Supplementary information

Exploring honey bee toxicological data as a proxy for assessing dimethoate sensitivity in stingless bees

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Supplementary Table 1: Estimation of the 24h-LD_{50} of dimethoate in eusocial bees considering oral and contact exposure, before and after adjusting for the bees' body weight

Species	Exposure	LD_{50} (μg/bee)	Safecty factor of 10x	Weight (mg)	LD_{50} (μg/mg of bee)	Safecty factor of 10x
Apis mellifera (Linnaeus, 1758)	Oral	0.04450	0.00445	74.1	0.00060	0.000060
	Contact	0.04054	0.00405		0.00055	0.000055
Cephalotrigona capitata (Smith, 1854)	Contact	0.05058		38.7	0.00131	
Friesella schrottkyi (Friese, 1900) *	Contact	0.00243		3.40	0.00071	
Friseomelitta varia (Lepeletier, 1836)	Oral	0.04223		10.8	0.00391	
	Contact	0.01961			0.00182	
Melipona mondury (Smith, 1863)	Oral	0.02601		75.1	0.00035	
	Contact	0.00729			0.00010	
Melipona quadrifasciata (Lepeletier, 1836)	Oral	0.03421		93.8	0.00036	
	Contact	0.04095			0.00044	
Melipona scutellaris (Latreille, 1811)	Oral	0.06432		87.2	0.00074	
	Contact	0.06823			0.00078	
Nannotrigona testaceicornis * (Lepeletier, 1836)	Oral	0.00388		6.20	0.00063	
	Contact	0.00249			0.00040	
Partamona helleri (Friese, 1900)	Oral	0.03853		17.6	0.00219	
	Contact	0.01273			0.00072	
Plebeia droryana (Friese, 1900) *	Oral	0.00170		4,98	0.00034	
Scaptotrigona bipunctata (Lepeletier, 1836)	Oral	0.03621		18.4	0.00197	
	Contact	0.03996			0.00217	
Scaptotrigona xanthotricha (Moure, 1950)	Oral	0.00877		20.5	0.00043	
	Contact	0.01945			0.00095	
Tetragona clavipes (Fabricius, 1804)	Oral	0.02717		15.3	0.00178	
	Contact	0.01165			0.00076	
Tetragonisca angustula (Latreille, 1811) *	Oral	0.00356		3.10	0.00113	
	Contact	0.00200			0.00064	
Trigona hyalinata (Lepeletier, 1836)	Oral	0.02637		25.2	0.00105	
	Contact	0.01893			0.00075	
Trigona spinipes (Fabricius, 1793)	Oral	0.03765		21.1	0.00178	
	Contact	0.02936			0.00139	

* Species not protected by the safety factor

Supplementary Table 2: General characteristics of the assays performed for 16 species of eusocial bees exposed to dimethoate

Species	Exposure temperature	Volume of insecticide solution (µL-contact exposure)	Body weight (mg)	Number of colonies	Origin of bees	Location	Category
Apis mellifera	34°C [1]	2	74.1	3	Rational boxes	Apiary	Foragers
Cephalotrigona capitata	28°C	1	38.7	1	Rational boxes	Apiary	Nurses and foragers
Friesella schrottkyi	25°C [2]	0.5	3.40	4	Rational boxes	Apiary	Nurses and foragers
Friseomelitta varia	30°C [3]	1	10.8	3	Rational boxes	Apiary	Foragers
Melipona mondury	28°C [4]	2	75.1	3	Rational boxes	Apiary and Mata do Paraíso (forest)	Foragers
Melipona quadrifasciata *	28°C [5]	2	93.8	3	Rational boxes	Coimbra e Apiário	Foragers
Melipona scutellaris *	28°C [6]	2	4.98	3	Rational boxes	Coimbra	Foragers
Nannotrigona testaceicornis * †	28°C [7]	0.5	6.16	1	Swarm	Campus – UFV	Drones
Partamona helleri	28°C [8]	1	17.6	3	Colonies collected in the nature	Apiary	Foragers
Plebeia droryana	28°C [9]	0.5	4.98	4	Rational boxes	Apiary	Nurses and foragers
Scaptotrigona bipunctata	28°C [10]	1	18.4	1	Rational boxes	Apiary	Foragers
Scaptotrigona xanthotricha	29°C [11]	1	20.5	3	Rational boxes and tree trunk	Apiary and campus	Foragers

Tetragona clavipes	28°C [12]	1	15.3	3	Rational boxes and tree trunks	Apiary	Foragers
Tetragonisca angustula *	25°C [13]	0.5	3.14	3	Rational boxes	Coimbra and apiary	Primarily Foragers
Trigona hyalinata	28°C [14]	0.5	25.2	1	Colony collected in the nature	Apiary	Foragers
Trigona spinipes *	28°C	1	21.1	3	Colonies collected in urban area of Viçosa	Campus - UFV and Coimbra	Foragers

* Species on IBAMA's list as priorities for pesticide risk analyzes in Brazil (Pires et al., 2018).

† Collected from unknown number of colonies during swarming.

Supplementary Methods

S1. Collection of bees

Honey bee workers were collected from three different queen-right colonies. Stingless bees were collected from three colonies, except for *P. droryana* and *F. schrottkyi*, collected from four colonies; and for *C. capitata*, *S. bipunctata*, and *T. hyalinata*, collected from one colony. Collecting these last three species was challenging, either due to the difficulty in locating their colonies, often situated in hard-to-access places, or because the colonies are not easily found in the Viçosa region. Due to limitations in the availability of workers, *P. droryana* (colonies with relatively few workers) were exclusively used in the oral exposure assessment, whereas *F. schrottkyi* and *C. capitata* was exclusively used in the contact exposure assessment. In the last case, the quantity of available workers was also small because there was only one colony available.

For species not inclined to exit their colonies after being stimulated to leave, various strategies were implemented according to the species. For *T. angustula*, the upper lid of the rational boxes was removed, and a transparent plastic bag (10 L) was affixed to the opening, guiding, and facilitating the collection of the bees. Upon opening the top of the rational boxes, a significant

number of *T. angustula* workers flew into the bag, ensuring a sample rich in foragers. *Nannotrigona testaceicornis* was captured using a butterfly insect net, while *C. capitata* was manually collected directly from the open box using tweezers. *Plebeia droryana* and *F. schrottkyi* were collected using an insect aspirator (Ecology Supplies, Glen Cove, NY, US). For *C. capitata*, *P. droryana*, and *F. schrottikyi*, workers of different casts (nurses and foragers) were collected. In the case of *N. testaceicornis*, the challenge arises from workers concealing themselves within colonies and the presence of a rigid involucrum, complicating in-colony collection. Males of *N. testaceicornis* were collected in a swarm close to a colony entrance in the campus of Universidade Federal de Viçosa (UFV) (Table 1S). Due to the swarm's mix of drones, determining the exact number of colonies they originated from was not feasible.

After collection, the bees were transported to the insectary of the Insect Molecular Biology Laboratory at UFV and anesthetized with carbon dioxide (CO_2). The exception was *C. capitata*, which was transferred one by one directly from the opened colony to the plastic pots without being anesthetized. Anesthetized bees were transferred to plastic trays, delicately removed one by one with the aid of tweezers and grouped into 500 mL plastic pots (for bees with a body weight greater than 70 mg) or 250 mL pots (for body weight below 70 mg). Each plastic pot contained 10 bees from the same colony, and each pot was considered one replicate. The plastic pots were prepared in advance[8] and placed in Biochemical Oxygen Demand (B.O.D.) incubators with controlled temperature adjusted according to the species (Table 1S), in complete darkness. These pots were transparent, featuring a bottom lined with filter paper and walls scratched with sandpaper to allow the bees to walk on their walls. Following the transfer of the bees, the pots were sealed with transparent plastic lids, perforated to facilitate air exchange.

Supplementary Table 3: Doses of the insecticide dimethoate used in oral and contact exposure. The doses are expressed in µg active ingredient (a.i.)/bee

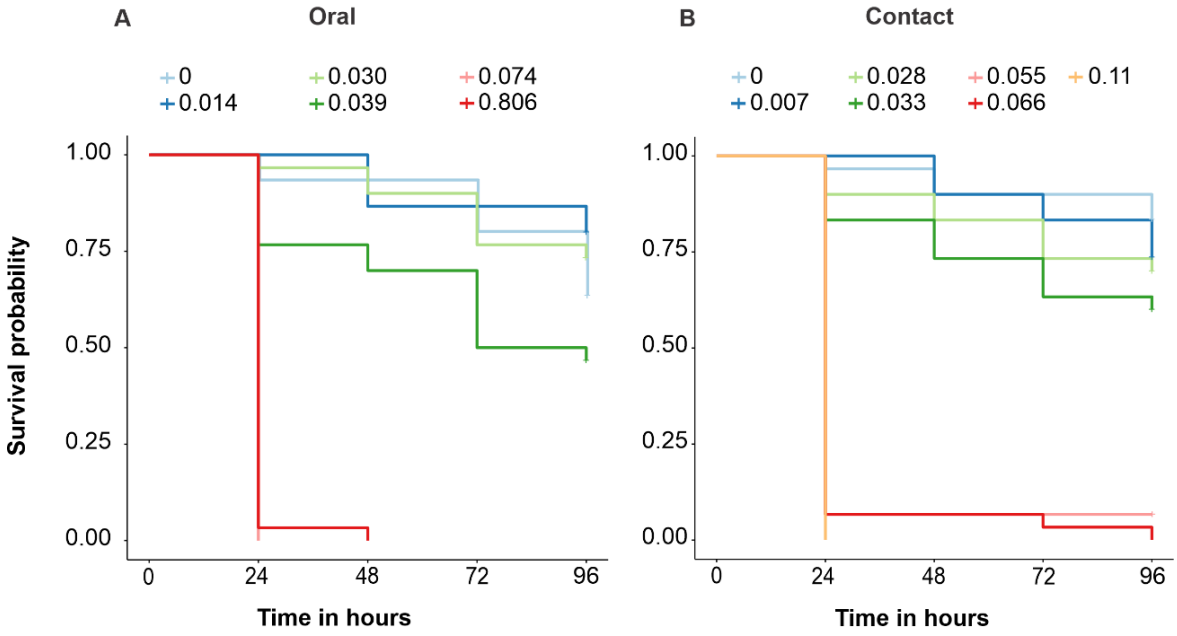
Species	Exposure	Doses
Apis mellifera	Oral	0.8059; 0.0743; 0.0393; 0.0304; 0.0140
	Contact	0.1100; 0.0660; 0.0550; 0.0330; 0.0275; 0.0068
Cephalotrigona capitata	Contact	0.1000; 0.0550; 0.0275; 0.0138; 0.0069; 0.0034
Friesella schrottkyi	Contact	0.0275; 0.0069; 0.0034; 0.0017
Friseomelitta varia	Oral	0.0694; 0.0599; 0.0456; 0.0100; 0.0068
	Contact	0.0550; 0.0275; 0.0138; 0.0069; 0.0034
Melipona mondury	Oral	0.0647; 0.0357; 0.0250; 0.0127; 0.0033
	Contact	0.0660; 0.0330; 0.0022; 0.0001
Melipona quadrifasciata	Oral	0.2335; 0.1180; 0.0702; 0.0249; 0.0181
	Contact	0.0825; 0.0550; 0.0275; 0.0138; 0.0069; 0.0034
Melipona scutellaris	Oral	0.1390; 0.0940; 0.0710; 0.0310; 0.0160
	Contact	0.1100; 0.0550; 0.0275; 0.0138; 0.0139
Nannotrigona testaceicornis	Oral	0.0654; 0.0319; 0.0040; 0.0013
	Contact	0.0138; 0.0069; 0.0014; 0.0007; 0.0002
Partamona helleri	Oral	0.3937; 0.0336; 0.0105; 0.0026
	Contact	0.1100; 0.0330; 0.0165; 0.0110; 0.0011
Plebeia droryana	Oral	0.0120; 0.0360; 0.0060; 0.0030; 0.0020; 0.0010
Scaptotrigona bipunctata	Oral	0.1429; 0.0517; 0.0267; 0.0137; 0.0047; 0.0042
	Contact	0.1000; 0.0880; 0.0660; 0.0550; 0.0275; 0.0220
Scaptotrigona xanthotricha	Oral	0.0224; 0.0208; 0.0177; 0.0064; 0.0036
	Contact	0.1000; 0.0550; 0.0069; 0.0034
Tetragona clavipes	Oral	0.0910; 0.0824; 0.0337; 0.0203; 0.0109
	Contact	0.1000; 0.0550; 0.0275; 0.0137; 0.0069
Tetragonisca angustula	Oral	0.0140; 0.0040; 0.0020; 0.0010
	Contact	0.0275; 0.0069; 0.0017; 0.0008; 0.0004
Trigona hyalinata	Oral	0.0690; 0.0410; 0.0302; 0.0130; 0.0080; 0.0030
	Contact	0.1000; 0.0550; 0.0275; 0.0137; 0.0069
Trigona spinipes	Oral	0.0845; 0.0488; 0.0235; 0.0160
	Contact	0.1000; 0.0550; 0.0275; 0.0137; 0.0069

Supplementary Table 4: Estimated median lethal concentration in a 24-hour (24h-LC_{50}) of dimethoate through oral exposure and contact exposure

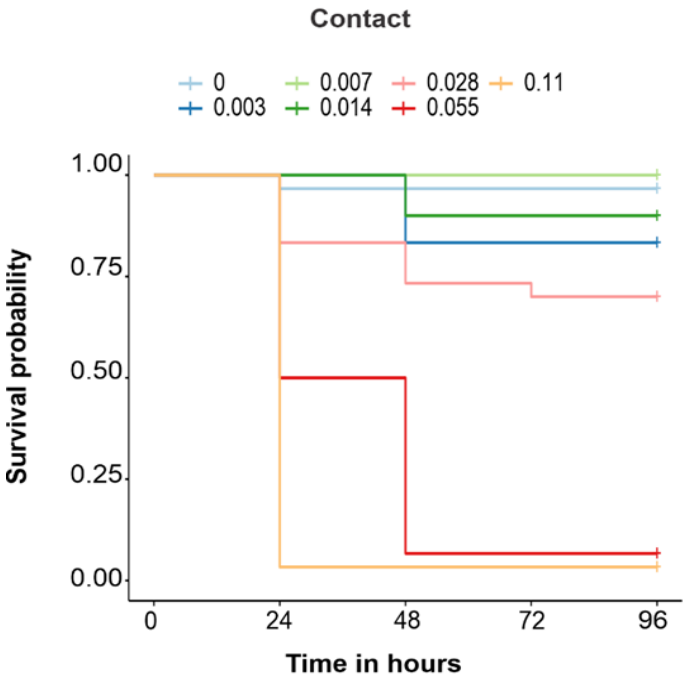
Species	Exposure	24h- LC_{50} µg/µL	χ2	DF	P-value	Slope (±SE)
Apis mellifera	Oral	0.00222 (0.00193 - 0.00261)	5.02	5	0.4	6.6 ± 1.1
	Contact	0.02027 (0.01862 - 0.02208)	2.73	4	0.6	8.5 ± 1
Cephalotrigona capitata	Contact	0.05058 (0.04257 - 0.05941)	4.93	4	0.3	5 ± 0.9
Friesella schrottkyi	Contact	0.00487 (0.00392 - 0.00580)	1.42	2	0.5	4.5 ± 0.9
Friseomelitta varia	Oral	0.00709(0.00555 - 0.00752)	0,00	3	1.0	17.7 ± 6.3
	Contact	0.01961 (0.01633 - 0.02380)	4.58	3	0.2	3.5 ± 0.5
Melipona mondury	Oral	0.0007804 (0.0006797 - 0.0008705)	6.13	2	0.1	6.4 ± 1
	Contact	0.00365 (0.00225 - 0.00559)	0.30	2	0.9	2.1 ± 0.3
Melipona quadrifasciata	Oral	0.00109 (0.0009406 - 0.00127)	1.21	3	0.8	5.8 ± 0.9
	Contact	0.02047 (0.01718 - 0.02424)	2.88	4	0.6	4 ± 0.5
Melipona scutellaris	Oral	0.00151 (0.00133 - 0.00170)	0.01	3	1.0	8.9 ± 1.7
	Contact	0.03411 (0.02958 - 0.03969)	3.89	3	0.3	6.1 ± 1
Nannotrigona testaceicornis	Oral	0.00141 (0.0004155 - 0.00290)	8.49	4	0.1	1.4 ± 0.3
	Contact	0.00497 (0.00374 - 0.00668)	2.81	3	0.4	2.4 ± 0.3
Partamona helleri	Oral	0.00181 (0.00137 - 0.00301)	0.59	2	0.7	2.9 ± 0.7
	Contact	0.01273 (0.01150 - 0.01392)	0.00	3	1.0	9.9 ± 2.1
Plebeia droryana	Oral	0.000815 (0.0003177 - 0.00133)	8.29	4	0.1	2.7 ± 0.6
Scaptotrigona bipunctata	Oral	0.00509 (0.00421 - 0.00623)	5.62	3	0.1	3.3 ± 0.4
	Contact	0.03996 (0.03015 - 0.05027)	8.41	4	0.8	5.5 ± 0.9
Scaptotrigona xanthotricha	Oral	0.00175 (0.00140 - 0.00213)	2.79	4	0.6	3.9 ± 0.7
	Contact	0.01945 (0.01366 - 0.02758)	0.87	2	0.9	4.2 ± 0.7
Tetragona clavipes	Oral	0.00272 (0.00210 - 0.00331)	4.31	3	0.2	3.8 ± 0.7
	Contact	0.01165 (0.00890 - 0.01439)	5.94	3	0.1	3 ± 0.5
Tetragonisca angustula	Oral	0.00170 (0.00136 - 0.00224)	2.10	2	0.4	3 ± 0.5
	Contact	0.00400 (0.00307 - 0.00536)	5.31	3	0.2	2.4 ± 0.3
Trigona hyalinata	Oral	0.00336 (0.00291 - 0.00382)	6.24	4	0.2	7.2 ± 1.3

	Contact	0.01893 (0.01562 - 0.02275)	0.38	3	0.9	3.5 ± 0.5
Trigona spinipes	Oral	0.00631 (0.00541 - 0.00736)	1.28	1	0.3	5.5 ± 1
	Contact	0.02936 (0.02441 - 0.03526)	5.62	3	0.1	3.6 ± 0.5

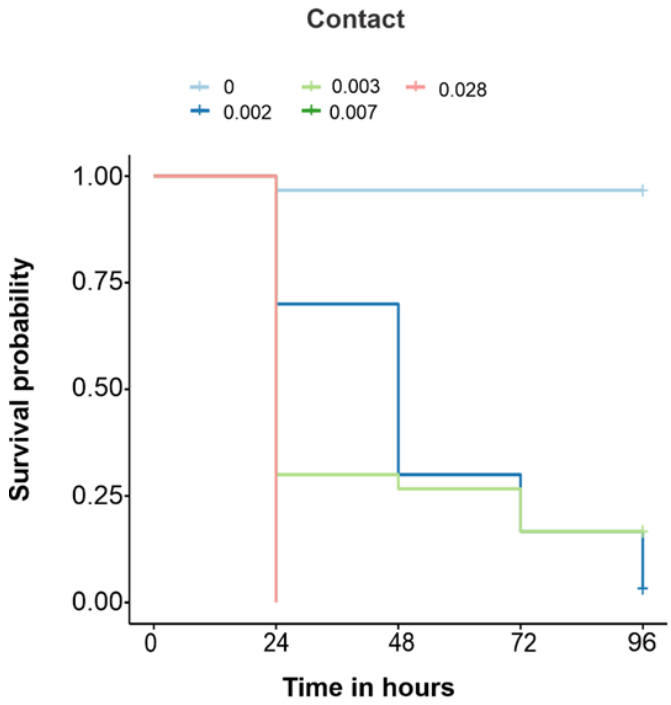
Supplementary Figures 1–16: Survival Curves. Following exposure to the dimethoate, the survival of stingless bees and Africanized *A. mellifera* was monitored daily for 96 hours (24h, 48h, 72h, and 96h) after both oral and contact exposures, in accordance with the Organization for Economic Co-operation and Development (OECD) protocols for acute oral exposure and contact exposure (OECD, 1998a,b). Survival data were subjected to survival analysis using the Kaplan-Meier estimate. The overall similarity between the survival curves was tested using the log-rank $\chi 2$ test ($P < 0.05$). Each color represents a distinct dose expressed in µg a.i./bee. (A) Oral exposure; (B) Contact exposure.



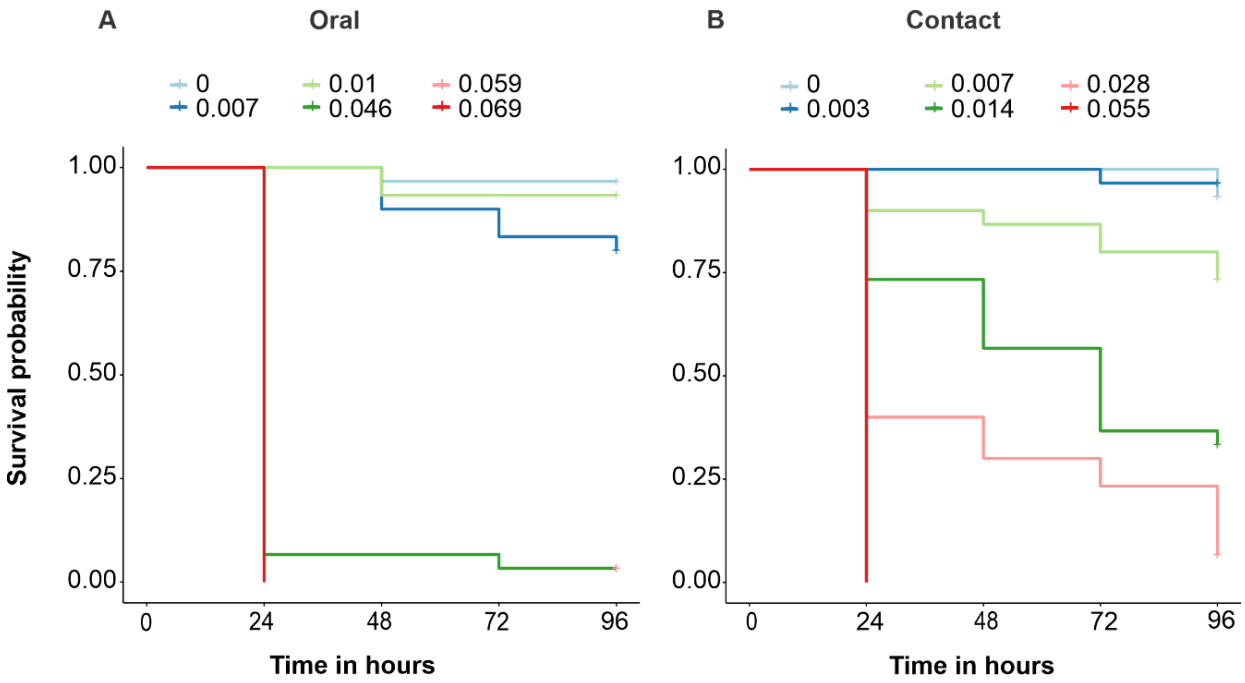
Supplementary Figure 1: Survival of *A. mellifera*.



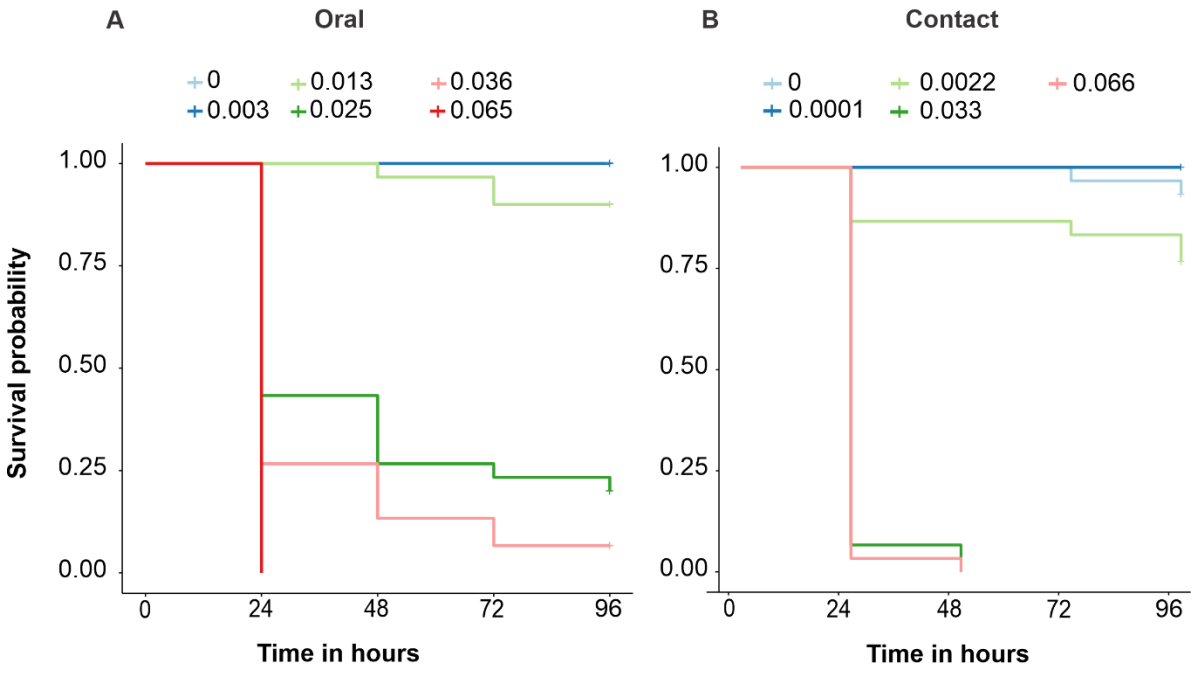
Supplementary Figure 2: Survival of *C. capitata*.



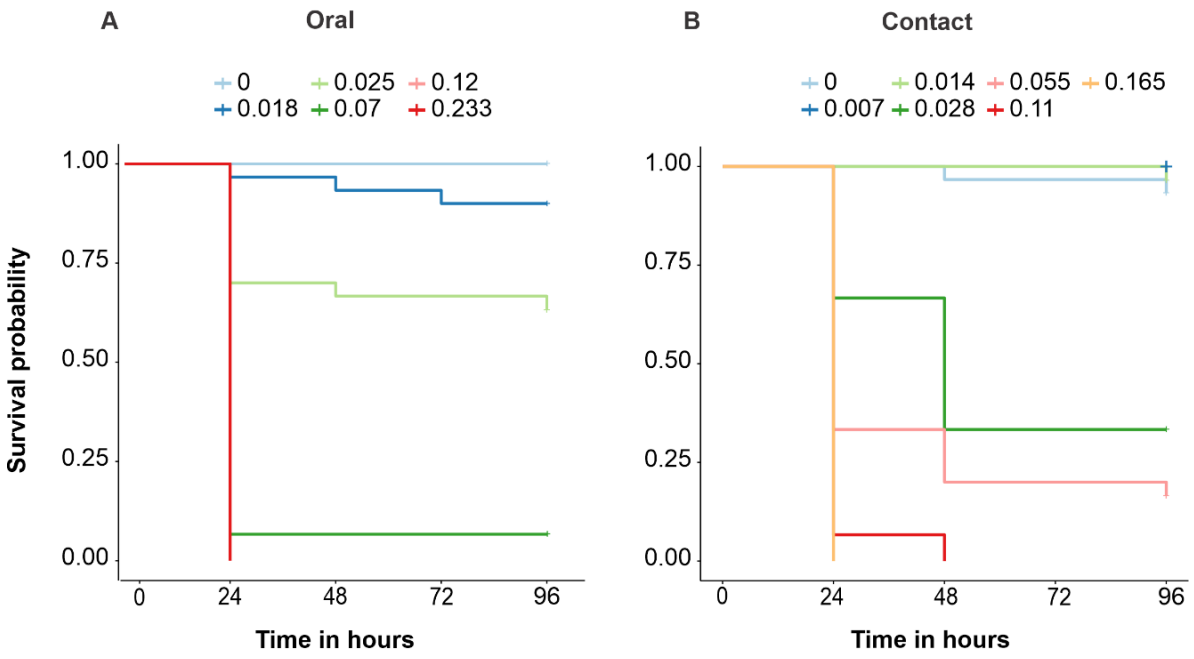
Supplementary Figure 3: Survival of *F. schrottkyi*.



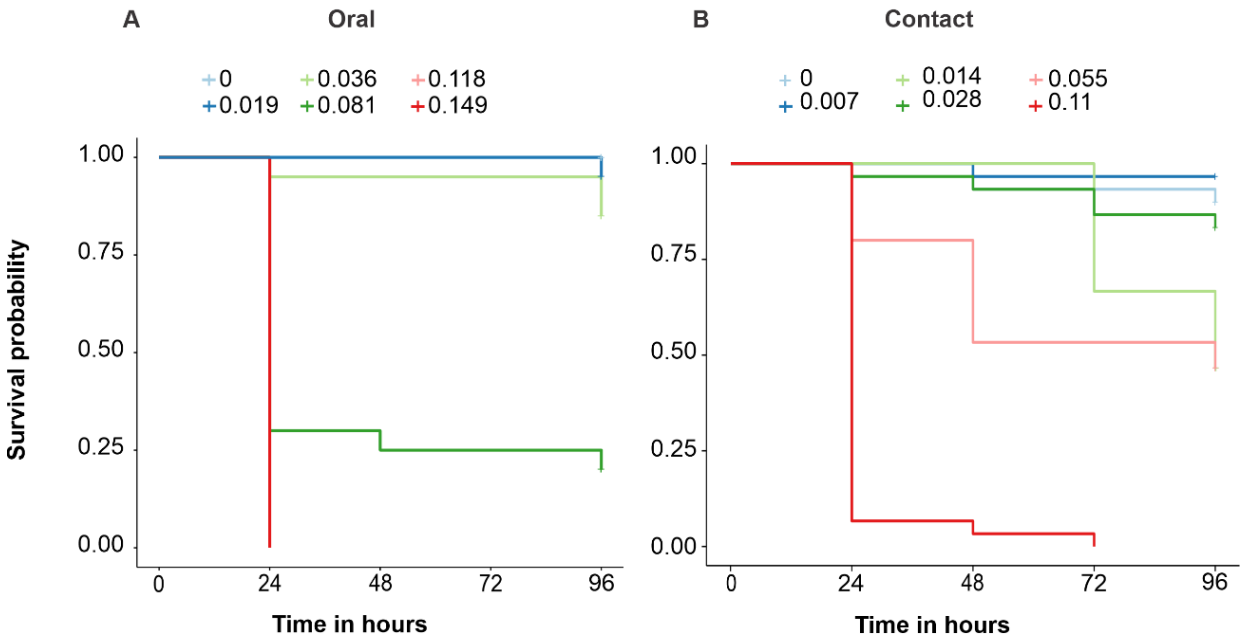
Supplementary Figure 4: Survival of *F. varia*.



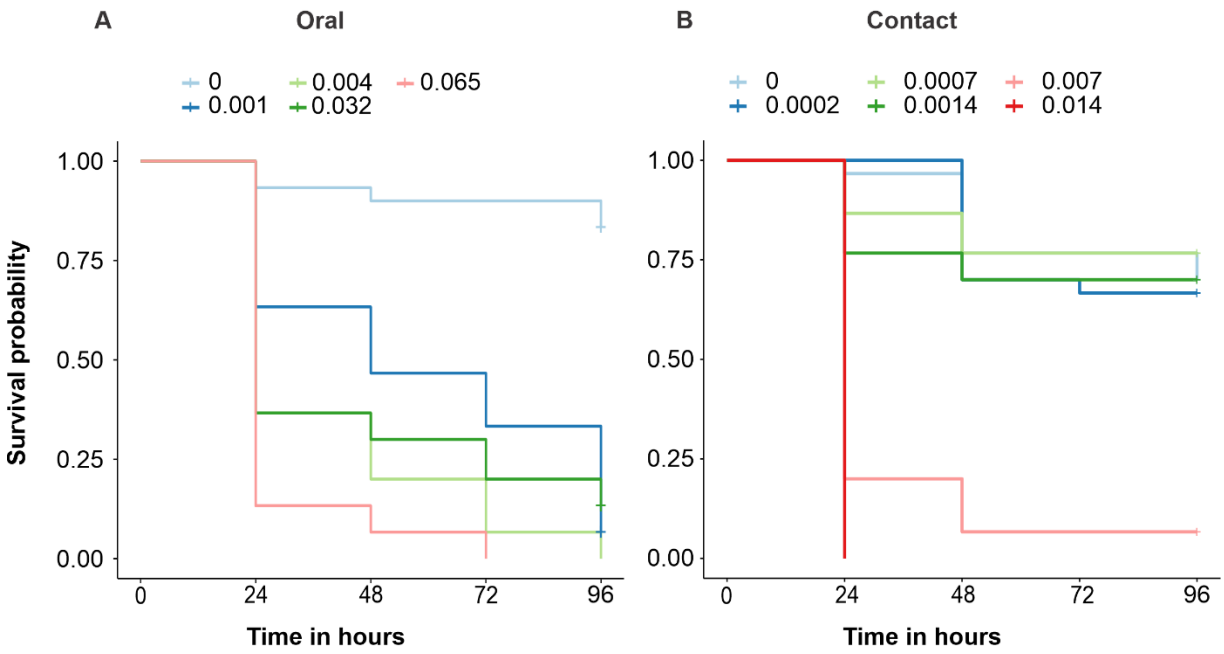
Supplementary Figure 5: Survival of *M. mondury*.



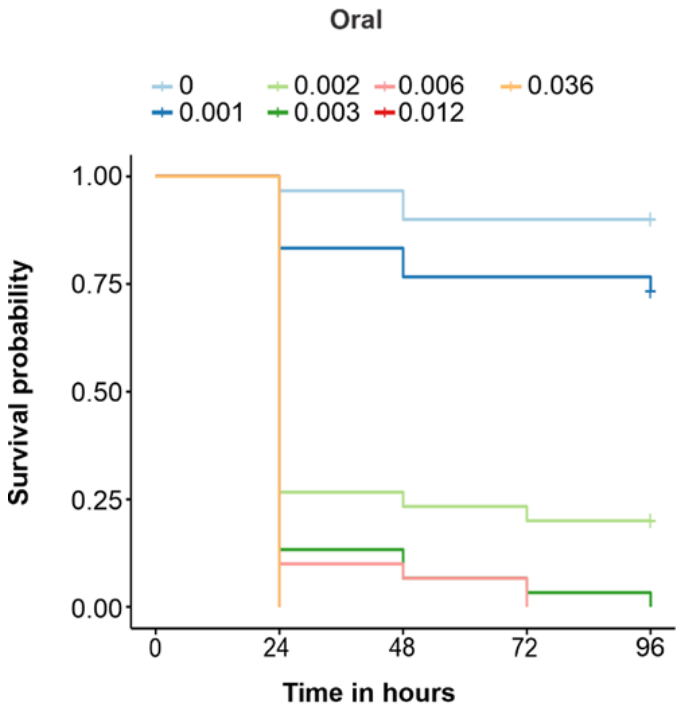
Supplementary Figure 6: Survival of *M. quadrifasciata*.



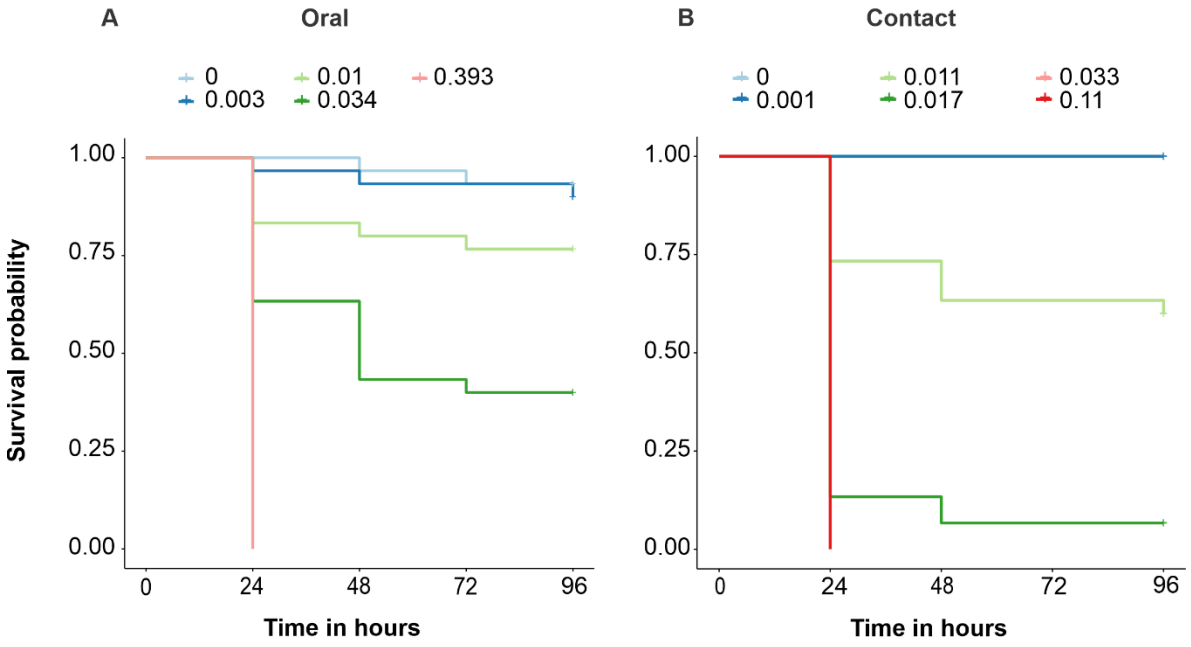
Supplementary Figure 7: Survival of *M. scutellaris*.



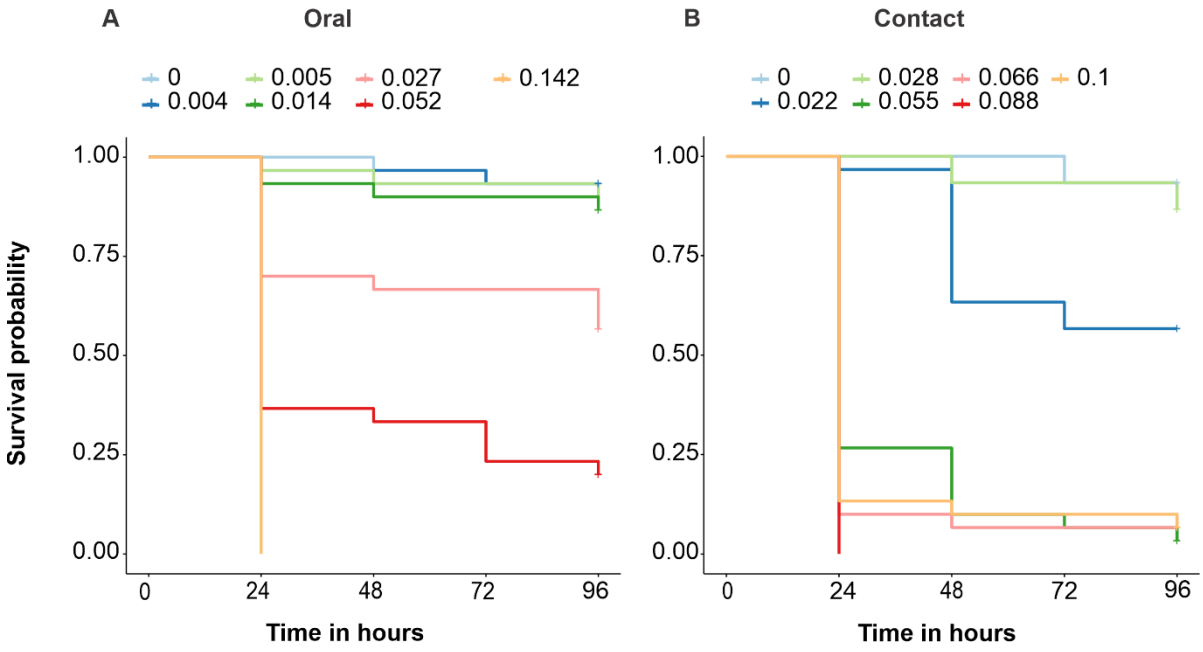
Supplementary Figure 8: Survival of *N. testaceicornis*.



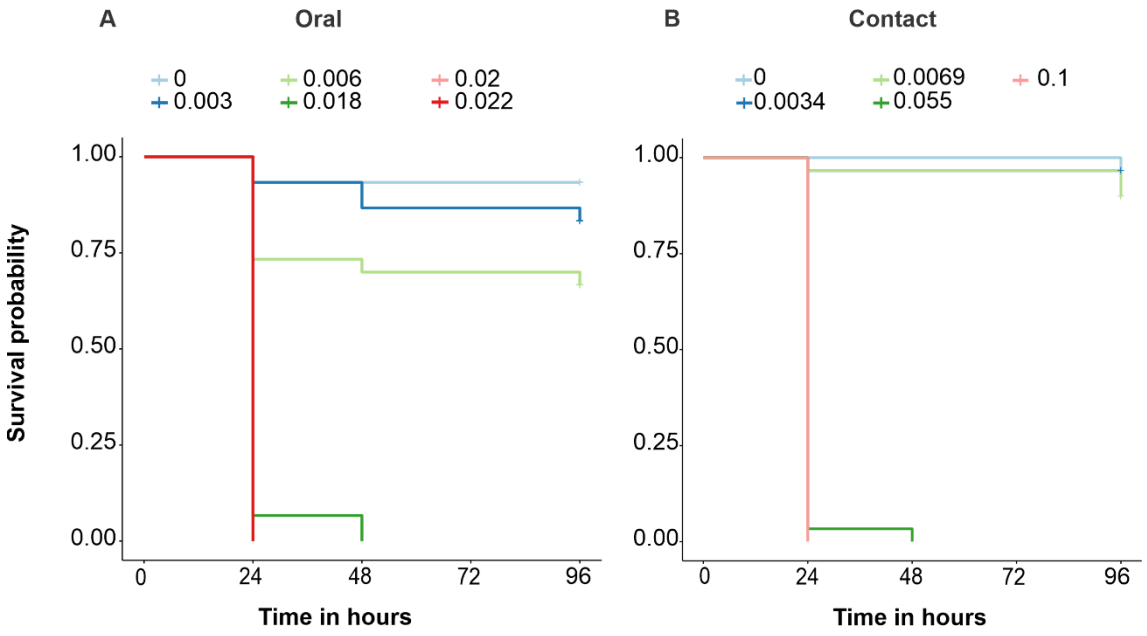
Supplementary Figure 9: Survival of the *P. droryana*.



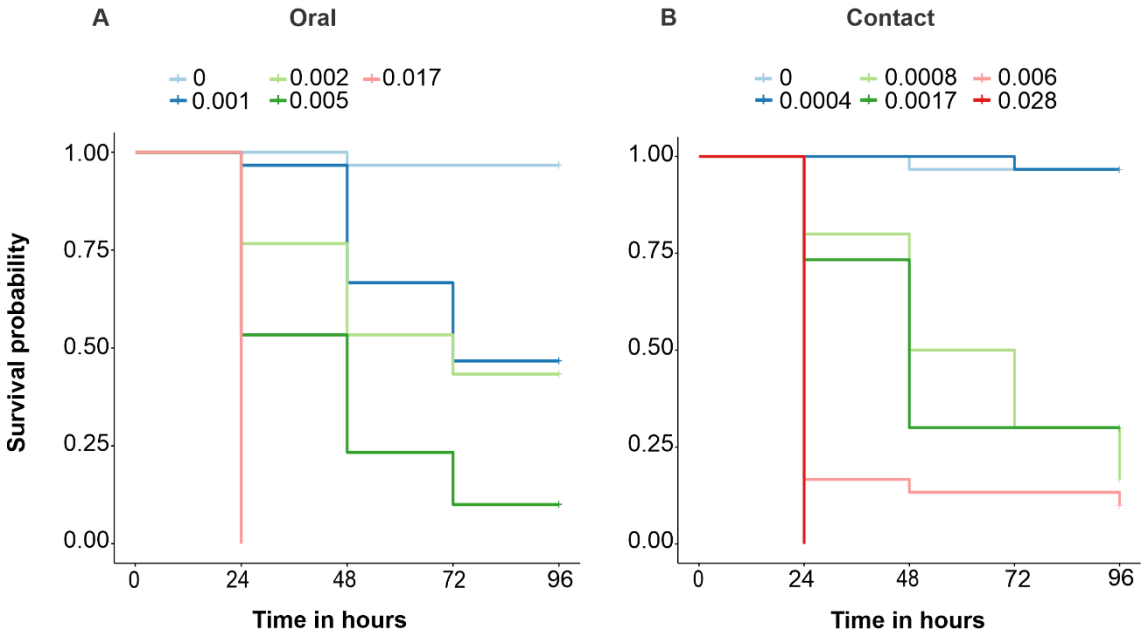
Supplementary Figure 10: Survival of *P. helleri*.



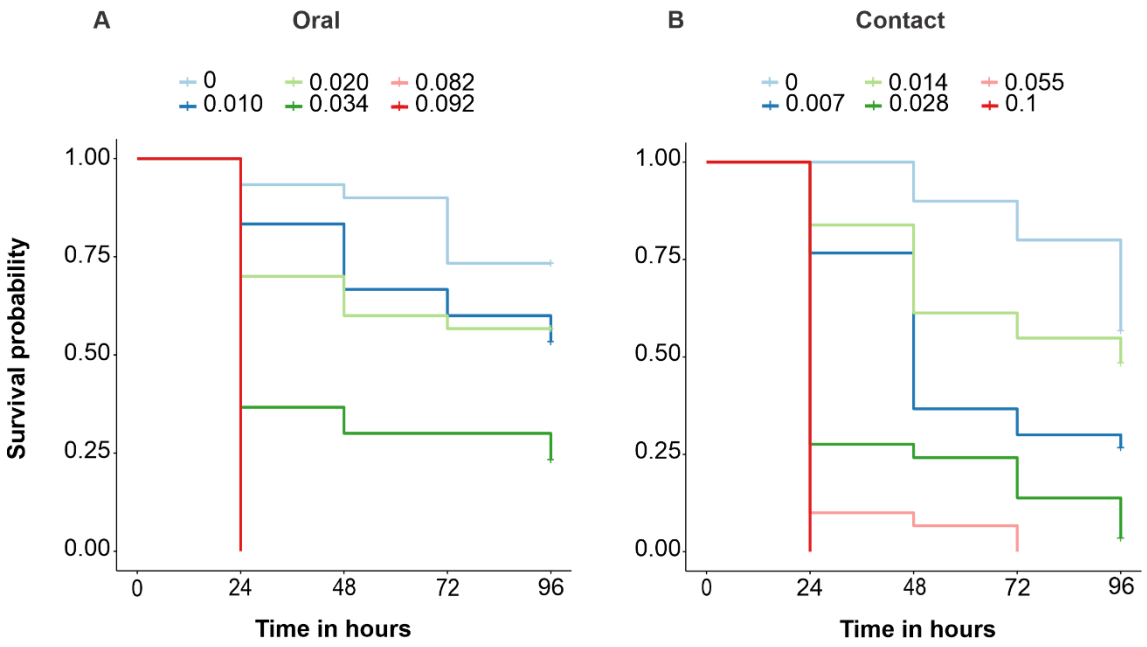
Supplementary Figure 11: Survival of *S. bipunctata*.



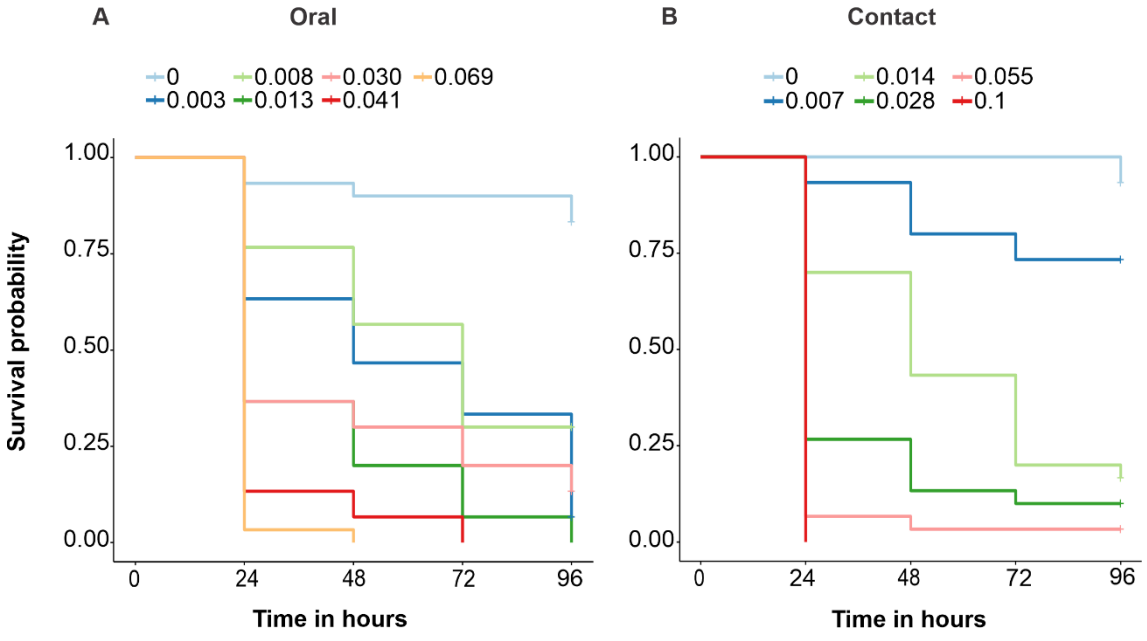
Supplementary Figure 12: Survival of *S. xanthotricha*.



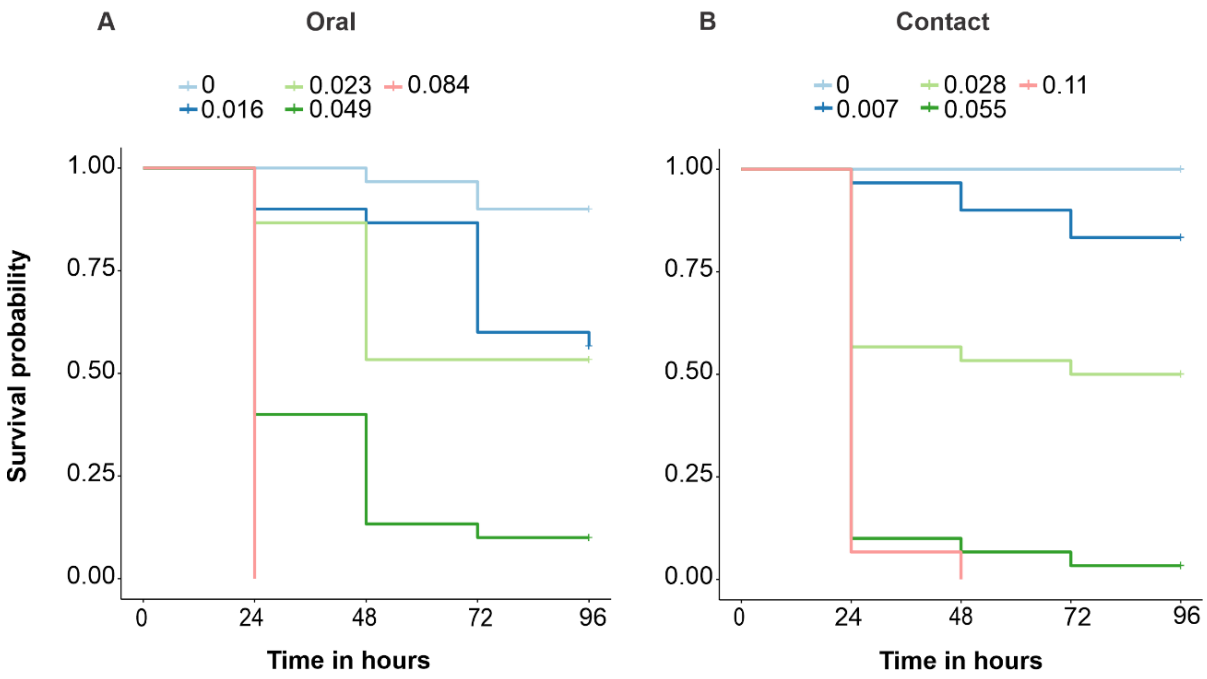
Supplementary Figure 13: Survival of *T. angustula*.



Supplementary Figure 14: Survival of *T. clavipes*.



Supplementary Figure 15: Survival of *T. hyalinata*.



Supplementary Figure 16: Survival of *T. spinipes*.

Supplementary References

1. Jones, J. C. & Oldroyd, B. P. Nest thermoregulation in social insects. *Adv. Insect Physiol.* **33**, 153–191 (2006). 10.1016/S0065-2806(06)33003-2.
2. Brito, P., Zama, U., Dolder, H. & Lino-Neto, J. New characteristics of the male reproductive system in the Meliponini bee, *Friesella schrottkyi* (Hymenoptera: Apidae): histological and physiological development during sexual maturation. *Apidologie* **41**, 203–215 (2010). 10.1051/apido/2009071.
3. da Silva, J. A., Barchuk, A. R. & Wolowski, M. Protocol for the *in vitro* rearing of *Frieseomelitta varia* workers (Hymenoptera: Apidae: Meliponini). *Zoologica* **39** (2022). 10.1590/s1984-4689.v39.e22003.
4. Lima, M. A. P. Aspectos da Biologia de *Melipona rufiventris* Lepeletier*, 1836* e de *Melipona Mondury* Smith, 1863 (Hymenoptera: Apidae, Meliponina), (2004).
5. Del Sarto, M. C. L. Toxicidade de Inseticidas para as abelhas *Melipona quadrifasciata* e *Apis mellifera* (Hymenoptera: Apidae), (2009).
6. Lourenço, C. T., Carvalho, S. M., Malaspina, O. & Nocelli, R. C. F. Oral toxicity of fipronil insecticide against the stingless bee *Melipona scutellaris* (Latreille, 1811). *Bull. Environ. Contam. Toxicol.* **89**, 921–924 (2012). 10.1007/s00128-012-0773-x, Pubmed:22886451.
7. Silva, I. C. da. Efeito tóxico de *Stryphnodendron polyphyllum* (Fabaceae) para as abelhas *Apis mellifera*, *Tetragonisca angustula*, *Scaptotrigona* aff. *depilis* e *Nannotrigona Testaceicornis* (Apidae). (2012). 10.11606/T.59.2012.TDE-27092012-152405.

8. Botina, L. L. et al. Toxicological assessments of agrochemical effects on stingless bees (Apidae, Meliponini). *MethodsX* **7**, 100906 (2020). 10.1016/j.mex.2020.100906, Pubmed:32426248.
9. Sbordoni, Y. S. R. Termorregulação em abelhas sem ferrão (Hymenoptera, Apidae, Meliponini): Produção ativa de calor e metabolismo energético, (2016). 10.11606/T.59.2016.TDE-04032016-104955.
10. Peruzzolo, M. C., Grange, L. & Ronqui, L. Mortalidade de abelhas sem ferrão *Scaptotrigona bipunctata* sob os efeitos dos herbicidas paraquat e diquat. *Arq. Cienc. Vet. Zool. Unipar* **24** (2021). 10.25110/arqvet.v24i1cont.2021.8408.
11. Araújo, V. A., Serrão, J. E., Antonini, Y., Dias, L. G. & Neto, J. L. Structural changes in the male reproductive tract of the stingless bee *Scaptotrigona xanthotricha* Moure, 1950 (Meliponini, Apidae) during sexual maturation. *Sociobiology* **67**, 526–534 (2020). 10.13102/sociobiology.v67i4.5513.
12. Castro, I. Obtenção artificial de rainhas e estabelecimento de novas colônias de *Tetragona clavipes*. vol. 99, (2012). 10.11606/D.59.2012.TDE-07082012-135102.
13. Almeida, F. C. Efeito do fungo entomopatogênico *Beauveria bassiana* no reconhecimento de companheiras de ninho da abelha *Tetragonisca angustula* (Apidae : Meliponini), (2020). 10.11606/D.11.2020.TDE-05102020-082240.
14. da Silva, I. M. et al. Selectivity of the botanical compounds to the *pollinators Apis mellifera* and *Trigona hyalinata* (Hymenoptera: Apidae). *Sci. Rep.* **10**, 4820 (2020). 10.1038/s41598-020-61469-2, Pubmed:32179793.