after exposure to the neonicotinoid imidacloprid was 2.35×10^{-8} g/bee for *Melipona quadrifasciata* (Tomé, Martins, Lima, Campos, & Guedes, 2012), 4.25×10^{-8} g L^{-1} after 24 hours and 1.43×10^{-8} g L^{-1} after 48 hours for *Scaptotrigona postica* (Soares, Jacob, Carvalho, Nocelli, & Malaspina, 2015) and $2.01\text{x}10^{-13}$ a.i. L^{-1} (24 hours) and $0.81\text{x}10^{-13}$ a.i. L^{-1} (48 hours) for *Melipona scutellaris* (++Costa, Grella, Barbosa, Malaspina, & Nocelli, 2015).

The LC_{50} for *Scaptotrigona bipunctata* after oral contamination with the neonicotinoid thiamethoxam were $2\text{x}10^{-6}$ g a.i. L^{-1} (Moreira et al., 2018) and the LC_{50} for *Scaptotrigona tubiba* after exposure to the pyrethroid deltamethrin were 0.70 ppm (Moraes, Batista, & Viana, 2000) and *Scaptotrigona bipunctata* showed an LD_{50} of 2.72×10^{-8} g a.i. L^{-1} (Pereira, Diniz, & Ruvolo-Takasusuki, 2021) after orally contaminated with the pyrethroid cypermethrin. Thus, it can be concluded that this species has a certain degree of tolerance to the insecticide Fastac Duo because although it is an agrochemical of synergistic effect, the LC_{50} was higher when compared to the common insecticides.

Neonicotinoids act as nicotinic acetylcholine receptor agonists (nAChR) (Pacífico-da-Silva, Melo, & Soto-Blanco, 2016). This binding is persistent since neonicotinoids are insensitive to the acetylcholinesterase enzyme and the activation of acetylcholine receptors is prolonged, causing hyperexcitability of the central nervous system of insects due to continuous and uncontrolled transmission of nerve impulses. Symptoms resulting from intoxication include tremors, seizures (eventually central nervous system collapse) and death (Faria, 2009).

On the other hand, pyrethroid insecticides act on sodium channels, interfering in their opening and closing, prolonging the entry time of Na^+ ions into the cell. At high concentrations, pyrethroids bind to the inotropic receptor complex of γ-aminobutyric acid (GABA) blocking chlorine channels and their activation, which leads to hyperexcitability of the insects' central nervous system (Santos et al., 2007).

Sublethal effects may induce behavioural changes in insects (Rossi, Roat, Tavares, Cintra-Socolowski, & Malaspina, 2013). Tison et al. (2016) studied chronic exposure to sublethal doses of thiacloprid in *A. mellifera* and found that this neonicotinoid caused foraging impairment, difficult to return to the colony, harmed navigation performance and bees communication. Moreover, thiacloprid residue levels increased on both foragers and nestmates over time.

Thany et al. (2015) studied the proboscis retraction of *A. mellifera* and showed that acetamiprid (neonicotinoid) impaired movement in the tested doses, interfering in their feeding behavior. Also, according to Freitas and Pinheiro (2010), the use of pyrethroids at recommended levels appear to affect the ability of honeybees to return to the colony.

Besides, Catae, Roat, Oliveira, Nocelli, and Malaspina (2014), verified that after thiamethoxam ingestion there was significant damage to the bee organism, affecting the distribution of specific proteins in the brain, indicating changes in oxygen supply, synapses, neuronal degeneration, which may explain deficiencies in learning and memory processes and that can compromise the behavior of individuals and colony health.

Oral contamination of insects at sublethal doses of agrochemicals can harm their organs (Catae et al., 2014; Diniz et al., 2020b). Catae et al. (2014) investigated the effects of the neonicotinoid thiamethoxam on the midgut and Malpighi tubule of *A. mellifera* and the results indicated that the insecticide is cytotoxic to bees. In the midgut, the damage was more evident in bees exposed to the insecticide on the first day. On the eighth day, the cells were ultrastructurally intact, suggesting its recovery. Malpighi's tubules showed severe alterations on the eighth day of exposure.

Diniz et al. (2020b) analyzed exposure of *Scaptotrigona bipunctata* with the insecticide Fastac Duo, showing several alterations in their midgut in all experimental periods, such as loosening of the circular and longitudinal musculature, disorganization of the epithelium, degradation of the peritrophic membrane and detachment of the epithelium of the basal lamina, in addition to the absence of regeneration nests, which indicate the degenerative effects of this agrochemical.

The action of agrochemicals on bees is considered adverse and may reflect on various aspects. Some responses occur through physiological and morphological changes, such as changes in the musculature, epithelium and peritrophic membrane, which results in harmful effects on survival and behavior, impairing foraging and compromising the colony survival (Roat et al., 2013).

Conclusion

Although the statistical results were presented under the Bayesian approach in dose-response studies with *Scaptotrigona bipunctata*, such application can be extended to other insect species. In the literature, studies using this species exposed Fastac Duo are scarce thus informational priors were not available. Therefore, new research must be carried out to include such information in future analyzes. However, the results found are informative to alert the high levels of agrochemicals that are being applied, without taking into account the environmental damage it can cause.

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References

Agência de Defesa Agropecuária do Paraná [Adapar]. (2021). Retrieved from http://www.adapar.pr.gov.br/

Arena, M., & Sgolastra, F. (2014). A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology, 23*(1), 324-334. DOI: http://dx.doi.org/10.1007/s10646-014-1190-1

Barbosa, D. B., Crupinski, E. F., Silveira, R. N., & Limberger, D. C. H. (2017). As abelhas e seu serviço ecossistêmico de polinização. *Revista Eletrônica Científica da UERGS, 3*(4), 694-703. DOI: http://dx.doi.org/10.21674/2448-0479.34.694-703

Betancourt, M. (2017). A conceptual introduction to Hamiltonian Monte Carlo. *arXiv: Methodology, 1*(1), 1-60. Retrieved from https://arxiv.org/pdf/1701.02434.pdf

Bridi, R., Larena, A., Pizarro, P. N., Giordano, A., & Montenegro, G. (2018). LC-MS/MS analysis of neonicotinoid insecticides: residue findings in chilean honeys. *Ciência e Agroecologia, 42*(1), 51-57. DOI: http://dx.doi.org/10.1590/1413-70542018421021117

Camargo, J. M. F., & Pedro, S. R. M. (2013). Meliponini Lepeletier, 1836. In J. S. Moure, D. Urban, & Melo, G. A. R. (Orgs), *Catalogue of bees (Hymenoptera, Apoidea) in the neotropical region*. Retrieved from http://www.moure.cria.org.br/catalogue

Catae, A. F., Roat, T. C., Oliveira, R. A., Nocelli, R. C. F., & Malaspina, O. (2014). Cytotoxic effects of thiamethoxam in the midgut and malpighian tubules of Africanized *Apis mellifera* (Hymenoptera: Apidae). *Microscopy Research & Technique, 77*(4), 274-281. DOI: http://dx.doi.org/10.1002/jemt.22339

Chen, M-H., & Shao, Q-M. (1999). Monte Carlo estimation of Bayesian credible and HPD intervals. *Journal of Computational and Graphical Statistics, 8*(1), 69-92. DOI: http://dx.doi.org/10.2307/1390921

Chuttonh, B., Chanbang, Y., Sringarm, K., & Burget, M. (2016). Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). *Food Chemistry, 192*(1), 149-155. DOI: http://dx.doi.org/10.1016/j.foodchem.2015.06.089

Costa, L. M., Grella, T. C., Barbosa, R. A., Malaspina, O., & Nocelli, R. C. F. (2015). Determination of acute lethal doses (LD50 and LC50) of imidacloprid for the native bee *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae). *Sociobiology, 62*(4), 578-582. DOI: http://dx.doi.org/10.13102/sociobiology.v62i4.792

Diniz, T. O., Pereira, N. C., Pizzaia, W. C. S., Sinopolis-Gigliolli, A. A., Silva, B. G., Borges, Y. M., & Ruvolo-Takasusuki, M. C. C. (2020a). Toxicity and genetic analysis of bees *Scaptotrigona bipunctata* after contamination with insecticide accephate. *Scientific Electronic Archives, 13*(8), 8-17. DOI: http://dx.doi.org/10.36560/ 13820201157

Diniz, T.O., Pereira, N. C., Silva, B. G., Pizzaia, W. C. S., Oliveira, F. G. M., Sinópolis-Gigliolli, A. A., & Ruvolo-Takasusuki, M. C. C. (2020b). Toxicity and effects of combined agrochemical in *Scaptotrigona bipunctata* bees. *Scientific Electronic Archives, 13*(12), 41-53. DOI: http://dx.doi.org/10.36560/131220201258

Dively, G. P., & Kamel, A. (2012). Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. *Journal of Agricultural and Food Chemistry, 60*(18), 4449-4456. DOI: http://dx.doi.org/10.1021/jf205393x

Dorneles, A. L., Souza Rosa, A., & Blochtein, B. (2017). Toxicity of organophosphorus pesticides to the stingless bees *Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*. *Apidologie, 48*(5), 612-620. DOI: http://dx.doi.org/10.1007/s13592-017-0502-x

Faria, A. B. C. (2006). Revisão sobre alguns grupos de inseticidas utilizados no manejo integrado de pragas florestais. *Ambiência, 5*(2), 345-357.

Freitas, B. M., & Pinheiro, J. N. (2010). Efeitos sub-letais dos pesticidas agrícolas e seus impactos no manejo de polinizadores dos agroecossistemas brasileiros. *Oecologia Australis, 14*(1), 282-298. DOI: http://dx.doi.org/10.4257/oeco.2010.1401.17

Giolo, S. R. (2017). *Introdução à análise de dados categóricos com aplicações*. São Paulo, SP: Blucher.

Hennessey, V. G., Rosner, G. L., Bast Jr, R. C., & Chen, M. Y. (2010). A bayesian approach to dose–response assessment and synergy and its application to in vitro dose–response studies. *Biometrics, 66*(4), 1275-1283. DOI: http://dx.doi.org/10.1111/j.1541-0420.2010.01403.x

Hoffman, M. D., & Gelman, A. (2014). The No-U-Turn sampler: adaptively setting path lengths in Hamiltonian Monte Carlo. *Journal of Machine Learning Research, 15*(1), 1593-1623.

Jacob, C. R. O, Zanardi, O. Z., Malaquias, J. B., Silva, C. A. S., & Yamamoto, P. T. (2019). The impact of four widely used neonicotinoid insecticides on *Tetragonisca angustula* (Latreille) (Hymenoptera: Apidae). *Chemosphere*, *224*(1), 65-70. DOI: http://dx.doi.org/10.1016/j.chemosphere.2019.02.105

Lee, P. (2013). *Bayesian statistics – an introduction*. Hoboken, N.J.: Wiley.

McCullagh, P., & Nelder, J. A. (1989). *Generalized linear models*. London, UK: Chapman and Hall.

Moraes, S. S., Batista, A. R., & Viana, B. F. (2000). Avaliação da toxicidade aguda (DL50 e CL50) de inseticidas para *Scaptotrigona tubiba* (Smith) (Hymenoptera: Apidae): via de contato. *Anais da Sociedade Entomológica do Brasil, 29*(1), 31-37. DOI: http://dx.doi.org/10.1590/S0301-80592000000100004

Moreira, D. R., Sinópolis Gigliolli, A. A., Falco, J. R. P., Julio, A. H. F., Volnistem, E. A., Chagas, F. D., & Ruvolo-Takasusuki, M. C. C. (2018). Toxicity and effects of the neonicotinoid thiamethoxam on *Scaptotrigona bipunctata* Lepeletier, 1836 (Hymenoptera: Apidae). *Environmental Toxicology*, *33*(4), 463-475. DOI: http://dx.doi.org/10.1002/tox.22533

Organisation for Economic Cooperation and Development [OECD]. (1998). *Test No. 213: honeybees, acute oral toxicity test, OECD guidelines for the testing of chemicals*, *Section 2*. Paris, FR: OECD Publishing. DOI: http://dx.doi.org/10.1787/9789264070165-en

O'Hagan, A. (2004). Bayesian statistics: principles and benefits. In R. J. Bogers (Ed.), *Wageningen UR Frontis Series 3* (p. 31-45). [S.l.]: Springer Science; Business Media.

Pacífico-da-Silva, I., Melo, M. M., & Soto-Blanco, B. (2016). Efeitos tóxicos dos praguicidas para abelhas. *Revista Brasileira de Higiene e Sanidade Animal, 10*(1), 142-157. DOI: http://dx.doi.org/10.5935/1981-2965.20160013

Pereira, N. C., Diniz, T. O., & Ruvolo-Takasusuki, M. C. C. (2021). Toxicity and genetic analysis of *Scaptotrigona bipunctata* Lepeletier, 1836 contaminated with the pyrethroid cypermethrin. *Scientific Electronic Archives*, *14*(1), 59-66. DOI: http://dx.doi.org/10.36560/14120211254

R Core Team. (2020). *R: a language and environment for statistical computing*. Vienna, AT: R Foundation for Statistical Computing.

Ramírez, V. M., Ayala, R., & González, H. D. (2018). Crop pollination by stingless bees. In Pot-pollen in stingless bee melittology. In P. Vit, S. Pedro, & D. Roubik (Eds.), *Pot-pollen in stingless bee melittology* (p. 139-153). Cham, CH: Springer.

Roat, T. C., Carvalho, S. M., Nocelli, R. C. F., Silva-Zacarin, E. C. M., Oalma, M. S., & Malaspina, O. (2013). Effects of sublethal dose of fipronil on neuron metabolic activity of Africanized honeybees. *Archives of Environmental Contamination and Toxicology, 64*(1), 456-466. DOI: http://dx.doi.org/10.1007/s00244-012-9849-1

Rosa-Fontana, A., Dorigo, A. S., Galaschi-Teixeira, J. S., Nocelli, R. C., & Malaspina, O. (2020). What is the most suitable native bee species from the Neotropical region to be proposed as model-organism for toxicity tests during the larval phase? *Environmental Pollution, 265*(Part B), 114849. DOI: http://dx.doi.org/10.1016/j.envpol.2020.114849

Rossi, C. A., Roat, T. C., Tavares, D. A., Cintra-Socolowski, P., & Malaspina, O. (2013). Effects of sublethal doses of imidacloprid in malpighian tubules of africanized *Apis mellifera* (Hymenoptera, Apidae). *Microscopy Research and Technique, 76*(5), 552-558. DOI: http://dx.doi.org/10.1002/jemt.22199

Sanchez-Bayo, F., & Goka, K. (2014). Pesticide residues and bees–a risk assessment. *PLoS ONE, 9*(4), e-94482. DOI: http://dx.doi.org/10.1371/journal.pone.0094482

Santos, M. D., Areas, M. A., & Reyes, F. G. R. (2007). Piretróides - uma visão geral. *Alimentos e Nutrição Araraquara*, *18*(3), 339-349

Shipe, M. E., Deppen, S. A., Farjah, F., & Grogan, E. L. (2019). Developing prediction models for clinical use using logistic regression: an overview. *Journal of Thoracic Disease, 11*(Suppl 4), S574-S584. DOI: http://dx.doi.org/10.21037/jtd.2019.01.25

Smith, B. J. (2007). Boa: an R package for MCMC output convergence assessment and posterior inference. *Journal of Statistical Software, 21*(11), 1-37. DOI: http://dx.doi.org/10.18637/jss.v021.i11

Soares, H. M., Jacob, C. R. O, Carvalho, S. M., Nocelli, R. C., & Malaspina, O. (2015). Toxicity of imidacloprid to the stingless bee *Scaptotrigona postica* Latreille,1807 (Hymenoptera: Apidae). *Bulletin of Environmental Contamination and Toxicology, 94*(6), 675-680. DOI: http://dx.doi.org/10.1007/s00128-015-1488-6

Sosa-Gómez, D. R., & Omoto, C. (2012). Resistência a inseticidas e outros agentes de controle em artrópodes associados à cultura da soja. In C. B. Hoffmann-Campo, B. S. Corrêa-Ferreira, & F. Moscardi (Eds.), *Soja - manejo integrado de insetos e outros artrópodes-praga* (p. 673-723). Brasília, DF: Embrapa. Retrieved from http://www.cnpso.embrapa.br/artropodes/Capitulo10.pdf

Stan Development Team (2020). *RStan: the R interface to Stan. R package version 2.21.2*. Retrieved from http://mc-stan.org/

Tison, L., Hahn, M. L., Holtz, S., Röβner, A., Greggers, U., Bischoff, G., & Menzel, R. (2016). Honey bees' behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. *Environmental Science & Technology, 50*(13), 7218-7227. DOI: http://dx.doi.org/10.1021/acs.est.6b02658

Thany, S. H., Bourdin, C. M., Graton, J., Laurent, A. D., Mathé-Allainmat, M., Lebreton, J., & Questel, J. Y. L. (2015). Similar comparative low and high doses of deltamethrin and acetamiprid differently impair the retrieval of the proboscis extension reflex in the forager honey bee (*Apis mellifera*). *Insects, 6*(4), 805-814. DOI: http://dx.doi.org/10.3390/insects6040805

Tomé, H. V. V., Martins, G. F., Lima, M. A. P., Campos, L. A. O., & Guedes, R. N. C. (2012). Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee *Melipona quadrifasciata anthidioides*. *PLoS ONE, 7*(6), e-38406. DOI: http://dx.doi.org/10.1371/journal.pone.0038406

Wickham, H. (2016). *Ggplot2: elegant graphics for data analysis*. Cham, CH: Springer International Publishing.