Ecotoxicology
https://doi.org/10.1007/s10646-020-02167-7

Toxicity of botanical extracts and their main constituents on the bees *Partamona helleri* and *Apis mellifera*

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Accepted: 18 January 2020

Abstract

Africanized and wild bees are sensitive to synthetic insecticides, but may not be sensitive to botanical extracts. In this work, we evaluated the toxicity of botanical extracts with homemade preparations used in agroecological crops and their constituents on the bees *Apis mellifera* and *Partamona helleri*. Toxicity bioassays of adult bees were done by means of oral exposure and ingestion, using the insecticide imidacloprid as a positive control. Dietary consumption, respiration rate and bee flight were evaluated as sublethal parameters. Although some extracts were toxic to bees, survival was always higher compared to the results obtained with the imidacloprid, which was lethal to 100% of bees. In dietary consumption, *P. helleri* consumed less (5 mg/bee) in 3 h than *A. mellifera* (11 mg/bee), and *P. helleri* consumed less (7 mg/bee) in 24 h than *A. mellifera* (22 mg/bee). There was no difference in consumption of food containing plant extracts or food containing water only. We did not detect any adverse effects of the botanical extracts on bee respiration rates or flight. The major constituent of *N. tabacum* is nicotine (8.4–15.1%), in *A. americana* it is β-caryophyllene (11.3%), and in *A. colubrina*, lupeol (12.2%). Imidacloprid and nicotine were more toxic to bees ($LC_{50} \leq 1.3$ and $LC_{50} \leq 44.3$). Botanical extracts were selective to *A. mellifera* and the native bee *P. helleri*, and therefore, have the potential for ecofriendly pest control.

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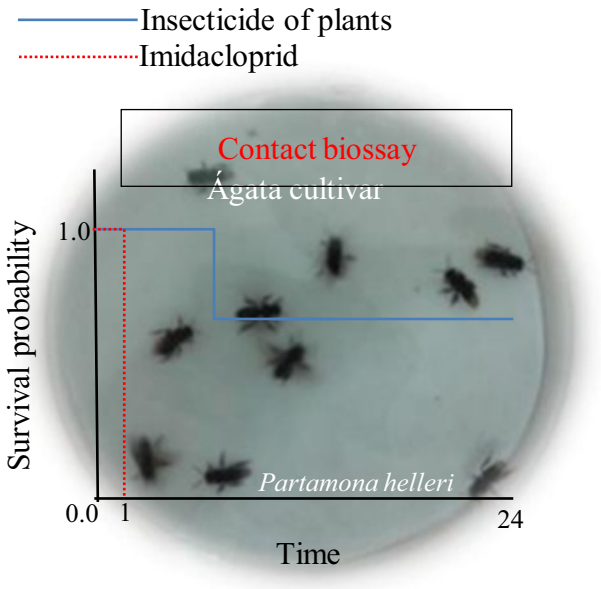
Published online: 13 March 2020

Graphical Abstract



Keywords *Agave americana* · *Anadenanthera colubrina* · Imidacloprid · Insecticide · Mortality · *Nicotiana tabacum*

Introduction

Approximately 90% of flowering species and 75% of the agricultural crops in the world require pollination, with bees being considered the most important pollinators (Brosi and Briggs 2013; Gianinni et al. 2015). The service of pollination is estimated to be worth 153 billion Euros, which corresponds to 9.5% of the value of worldwide agricultural production for human food (Gallai et al. 2009). While pollinators, especially bees, favor agricultural crops (Gianinni et al. 2015), other insects are considered pests since they damage crops and increase production costs (Gontijo et al. 2013, 2015). Conventional production systems use synthetic pesticides as the principal method of controlling these insects (Gontijo et al. 2013), despite the elevated risks of human and environmental contamination (Sheahan et al. 2017) and intoxication of non-target organisms (Sanchez-Bayo and Goka 2014). Bees are among non-target organisms impaired by pesticide exposure and their association with the decline of pollinators has been discussed worldwide (Feltham et al. 2014; Johnson 2015).

In areas of organic and agroecological production, where the use of the pesticides is not permitted, the use of botanical extracts is an option for pest insect management (Isman 2006; Pereira 2014; Pereira et al. 2018). The use of botanical extracts can make it more sustainable to control key insect pests, which usually require extensive pesticide applications and become resistant to insecticides (Campolo et al. 2017; Soares et al. 2019). Pyrethrum, rotenone, neem and essential oils are the principal botanical products used in insect control. Ryania, nicotine and sabadilla are also used but have limited utility. In addition, extracts from various plants used in homemade preparations are still used in many regions and countries (Isman 2006; Barbosa et al. 2015a).

Agroecological farmers have used homemade preparations of extracts from *Nicotiana tabacum* L., *Anadenanthera colubrina* Vell. and *Agave americana* L. with satisfactory results for controlling pest insects in vegetable crops (Pereira 2014; Pereira et al. 2018). However, little is known about the toxicity and sublethal effects of botanical extracts on non-target organisms (Burden et al. 2016; Tomé et al. 2014). The fact that these are extracts does not mitigate the toxicological risks to beneficial organisms (Gontijo et al. 2015; Tomé et al. 2015). Thus, there is a need to perform appropriate assessments of botanical extracts on beneficial organisms.

The objective of this study was to evaluate the selectivity of botanical extracts with homemade formulations of *Nicotiana tabacum* L., *Anadenanthera colubrina* Vell and *Agave americana* L., as well as their chemical constituents, on the bees *Apis mellifera* and *Partamona helleri*. These bees were selected for the study because honey bees and stingless bees constitute the principal pollinators of native and cultivated plants in Brazil (Gianinni et al.

2015). We also studied the sublethal effects of botanical extracts dietary consumption, respiration rate and flight of bees.

Materials and methods

Plants, insects and solvents

The tobacco species (leaf and rolled-tobacco leaves pressed and converted into smoke rolls) (*Nicotiana tabacum* L.), red angico (*Anadenanthera colubrina* Vell) and maguey (*Agave americana* L.) were selected because of their use by agroecological farmers for pest control (Pereira 2014; Pereira et al. 2018). Tobacco, maguey and angico leaves were collected at a property in the region of Viçosa, Minas Gerais State, Brazil (20°43′58.37″ S, 42°49′23.50″ W), altitude 738 m. The extracts from these plants are used by agroecological farmers to control rose-grain aphid (*Metopolophium dirhodum* Walker, 1849), cochineals (*Dactylopius coccus* Costa, 1835), caterpillars and beetles in the production of vegetable crops such as *Lactuca sativa* L. (lettuce), *Cichorium intybus* L. (chicory), *Brassica oleracea* L. (cabbage), *Allium fistulosum* L. (chive), *Eruca sativa* Mill. (arugula), *Rumex acetosa* L. (sorrel) and others. The tobacco roll and imidacloprid (Evidence 700 WG) were acquired commercially in the city of Viçosa, Minas Gerais State.

To mount the bioassays, adult workers of *A. mellifera* (honey bee) and *P. helleri* (stingless bee) were used. The bees were collected at the apiary and meliponary at the Federal University of Viçosa (UFV), 20°45′32.71″ S, 42°52′04.10″ W and altitude of 815 m. For *P. helleri* collection an Erlenmeyer flask (1000 mL) was used, with its opening inserted at the entrance of the hive for the bees to enter the flask. Later, the bees were released into organza cages (0.4 × 0.4 × 0.4 m) in a dark room, with white light in the background to prevent escape and facilitate bee transfer to the plastic pots (500 mL). *Apis mellifera* bees were collected manually from the colonies with the help of entomological forceps (Papilon, number 13) and transferred directly to the plastic pots (500 mL).

The solvents used to prepare the extracts were water with alcohol (96° GL) for the tobacco extracts (roll and leaf) and water alone for the maguey and angico extracts. Botanical extract preparation followed the protocols by Pereira (2014) and Pereira et al. (2018). The tobacco roll was cut into pieces 10 cm in length, with 100 g of tobacco added to a glass flask (1000 mL) followed by 250 mL of alcohol and 250 mL of water. After 10 days the solution was filtered through cotton and diluted at the concentration of 33.33 mL/L (v/v). The same procedure was done to prepare the tobacco-leaf based botanical extract. The red angico peel was collected using a machete to remove rectangular pieces from an already mature tree, with 250 g of these pieces inserted into a plastic bottle containing 250 mL of water. After 30 days the botanical extract was filtered through cotton and diluted in water at the concentration of 10 mL/L (v/v). The maguey leaves were cut off with a machete, the thorns were removed and the leaves were sliced with a knife. In a blender, 100 g of the leaves and 100 mL of water were combined and processed for 3 min. To remove the larger fragments, the extract was filtered through a sieve and then cotton. The maguey botanical extract was diluted at the concentration of 3000 mL/L (v/v) (Pereira 2014; Pereira et al. 2018). The quantity of imidacloprid used (700 WG) was calculated based on the spray volume by hectare at the concentration of 3.00 mg a.i./m^2 (300 g/ha), in accordance with the Brazilian Ministry of Agriculture (MAPA 2019). The solution was diluted in distilled and deionized water to perform the contact bioassays and then in 50% sucrose solution (sugar/water syrup 50% v/v) for the ingestion bioassays (Tomé et al. 2015).

GC–MS analysis

The tobacco, maguey, and angico botanical extracts were diluted in 10 mL of ethanol and filtered through 47 mm membrane filters (Millex) prior to GC–MS analyses. The GC–MS analysis was performed on the extracts of the tobacco leaves and roll samples using a GC–MS (GCMS 2010-Plus, Shimadzu), equipped with a "split/splitless" type injector (200 °C). Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperatures were set at 250 and 300 °C. The oven temperature was programmed from 60 to 250 °C/min, then held isothermal for 20 min and finally raised to 300 °C at 10 °C/min. Diluted samples (1/45 v/v, in ethanol) of 0.3 mL were manually injected in splitless mode.

The chemical compounds were identified by a gas chromatograph coupled with a mass detector GC-MS (GC-MS 2010-Plus, Shimadzu). The injector and detector temperatures were 220 and 300 °C. The initial column temperature was 40 °C for 3 min, with a programmed temperature increase of 3 °C/min to 300 °C for 25 min. The split mode ratio was 1:10. One microliter of the sample containing 1% (w/v in dichloromethane) was injected and helium was used as the carrier gas with a constant flow rate of 1.5 mL on the Rtx®-5MS capillary column (30 m, 0.25 mm × 0.25 µm). Compounds were identified by comparing mass spectra with those available in the NIST08 and NIST11 libraries and Wiley Spectrotech Database (7th edition), as well as by the retention indices. The nicotine and β-Caryophyllene compounds

identified as plant constituents were obtained from Sigma Aldrich (Darmstadt, Germany). The compounds were selected because they are the most concentrated in the samples of the plants.

Bioassays with botanical extracts and chemical constituents

The bioassays were conducted in a completely randomized design, with seven treatments and five repetitions. The treatments were the botanical extracts (*A. americana leaves*, *A. colubrina* peel, *N. tabacum* leaves and roll), the insecticide imidacloprid (positive control) and the controls (solvents: water and water with alcohol) (Table 1). Each experimental unit was composed of 20 adult bees of each species. Pure nicotine, β-Caryophyllene and imidacloprid standards were purchased from Sigma-Aldrich Ltd (São Paulo, Brazil). A preliminary assay was performed to determine the "all or none" response, to establish a range of concentrations for concentration–response lines. Concentration varied from 0.446 to 122.541 ng/µL honey bee for nicotine, 22.856 to 252.144 ng/µL honey bee for β-Caryophyllene, 0.0002 to 3.988 ng/µL honey bee for imidacloprid (ingestion test), 3.242 to 98.442 ng/bee for nicotine, from 12.673 to 440.626 ng/bee for β-Caryophyllene and 0.004 to 2.155 ng/bee for imidacloprid (contact test) (Table 1). The compounds were diluted in acetone, with only acetone for the control. The number of dead bees in each repetition was counted after essential oil exposure for 48 h after application.

Exposure by contact

In contact bioassays, we used plastic pots with transparent polyethylene and a volume of 500 mL for short term exposures (Nerin et al. 1996). These pots contained holes in the lid to circulate air and a circular opening in the side for the bee feeders. In each 500 µL pot of the respective treatment was sprayed (395 µL on the side and bottom and 105 µL on the cover), using a compressor at 50 psi (Sagyima Pro, model ASW 186), to cover the internal surface of the pot. For the controls the pots were treated with only the solvent used in the botanical extract process. The pots were left to dry at 25 ± 3 °C for 2 h in a closed and dark environment. To each previously treated pot we added 20 bees and a feeder with a hole at the end (Eppendorf®), where food was provided (sugar/water syrup 50% v/v). After 3 h of contact, the bees were transferred to non-treated containers and the contaminated pots were discarded. Bee mortality was recorded after 1, 2, 3, 6, 12 and 24 h and bees were considered dead when incapable of moving (Tomé et al. 2015). The pots with the bees were maintained in a greenhouse (28 ± 2 °C, 65 ± 5% R.H.). The surviving bees from each treatment were subjected to respiration and flight bioassays.

Respiration rate

Bee respiration was evaluated under laboratory conditions with a respirometer of type CO_2 Analyzer TR 2 (Sable Systems International, Las Vegas, USA) (Pimentel et al. 2007). At the end of the bioassay, one bee from each

Table 1 Treatments, concentrations, solvent and parts of the plant used in contact and ingestion exposures

Component	Plants of the plant	Solvent	Exposure		Treatment
			Contact	Ingestion	
Extracts (mL/L)					
N. tabacum	Leaves roll	Water + alcohol (33.3)	x	x	Extract
A. americana	Leaves	Water (10.0)	x	x	Extract
A. colubrina	Peel	Water (3000)	x	x	Extract
Imidacloprid[a]	–	Water (300 g/ha)	x	x	Control
Constituent ingestion (ng/µl)[b]					
Nicotine	–	Acetone (0.45–122.5)	x	x	Standard
β-Caryophyllene	–	Acetone (22.9–252.1)	x	x	Standard
Imidacloprid	–	Acetone (0.0002–4.0)	x	x	Standard
Constituent contact (ng/bee)[b]					
Nicotine	–	Acetone (3.2–98.4)	x	x	Standard
β-Caryophyllene	–	Acetone (12.7–440.6)	x	x	Standard
Imidacloprid	–	Acetone (0.004–2.2)	x	x	Standard

[a]Synthetic insecticide (control)

[b]Sigma standard

treatment was transferred to a glass respirometric chamber, with a volumetric capacity of 25 mL. These chambers were connected to a completely closed system with an infrared reader, where air without CO_2 circulates into the chamber for 2 min at a flow rate of 600 mL/min. Measurements were made on the equipment for 3 h on the quantity of CO_2 (μmL/CO_2/h/bee) (Tomé et al. 2014). Five repetitions were done.

Flight assessment

To evaluate flight after 24 h of exposure to the botanical extracts, all surviving bees were freed at the base of a wood tower (1.05 m tall, formed by three stacked cages of wood 0.35 × 0.35 × 0.35 m each). The cages were wrapped with an organza fabric, with an interior open to allow free flight. The flight test was done in a dark room, with only a fluorescent light fixture suspended 50 cm from the top of the tower. The time spent in flight from the base to the light was recorded on a chronometer for 1′30″ (1 min and 30 s). After that time the bees that remained at the base of the tower were considered incapable of flight (Tomé et al. 2015).

Exposure by ingestion

Twenty bees were added to plastic pots of transparent polyethylene with a volume of 500 mL, with holes in the lid for air circulation and an opening on the side to add the feeder (Tomé et al. 2015). The bees fasted for 1.5 h and then a feeder was added to each pot, with the feed previously contaminated with the treatments. The feeders were weighed on an analytical balance (Shimadzu: AUW 220 D: 0.01 mg) before and after the experiment to verify the quantity of food ingested by the bees. The supplied food and botanical extracts were diluted to the same concentration of the contact bioassays, but the dilution was done in the saccharose solution (sugar/water syrup 50% v/v). The water control used sugar/water syrup 50% (v/v) and the water with alcohol control used 48.26% of water, 1.74% of alcohol and 50% sugar (v/v/v). After 3 h the contaminated feed was substituted with pure food (sugar/water syrup 50% v/v). The pots, containing the bees, were kept in a greenhouse (28 ± 2 °C, 65 ± 5% R.H.). Mortality was evaluated after 1, 2, 3, 6, 12 and 24 h (Tomé et al. 2015), with bees that remained immobile considered dead. Surviving bees were subjected to the flight and respiration tests, as described for the contact bioassay.

Statistical analyses

All statistical tests were performed in SAS version 9.0 (SAS Institute, Cary, NC, USA). The survival data for contact and ingestion were subjected to survival analyses using the Kaplan–Meier estimators. Bees that were still living at the end of the bioassays (24 h) were treated as censored data, since the exact survival time of these bees is unknown. The overall similarity among the survival curves was analyzed by the log-rank χ^2 test and the paired comparisons among the curves were tested using the Holm–Sidak method. The dietary consumption data in the ingestion assays were subjected to analysis of variance of the repeated measurement to test the effect of dietary consumption in relation to time, with the differences in the time intervals tested by the F test. The flight time data were subjected to analysis of variance and the respiration data used the non-parametric Kruskall–Wallis test. Adult mortality results were subjected to probit analysis, correcting the data for natural mortality (Abbott 1925). The 95% confidence intervals of LC_{50} were estimated.

Results

In the contact bioassays, survival curves obtained by the Kaplan–Meier estimators showed significant differences between the treatments for *P. helleri* (log-rank test: $\chi^2 = 767.97$, df = 6, $p < 0.001$) and *A. mellifera* (log-rank test: $\chi^2 = 728.69$, df = 6, $p < 0.001$) (Fig. 1). The *N. tabacum* (leaf) and *A. colubrina* extracts did not alter the survival rates of the two species compared to the water only control, but the *N. tabacum* (roll) extract reduced the survival of the two bee species evaluated (Fig. 1). Contact with the *A. americana* extract significantly reduced the probability of survival for *A. mellifera* (Fig. 1c), but did not alter the survival of *P. helleri* (Fig. 1a). This was observed even if the *A. mellifera* bees showed a lower probability of survival in all treatments when compared to the probability of survival for *P. helleri*. The contact with imidacloprid caused 100% bee mortality for both species after 20 min of exposure (Fig. 1a–c).

The ingestion bioassays survival curves obtained by the Kaplan–Meier estimators indicated that ingestion of botanical extracts changed the survival of *P. helleri* (log-rank test: $\chi^2 = 619.49$, df = 6, $p < 0.001$) and *A. mellifera* (log-rank test: $\chi^2 = 768.96$, df = 6, $p < 0.001$) (Fig. 2). The *A. americana* extract affected the survival of both bee species. The *A. colubrina* extract affected only the survival of *A. mellifera* (Fig. 2c). With the exception of the *A. americana* extract, *A. mellifera* showed a lower probability of survival when compared to *P. helleri*. The ingestion of imidacloprid killed 100% of *P. helleri* and *A. mellifera* after 20 min of exposure (Fig. 2a, c). In general, the botanical extracts that showed the lowest selectivity to bees was *A. americana* (Figs. 1c and 2a–c), with the highest selectivity shown by the *A. colubrina* extract. No bees

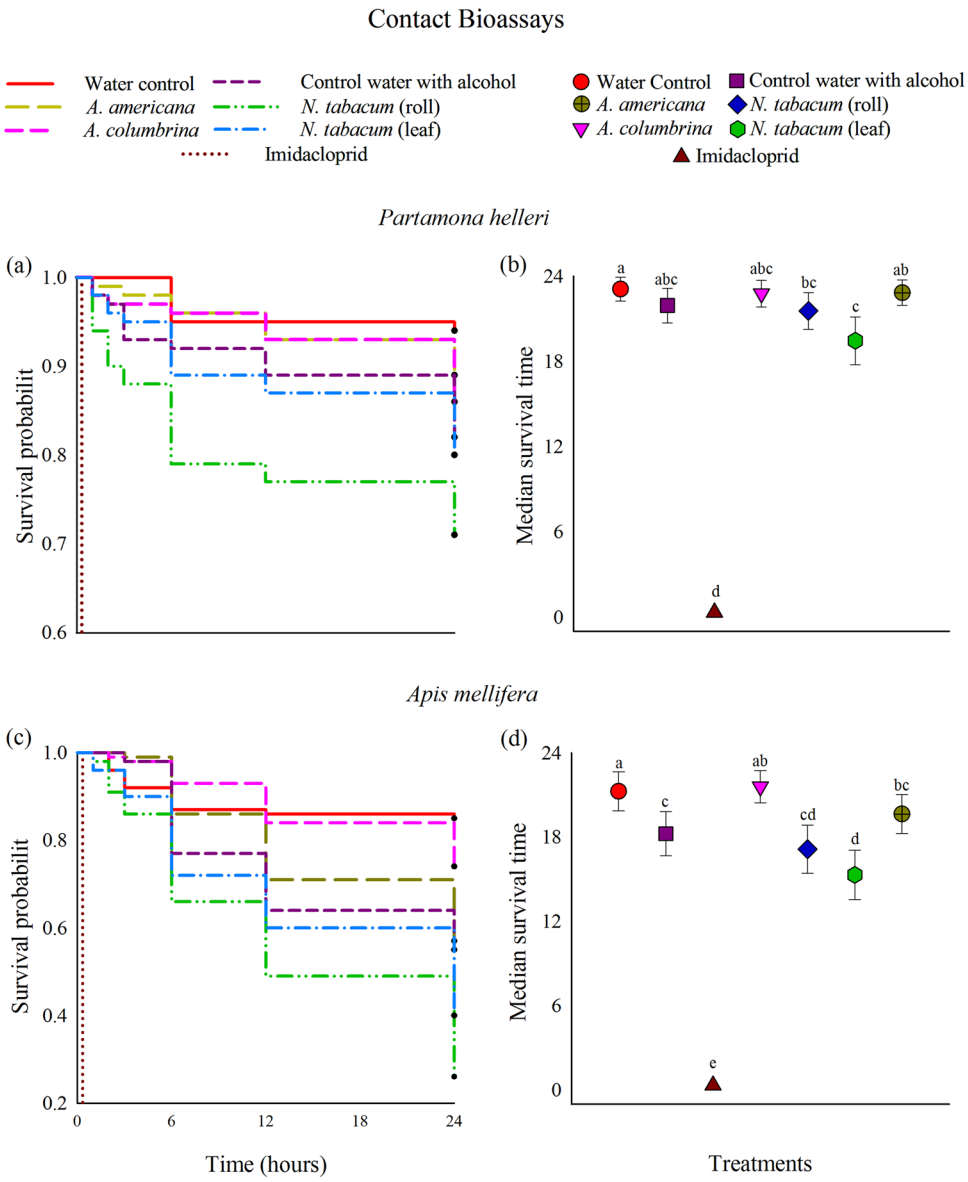


Fig. 1 Survival curves for *Partamona helleri* (**a**) and *Apis mellifera* (**c**) exposed to the botanical extracts by contact. The curves were generated by Kaplan–Meier estimators and compared by the log-rank test ($P < 0.05$). The box plots represent the mean time (hours of life) and survival confidence interval of the bees *Partamona helleri* (**c**) and *Apis mellifera* (**d**). Different letters indicate significant differences among the treatments based on the Holm–Sidak test ($P < 0.05$)

survived after being exposed to imidacloprid in either the contact or ingestion assays. Therefore, the flight and respiration tests were not done on the bees from this treatment.

There was no difference in the time × treatment interaction of the food consumption of *A. mellifera* bees (Wilk's Lambda = 0.76, $F_{5,\ 24} = 1.48$, $p = 0.234$), but there was a difference in time (Wilk's Lambda = 0.03, $F_{1,\ 24} = 732.90$, $p < 0.001$) and in treatments ($F_{5,\ 24} = 4.00$, $p < 0.001$) (Fig. 3a). As for *P. helleri* bees, differences were observed in the interaction of time × treatment (Wilk's Lambda = 0.63; $F_{5,\ 24} = 2.77$, $p = 0.041$), in time (Wilk's Lambda = 0.51, $F_{1,\ 24} = 23.47$, $p < 0.001$) and treatments ($F_{5,\ 24} = 5.33$, $P = 0.002$) (Fig. 3b). There was no difference in consumption of food containing plant extracts or food containing water only (control) (Fig. 3).

In relation to tests evaluating the sublethal effects of the extracts, we observed no differences in the consumption of pure food offered 3 h after exposure to the extracts and no differences were observed in relation to the treatments. An inverse behavior was observed between *P. helleri* and *A. mellifera*. In 3 h, *P. helleri* consumed mean 5 mg/bee and *A. mellifera* consumed mean 11 mg/bee. On the other hand, in 24 h *P. helleri* consumed mean 7 mg/bee and *A. mellifera* consumed mean 22 mg/bee. The *P. helleri* bees consumed more food contaminated with the extracts than the pure food (saccharose solution without the treatments), except in the tobacco *N. tabacum* (roll) treatment. When offered contaminated food, *A. mellifera* consumed less, but consumption increased when offered the pure food (Fig. 3).

At the end of 24 h, the contact of the bees with the treatments did not affect the flight of *P. helleri*

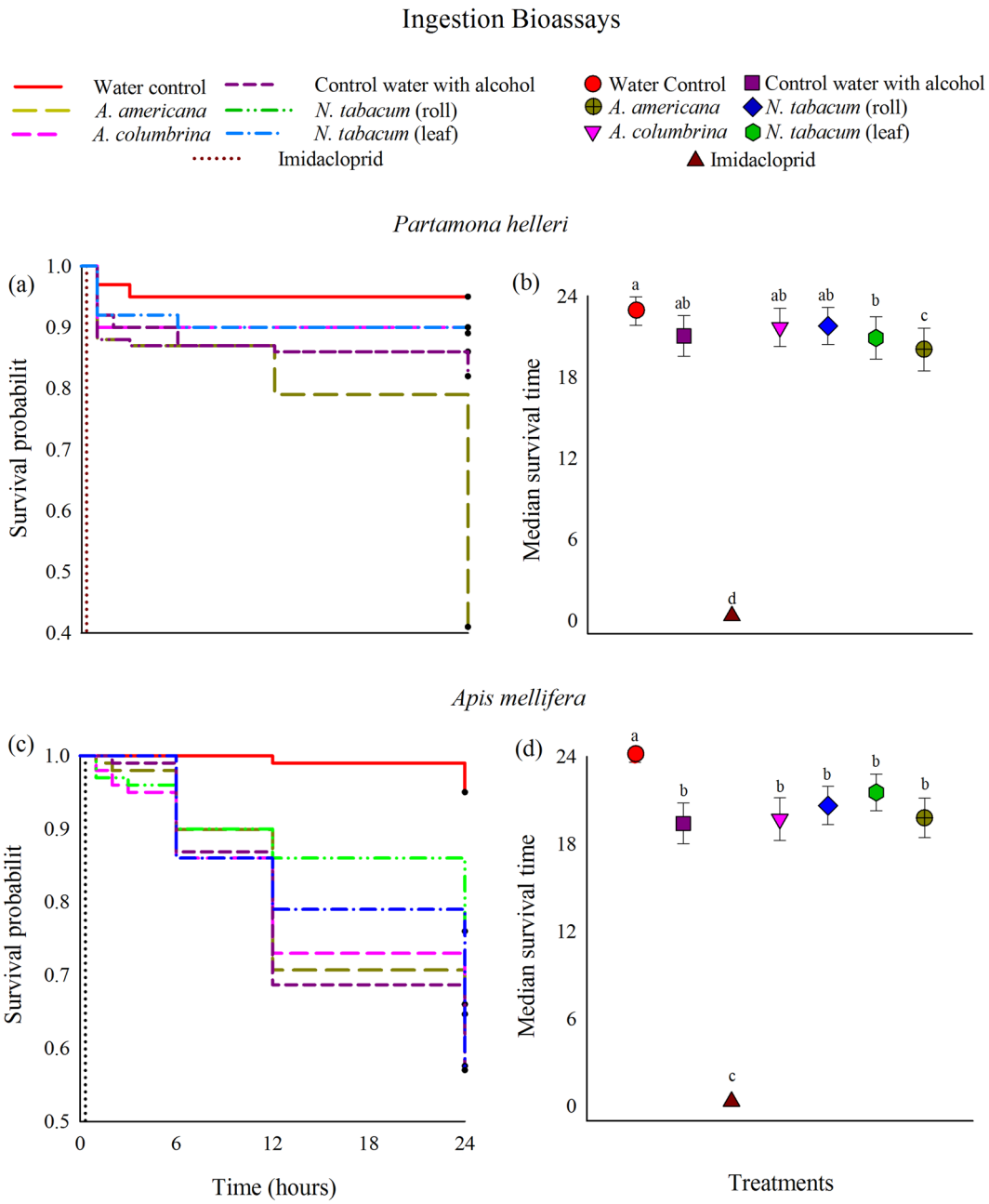


Fig. 2 Survival curves of *Partamona helleri* (**a**) and *Apis mellifera* (**c**) exposed to the botanical extracts by ingestion. The curves were generated by Kaplan–Meier estimators and compared by the log-rank test ($P < 0.05$). The box plots show the mean time (hours of life) and survival confidence interval of the bees *Partamona helleri* (**c**) and *Apis mellifera* (**d**). Different letters indicate significant differences among the treatments based on the Holm–Sidak test ($P < 0.05$)

($F_{5, 24} = 1.69$, $p = 0.174$) or *A. mellifera* ($F_{5, 24} = 2.27$, $p = 0.079$). This was also observed for the ingestion bioassays, where the treatments did not affect the flight of *P. helleri* ($F_{5, 24} = 2.25$, $p = 0.081$) or *A. mellifera* ($F_{5, 24} = 0.70$, $p > 0.05$). For the contact bioassays, the bee respiration rate did not differ among the treatments (*P. helleri*: $\chi^2 = 4.82$, df = 5, $p = 0.438$; *A. mellifera*: $\chi^2 = 1.08$, df = 5, $p = 0.956$). The mean ± standard error of the respiration rate for *P. helleri* was 43.85 ± 5.70 µl/CO_2/h/bee and for *A. mellifera* was 116.80 ± 15.77 µl/CO_2/h/bee. There were no differences observed in the respiratory rates of the bees (*P. helleri*: $\chi^2 = 9.48$, df = 6, $p = 0.091$; *A. mellifera*: $\chi^2 = 4.42$, df = 6, $p = 0.491$). The mean ± standard error of the respiration rate of *P. helleri* was 62.89 ± 13.73 µl/CO2/h/bee and for *A. mellifera* it was 105.76 ± 19.43 µl/CO_2/h/bee.

Nicotine, β-Caryophyllene and lupeol were the major components of the plants and it was quantified by the retention time of 10.05, 20.41 and 39.22 min at

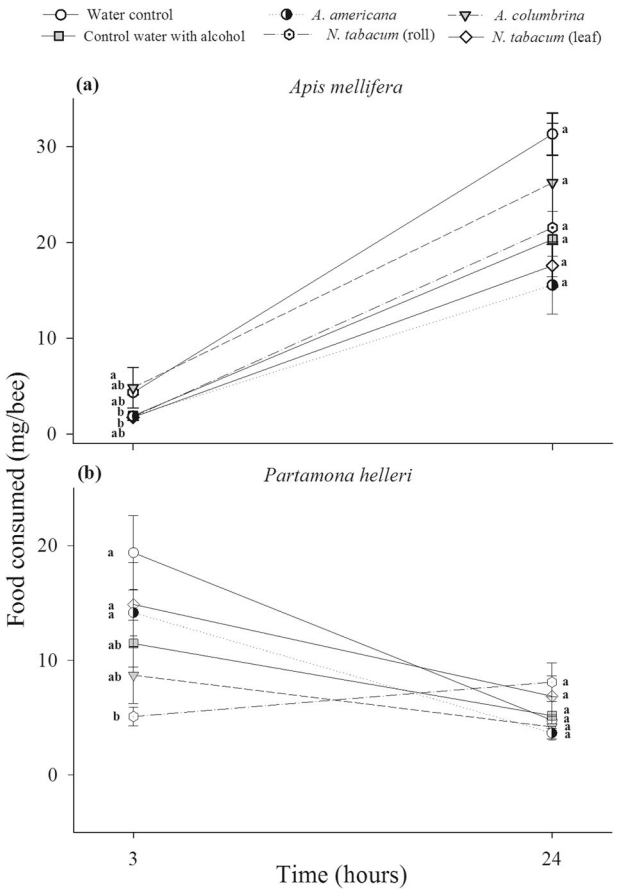


Fig. 3 Quantity of food consumed by *A. mellifera* (**a**) and *P. helleri* (**b**) in the ingestion assay. In the first 3 h the graph shows the consumption of food contaminated with the extracts followed by the consumption of food containing the saccharose solution without the contaminants. The symbols represent the average and the standard error. The different letters at the same time (hour) indicate significant differences between the treatments based on the Tukey test ($P < 0.05$)

concentrations of 15.14, 11.32 and 12.22% (Table 2). The concentration–mortality curves for each compound varied significantly among the species and the exposition method (ingestion/contact). For *A. mellifera*, imidacloprid had the highest toxicity ($LC_{50} = 0.09$ ng/bee). The nicotine had intermediate toxicity ($LC_{50} = 32.45$ ng/bee). Both showed higher toxicity to *A. mellifera* (ingestion). β-Caryophyllene and lupeol were less toxic, with the LC_{50} being 100 times greater than for imidacloprid and nicotine (Table 3).

Discussion

Results showed bee susceptibility to botanical extracts of *A. americana*, *A. colubrina* and *N. tabacum* (roll and leaf) varied between *A. mellifera* and *P. helleri*, in accordance with the type of extract used in the bioassays and in response to the type of exposure. Plant extracts can cause adverse effects to nontarget insects such as bees (Melathopoulos et al. 2000; Koskor et al. 2009; Xavier et al. 2015).

Table 2 Chemical constituents of *Nicotiana tabacum* (leaves and roll), *Anadenanthera colubrine* (leaves) and *Agave americana* (leaves) ethanol fraction

Compounds	Retention time	Area (%)
Nicotiana tabacum leaves		
N-Dimethylglycine	2.21	6.21
Glycerine	3.01	3.24
n-Hexadecanoic	3.11	5.12
Orcinol	4.65	6.41
1,3-Methylene-d-arabitol	5.42	1.14
2,4-Dihydroxy-5,6-dimethylpyrimidine	9.32	0.15
Nicotine	10.05	8.35
Nicotiana tabacum roll		
2 Phenyl ethanol	2.10	1.02
Phenyl acetaldehyde	2.14	3.25
Ácido vanílico	6.12	2.21
p-Xylene	8.22	3.66
7-Deca, hexadecanol	9.44	0.25
Nicotine	10.06	15.14
β-sitosterol	17.45	9.45
Octacosan-1-ol	20.47	2.15
Tetracosan-1-ol	22.51	3.74
1-Hexadecanol	44.11	1.07
Phytol	55.22	0.12
Agave americana leaves		
n-Pentadecane	4.98	1.25
(E)-1-Methoxymethoxy-1-tetradecane-3-ol	5.11	9.24
2-Undecanone	8.10	10.32
3,2-Benzenedicarboxylic acid	8.52	9.54
n-Heptadecane	8.64	1.25
Mono-2-ethylhexyl phthalate	10.22	3.45
Nonacosane	12.54	10.44
Tetracosane	13.57	2.52
2,5-Cyclohexadiene-1,4-dione	18.39	1.11
Nonadecane	19.10	1.02
β-Caryophyllene	20.41	11.32
Anadenanthera colubrina		
Propanal	1.21	1.11
Trans-2-hexenal	3.44	0.32
Butanal	3.78	1.74
Acetaldeide	5.66	2.54
2-octenal	6.87	2.31
Nonanal	7.45	1.65
Trans-2-devenal	9.44	3.54
2-propenal	11.12	6.77
2,7-Dimetiloct-2,7-dienol	11.65	9.21
β-Citral	15.24	3.21
Nerol	16.22	1.98
Acid Nerolic	19.32	3.44
5-OH-dimetiltriptamine	22.54	2.14
5-metoxi-dimetiltriptamine	30.11	10.12
Apigenin	32.11	6.54
Acid 4-hidroxibenzoic	35.66	12.11
Lupeol	39.22	12.22
Acid 4-hidroxibenzoic	41.65	10.64

Table 3 Mortality-response of *Partamona helleri* and *Apis mellifera* to nicotine, β-Caryophyllene, lupeol and imidacloprid

Probit parameters	Nicotine	β-Caryophyllene	Lupeol	Imidacloprid
Partamona helleri (contact) ng/μL honey bee				
LC_{50}	44.32	122.2	200.1	0.65
χ^2	8.41	6.54	15.12	7.11
p value	0.12	0.10	0.06	0.23
Inclination	1.01	0.22	0.07	3.21
Confidence interval	39.2–47.1	111.1–135.2	196.3–210.1	0.55–0.68
Apis mellifera (contact) ng/μL honey bee				
LC_{50}	60.15	127.4	222.5	1.35
χ^2	9.36	6.17	13.21	12.05
p value	0.09	0.92	0.06	0.44
Inclination	0.09	0.15	0.03	3.76
Confidence interval	55.1–67.9	119.3–137.5	215.1–229.2	0.98–2.13
Partamona helleri (ingestion) ng/bee				
LC_{50}	38.76	117.1	212.2	0.11
χ^2	10.02	9.10	8.11	6.77
p value	0.42	0.81	0.10	0.31
Inclination	1.34	0.33	0.02	3.01
Confidence interval	34.1–41.2	111.2–124.6	215.1–229.2	0.05–0.20
Apis mellifera (ingestion) ng/bee				
LC_{50}	32.45	111.2	210.1	0.09
χ^2	12.86	9.43	8.53	10.98
p value	0.24	0.54	0.26	0.64
Inclination	1.33	0.18	0.04	4.12
Confidence interval	30.1–35.3	105.1–115.2	200.2–217.6	0.01–0.16

The difference in susceptibility of the bees to the botanical extracts may be related to various aspects beyond species and body size, such as genetics, lifecycle, feeding, foraging behavior, type of exposure and detoxifying enzymes (Arena and Sgolastra 2014; Johnson 2015). Results showed the alcohol solvent used to prepare *N. tabacum* (leaf and roll) extracts had a negative effect on the survival of the bees at contact and ingestion assays. This difference in the toxicity of the alcohol solvent may be due to the quantity and type of compounds extracted (Azwanida 2015). Moreno et al. (2011) observed more toxicity from *Calendula officinalis* extracts than hexane extract on the pest *Tuta absoluta* (Meyrich) (Lepidoptera: Gelechiidae).

In a general way, the botanical extracts were more selective to *P. helleri* than *A. mellifera* because *P. helleri* ingested large quantities of food immediately after fasting and reduced pure food consumption over time (except *N. tabacum* roll). Bees *A. mellifera* and *Bombus terrestris* show preference for nectar contaminated with imidacloprid (Kessler et al. 2015) and *A. mellifera* show preference for contaminated pollen (Han et al. 2012). Thus, the greater consumption of contaminated food may have increased the potential toxicity of the constituents present in the plants. Therefore, the ingestion exposure method shows the consequences of foraging on contaminated plants. For example, there are neonicotinoid insecticides that are systemic in plants (Blacquière et al. 2012) and may be translocated to grains of pollen and nectar (Goulson 2013).

The high mortality of the two bee species caused by imidacloprid (100%) has been reported by numerous studies (Goulson 2013; Laycock et al. 2014; Tomé et al. 2015; Johnson 2015). The high toxicity is explained by the presence of the nitro functional group that gives this pesticide great affinity to the neonicotinoids acetylcholine receptor (Tomizawa and Casida 2003). When they come into contact with bees, the neonicotinoids may be transported to the interior of the colonies by ingestion (Mullin et al. 2010) or contact with grains of contaminated pollen and nectar (Fairbrother et al. 2014). In addition to *P. helleri* and *A. mellifera*, imidacloprid can exhibit detrimental side effects on other bee species (e.g. *Bombus terrestris*) and

hymenopteran beneficials, such as insect parasitoids (Desneux et al. 2007; Mansour et al. 2018).

Extracts of *N. tabacum* are not selective to bees, since nicotine and neonicotinoids have similar mechanisms of action in insects (Blacquière et al. 2012). In fact, that selectivity was not observed for honey bees in the contact bioassay (mortality > 60%). For *P. helleri*, extracts of *N. tabacum* (leaf and roll) present themselves as being as selective to the bioassays by contact as if they had been ingested (mortality < 30%). Bees of greater body volume are generally more tolerant to pesticides, whether through contact or ingestion (Johansen et al. 1983) and some authors report that stingless bees (Meliponini) are more sensitive to the pesticides (Del Sarto et al. 2014; Tomé et al. 2012). But our results showed that *P. helleri* was more tolerant to the botanical extracts than *A. mellifera*.

The higher toxicity of *A. americana* extract to bees can be attributed to its high concentrations (3000 mL/L v/v). Although under laboratory conditions *A. americana* showed low selectivity when ingested by *P. helleri* and in contact with *A. mellifera*, selectivity may be greater under field conditions. This is because botanical extracts generally degrade or dissipate more rapidly than formulated synthetic pesticides (Fantke et al. 2014; Tomé et al. 2015). The difference in food consumption between the species may result from their natural feeding behavior, since there were no differences between the controls and the treatments. Foraging honey bees are capable of perceiving risks (i.e. danger), so they carry a greater amount of food when leaving the nest to forage (Tan et al. 2015). This strategy of *A. mellifera* may explain the lower consumption of the contaminated diet after the fasting and when offered food without the botanical extracts, with increased consumption to compensate for low ingestion after starvation.

The flight and respiration of *A. mellifera* and *P. helleri* bees was not affected by the botanical extracts in either bioassay (contact or ingestion). This may be related to the lack of bioavailability of the botanical extracts to the bee's muscles or nervous system (Zafeiridou and Theophilidis 2006). Some pesticides affect bee flight and can alter the foraging behavior and colony survival of *A. mellifera* (Balbuena et al. 2015) and *Melipona quadrifasciata anthioides* bees (Tomé et al. 2012).

Commercial botanical extracts often show selectivity to non-target organisms, such as bees (Castillo 2009), but there are some exceptions. There are reports of lethal or sublethal effects on bees *Melipona quadrifasciata* (Barbosa et al. 2015b), *A. mellifera* (Xavier et al. 2015), *P. helleri* and *Scaptotrigona xanthotrica* (Tomé et al. 2015). To our knowledge no previous works have evaluated the selectivity of botanical extracts with homemade formulations on wild and honey bees. What is known regarding the tolerance of bees to natural and synthetic toxins is that one of the principle mechanisms involved is metabolic resistance. The principal enzymes responsible for the metabolism or detoxification of the toxins are the carboxylesterases (COEs), glutathione S-transferase (GSTs) and cytochrome P450 (Du Rand et al. 2015). In spite of this, further research is needed to know the active principles present in the extracts used to better clarify the mechanisms that confer selectivity of these botanical extracts to bees, since the mechanisms that allow the bees to tolerate the toxic secondary metabolites remain unknown (Du Rand et al. 2015).

In our work the most common constituents nicotine (Walia et al. 2017), β-Caryophyllene (Cárdenas-Ortega et al. 2015), lupeol (Ningombam et al. 2017) have documented insecticidal action. However, this does not mean that a constituent with a low concentration is not an insecticide. The effects of nicotine on insects are well documented, but little is known about the effects of β-Caryophyllene and lupeol. This study showed that the susceptibility of the bees to botanical extracts varies with bee species, extract and type of exposure. In addition, this research demonstrated that botanical extracts are safer for bees than the synthetic pesticide. Imidacloprid and nicotine were more toxic to *A. mellifera* and *P. helleri* bees. This means that if the extracts are effective against target insects, they may be used as an alternative to synthetic compounds in a way that helps preserve stingless and honey bees.

Acknowledgements This research was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). We thank Journal Editors of America LLC for corrections and English editing of this manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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