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Original article

Toxicity of insecticides used in the Brazilian melon crop to the honey bee *Apis mellifera* under laboratory conditions

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Abstract – This study aimed at evaluating the toxicity of insecticides used in melon crop (*Cucumis melo* L.) on adults of *Apis mellifera* L. (Hymenoptera: Apidae) under laboratory conditions. Three ways of exposure were used: direct spraying, feeding with insecticide contaminated diet, and contact with sprayed leaves. Bees were exposed to the insecticides abamectin, acetamiprid, cartap chloride, chlorfenapyr, cyromazin, deltamethrin, thiamethoxam, flufenoxuron, and pyriproxyfen at the highest dosages recommended by the manufacturers for the melon crop in Brazil. Results indicated that, regardless of how the bees were exposed to insecticides, thiamethoxam, abamectin, and chlorfenapyr were extremely toxic to adults of *A. mellifera*. Acetamiprid, deltamethrin, and cartap chloride were most toxic when directly sprayed on the bees. Cyromazin and pyriproxyfen caused low mortality rates to *A. mellifera*, whereas flufenoxuron caused moderate mortality when fed to adult bees.

conservation / mortality / nontarget organisms / pollinator / phytosanitary treatment

1. INTRODUCTION

Most angiosperms (87 %) are partially or totally dependent on insect pollination for fruit set, particularly in the tropical region where 94 % of wild and cultivated plants depend directly on insect pollination (Ollerton et al. 2011). According to Klein et al. (2007), 70 % of the 124 main crops directly used for human consumption worldwide are dependent on insect pollinators. Honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) are essential pollinators and play both a functional and ecological role that is paramount for the maintenance of native plant communities as well as agricultural productivity (McGregor 1976; Malerbo-Souza et al. 2003; Gallai et al. 2009, Bernal et al. 2010, Potts et al. 2010). In a recent study carried by Lautenbach et al. (2012), authors state that global pollination benefits are dominated by a small number of countries, showing Brazil in fourth place.

In Brazil, melon (*Cucumis melo* L.) is one of the most cultivated cucurbit crops with 19,701 ha grown and annual production of 499,330 tons in 2011 (IBGE Brazilian Institute of Geography and Statistics 2012). Melon production in Brazil's high-production areas is dependent on workers of *A. mellifera* for pollination and fruit set. The importance of honey bees to melon production has been demonstrated previously (Sousa et al. 2009). Brazilian melon production is concentrated in the

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semiarid region, mainly in the states of Rio Grande do Norte and Ceará. These two states are responsible for 80 % of Brazilian melon production, where 90 % are exported (IBGE 2012). Due to the regional climatic conditions, the crop cycle is extremely short, approximately 75 days with blooming periods that last about 10 days. Melon fruiting occurs during the dry season when native pollinators are low in number.

However, this crop is attacked by several pests of economic importance such as the serpentine leafminer *Liriomyza* spp. (Diptera: Agromyzidae), the whitefly *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae), the pickleworm *Diaphania nitidalis* Cramer, and the melonworm *Diaphania hyalinata* L. (Lepidoptera: Crambidae). Thus, insecticide spraying has become indispensable for the maintenance of phytosanitary conditions and crop productivity. Moreover, the phytosanitary management of the melon crop deserves special attention since it is attacked by insects in practically all crop phenological phases (Guimarães et al. 2008).

The adverse impact that broad spectrum insecticides also have on nontarget beneficial insects is widely known to be a major cause of pollinator decline in cultivated areas, especially in vast areas devoted to monocultures (Kearns and Inouye 1997; Fletcher and Barnett 2003; Devine and Furlong 2007; Freitas et al. 2009). Neonicotinoids, the widespread and fastest-growing class of chemical insecticides, have been demonstrated to be highly toxic to *A. mellifera* (Iwasa et al. 2004; Laurino et al. 2011). As well, among the agrochemicals known to be most toxic to bees are abamectin, chlorfenapyr, deltamethrin, and thiamethoxam (Rhodes and Scott 2006), either by topical or oral administration (Carvalho et al. 2009).

In a general sense, knowledge on the different effects that insecticides can have on pollinators are a worldwide concern especially in agricultural systems (Desneux et al. 2007; Barnett et al. 2007; Johnson et al. 2010; Pinheiro and Freitas 2010; Van Engelsdorp and Meixner 2010; Blacquiere et al. 2012).

Information on the toxicity of insecticide doses used in the melon crop in Brazil on the honey bee *A. mellifera* is very scarce despite the economic importance of this crop. However, this kind of information is necessary for the implementation of integrated pest management programs, which can assure the maintenance of these pollinators in the field. Therefore, this study was undertaken to evaluate the toxicity of insecticides commonly used in the melon crop in Brazil on adults of *A. mellifera*, in the maximum dosage for this crop allowed by the Ministry of Agriculture. We investigated the effect of nine insecticides and three exposure methods on bee mortality and behavior under laboratory conditions: abamectin, acetamiprid, cartap chloride, chlorfenapyr, cyromazin, deltamethrin, thiamethoxam, flufenoxuron, and pyriproxyfen.

2. MATERIAL AND METHODS

This study was carried out at the Laboratory of Applied Entomology at the Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, in the state of Rio Grande do Norte, Brazil. The bees used in the experiments were collected from a single colony at the apiary of the Cooperative APISMEL, in Serra do Mel, also in the state of Rio Grande do Norte and transported to the laboratory in screen-topped acrylic boxes. Three ways of exposure were used to evaluate the toxicity of insecticides on bees: directly spraying on adult bees, feeding bees with insecticide contaminated diet, and bee contact with insecticide residues on treated melon leaves.

The bees used in the experiments were placed in plastic containers (cylinders of 12.0 cm diameter× 9.0 cm height) (hereon called arenas) covered with voile cloth secured with rubber bands. Each arena constituted an experimental unit. Bees were offered a solution of honey and sugar in a plastic vial and also a cotton wick saturated with distilled water. Bees were anesthetized by cooling (4 °C for 1 min) before each experiment (for handling during spraying and/or placing inside the arenas). The tests were carried out in a climate-controlled growth chamber at 25±2 °C, 50±10 % RH, and photophase of 12 h.

We used the commercial products Mospilan®, Vertimec®, Thiobel®, Pirate®, Trigard®, Decis®, Actara®, Cascade®, and Tiger® as sources of the insecticides listed in the introduction. All products were soluble in water and solutions were prepared in distilled water

except abamectin (Vertimec®) that was mixed first with mineral oil (0.25 %), to reproduce exactly the field conditions. Insecticides were used at the maximum dosages recommended by the manufacturers (Table I). All solutions were prepared using distilled water. As control treatments, in the first assay, water was directly sprayed on the bees; in the second assay, the control treatment comprised only the solution of honey and sugar, and in the third assay, water was sprayed over the leaves. The experiment was carried out using a completely random design and each way of exposure comprised 10 treatments and 10 replications. Each replicate comprised 10 adult bees.

The mortality was assessed at 1, 2, 3, 4, 5, 6, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60, and 72 h after insecticide treatment, and the behavior (e.g., prostration, tremors, paralysis, etc.) of the bees was monitored and recorded from the first 30 min after spraying until the end of the experiment. Bees that did not respond to mechanical stimuli were scored as dead; nevertheless, they were maintained inside the arenas until the end of the experiment. The mechanical stimuli were applied by touching the body of the bees upon each evaluation, using a thin paint brush.

2.1. Direct spraying of insecticides on *A. mellifera*

After being anesthetized, groups of 10 bees were directly sprayed with the respective insecticides tested using a manual sprayer at 0.58 mL/s and an average spraying rate of 0.00583 mL/cm^2, simulating a field spraying. Bees were then placed in the arenas to assess the effects of insecticides until the end of the 72 h period.

2.2. Feeding *A. mellifera* insecticide contaminated diet

The diet (bee candy) was prepared using 20 mL of honey and 50 g of sugar, which were mixed and homogenized to form a paste. The insecticides were applied to the diet surface (7.06 cm^2) to simulate a field spraying. After the bees were placed in the arenas, they were fed the insecticide contaminated diet and water and were observed continually to confirm that they had ingested the food.

2.3. Contact of *A. mellifera* with insecticide contaminated melon leaves

For this way of exposure, some melon plants of the variety Orange Flesh were cultivated in a greenhouse. Plants with a minimum of four true leaves were selected, and five plants were used for each treatment. Using a manual sprayer at 0.58 mL/s and an average spraying rate of 0.00583 mL/cm^2, a field spraying was simulated so that the insecticide drops uniformly covered the entire foliar surface. The plants were then transferred to an airy and shaded room for 1 h to allow for the insecticides to dry. Three dry leaves were placed in each arena with the regular diet and water before the insects were released.

2.4. Statistical analysis

Data for survival of adults were analyzed using the package "survival" (Therneau and Lumley 2010) for the R software (R Development Core Team 2010) and subjected to a Weibull distribution analysis. Treatments with similar effects (toxicity and mortality speed) were grouped using contrasts. The lethal time 50 (LT_{50}) was also calculated for each group. Mortality percentages were calculated for each treatment in the three exposure methods and corrected using Abott's equation (Abbott 1925).

3. RESULTS

3.1. Effect of insecticides directly sprayed on *A. mellifera*

Thiamethoxam, deltamethrin, abamectin, acetamiprid, chlorfenapyr, and cartap chloride were highly toxic when directly sprayed on bees, killing between 80 and 100 % of the bees (Table II). However, there was a remarkable difference in the LT_{50} values for the different insecticides tested. Group 7 (thiamethoxam and deltamethrin) showed the lowest LT_{50} (1.00 h) (Figure 1). Abamectin (group 6) caused 100 % mortality in <10 h after spraying with a LT_{50} of 3.16 h (Figure 1). Group 5 (acetamiprid and chlorfenapyr), with a LT_{50} of 6.11 h, caused 95 and 100 % mortality in the first 15 h of assessment (respectively) (Figure 1). Cartap chloride (group 4), with a LT_{50} of 31.66 h, caused high mortality and killed 81.4 % of all bees 72 h

Table I. Insecticides used in the melon crop assessed to verify their toxicity to *Apis mellifera.*

Active ingredient	Chemical group	Actionmode	Dosage (g i.a./L)	Target pest
Abamectin[a]	Avermectin	Contact and ingestion	0.0180	*Liriomyza* spp.
Acetamiprid	Neonicotinoid	Sistemic	0.0600	*B. tabaci* biotype B
Cartap chloride	Bis (Thiocarbamate)	Contact and ingestion	1.2500	*Diaphania* spp.
Chlorfenapyr	Pyrazoleanalog	Contact and ingestion	0.2400	*Thrips palmi*
Cyromazin	Triazinamine	Sistemic and ingestion	0.9000	*Liriomyza* spp.
Deltamethrin	Pyretroid	Contact and ingestion	0.0075	*Diaphania* spp.
Thiamethoxam	Neonicotinoid	Sistemic	0.1500	*B. tabaci* biotype B
Flufenoxuron	Benzoylphenylurea	Contact and ingestion	0.1000	*Liriomyza* spp.
Pyriproxyfen	Pyridyloxypropylether	Contact	0.1000	*B. tabaci* biotype B

[a] Abamectin was mixed with mineral oil Assist (R) at 0.25 %

after the initial exposure (Figure 1). Unlike the other insecticides tested in this study, group 3 (pyriproxyfen) caused 30.2 % mortality and group 2 (cyromazin and flufenoxuron) caused <20 % mortality of *A. mellifera* (Table II); both groups had a LT_{50} higher than 100 h (Figure 1), but significantly different from control.

In the first hour after exposure to thiamethoxam, 100 % of honey bees showed prostration followed by death. The bees sprayed with deltamethrin immediately presented tremors followed by paralysis, characterizing knockdown effect, dying within the first hour. Abamectin caused a reduction in mobility, and bees showed slow movements until all were dead 6 h after spraying. Acetamiprid caused prostration followed by paralysis similar to the symptoms caused by thiamethoxam, but bees' probability of survival was significantly longer than the latter. Insects contaminated with chlorfenapyr showed no apparent motor disturbance within the first 30 min after spraying, but after the first hour 98 % of the bees started to move slowly, evolving to paralysis and subsequent death of all insects after 15 h from spraying.

After spraying of cartap chloride, paralysis was observed on 100 % of bees, with 57 % mortality within 2 h. The lasting 43% remained paralyzed for 10 h and then recovered movements, but continued

Table II. Mortality of *Apis mellifera* corrected using Abbott´s equation in each exposure method.

Active Compound	Mortality (%)		
	Direct spraying	Contaminated diet	Contaminated leaves
Abamectin	100	100	100
Acetamiprid	100	47.6	60
Cartap chloride	81.4	21.4	57.3
Chlorfenapyr	100	100	92
Cyromazin	19.8	28.6	30.7
Deltamethrin	100	45.2	72
Thiamethoxam	100	100	100
Flufenoxuron	17.4	64.3	54.7
Pyriproxyfen	30.2	16.7	34.7

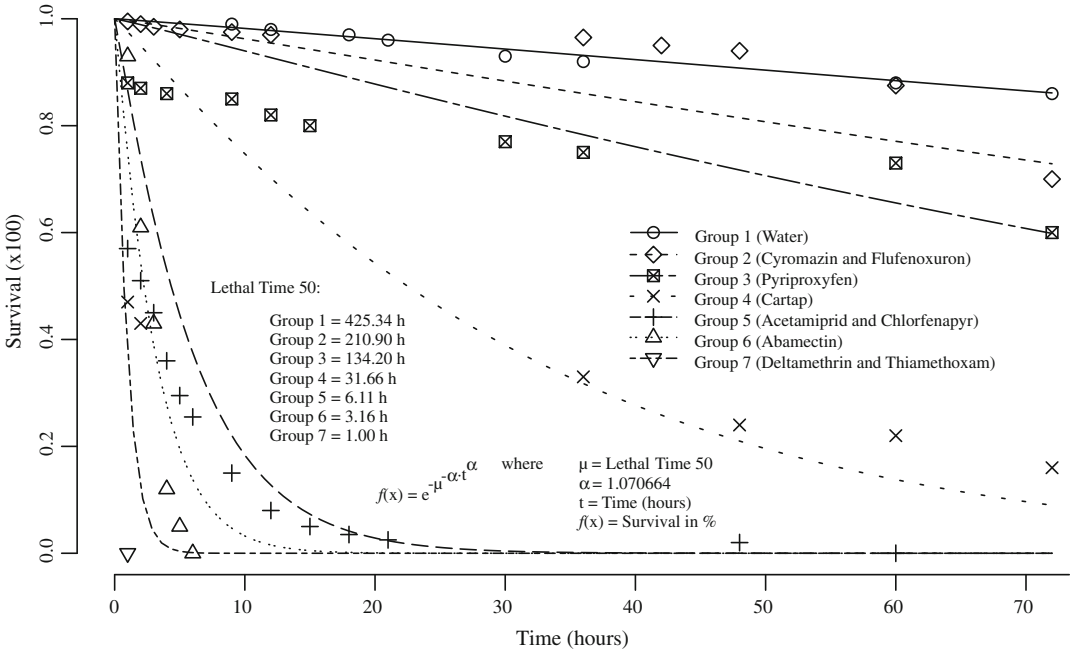

Figure 1. Survivorship (%) of *Apis mellifera,* after exposure to direct spraying with different insecticides, and lethal times (LT_{50}) in hours.

to die until the end of 72 h, where only 18.6 % remained alive. Regarding to pyriproxyfen, cyromazin, and flufenoxuron, there was no apparent disturbance. Bees kept moving and feeding normally.

3.2. Effect of insecticide contaminated diet on *A. mellifera*

In this way of exposure, group 6 (thiamethoxam) and group 5 (abamectin and chlorfenapyr) were the most toxic of all insecticides tested causing 100 % mortality to bees with LT_{50} of 1.51 and 7.77 h, respectively (Table II and Figure 2). This result demonstrated that these three insecticides were equally as toxic to *A. mellifera* by oral administration as by spraying. Group 4 (flufenoxuron) and group 3 (acetamiprid and deltamethrin) showed medium toxicity to bees, causing mortality rates between 45.2 and 64.3 % (Table II), with LT_{50} of 48.91 h for group 4 and 79.84 h for group 3 (Figure 2). Group 2 (cartap chloride, cyromazin, and pyriproxyfen) comprised the least toxic insecticides in this assay, with a LT_{50} of 166.83 h and significantly different from control (Table II and Figure 2).

The same behavioral symptoms observed in the direct spraying methodology were also recorded for ingestion, although their intensity and percent mortality were different for some insecticides in the two ways of exposure. For abamectin, chlorfenapyr, cyromazin, and thiamethoxam, symptoms had similar intensity and these insecticides also caused equivalent mortality as direct spraying.

Flufenoxuron scored a mortality of 64.3 %, being three times more toxic when ingested than when directly sprayed on the bees. Acetamiprid was much less toxic by ingestion than by direct spraying, with only 19 % of bees showing prostration and paralysis followed by death within the first 3 h after ingestion of the contaminated diet. After this period, no apparent disturbances were observed, but death continued to occur along the assessments. Regarding to deltamethrin, after ingesting the contaminated diet, 13 % of the bees rapidly showed paralysis, followed by death. The

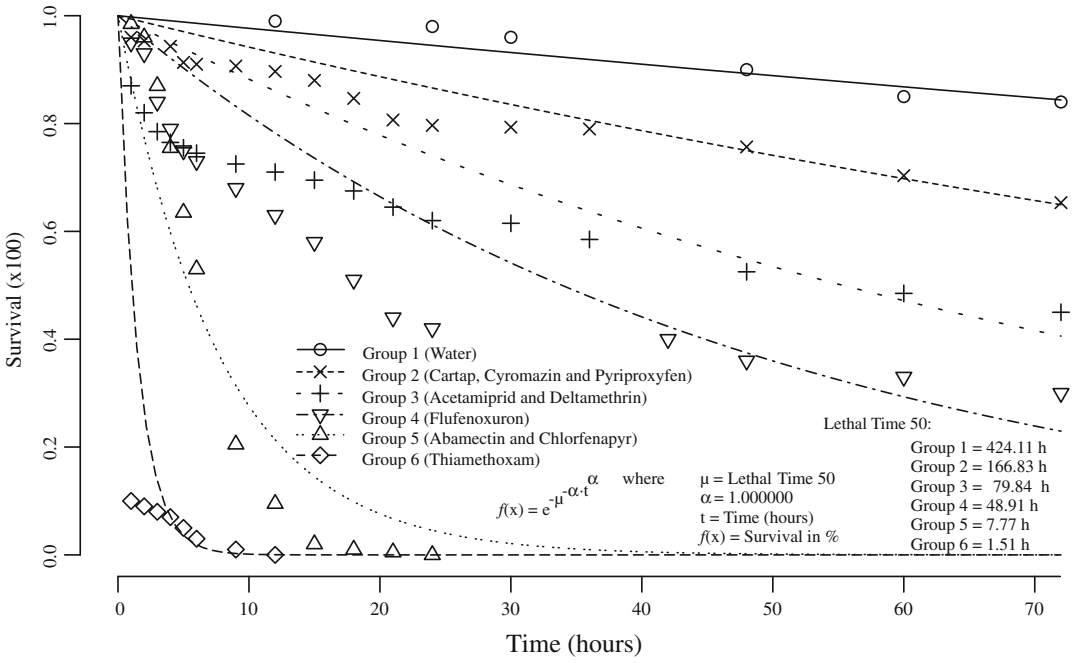

Figure 2. Survivorship (%) of *Apis mellifera* after ingestion of insecticide contaminated diet and lethal times (LT_{50}) in hours.

remaining insects (87 %) only displayed slow movements and kept dying until the end of the assessments, where only 54.8 % remained alive. Cartap chloride, although less toxic when ingested, also caused paralysis in the insects. After ingesting the contaminated diet with cartap chloride, bees exhibited slow movements, followed by tremors and eventually paralysis with 15 % death; however, they recovered their movements about 6 h later, but some bees still died until the end of evaluations. Pyriproxyfen was less toxic than in direct spraying, with only 16.7 % mortality and again did not cause any behavioral disorders.

3.3. Effect of insecticide contaminated leaves on *A. mellifera*

Group 7 (thiamethoxam), group 6 (abamectin), and group 5 (chlorfenapyr and deltamethrin) were the most toxic of all insecticides tested (Table II). However, thiamethoxam had the lowest LT_{50} of 2.61 h (Figure 3). Abamectin residues were also highly toxic and caused 100 % mortality after 36 h. However, the effect of abamectin was slower than that of thiamethoxam, and lethal effects were only observed from the 11th hour of observation with a LT_{50} of 18.45 h (Figure 3). Chlorfenapyr and deltamethrin killed, respectively, 92 and 72 % of the insects with a LT_{50} of 44.12 h (Figure 3).

Group 4 (acetamiprid and cartap chloride), with a LT_{50} of 60.89 h, and group 3 (flufenoxuron), with a LT_{50} of 78.49 h, caused mortality throughout the observation period, showing medium mortality rates ranging from 54 to 60 % (Figure 3). Cyromazin and pyriproxyfen, both from group 2 with a LT_{50} of 100.58 h (Figure 3), showed mortality rates of 30.7 and 34.7 %, respectively (Table II).

In this exposure method, we observed the same behavioral symptoms recorded for direct spraying and contaminated diet methodologies, although percent mortality was different for some insecticides. Similar mortality as in the previous exposures was observed for abamectin, chlorfenapyr, cyromazin, and thiamethoxam, and symptoms also had the same intensity. Regarding to deltamethrin, right after the contact with the contaminated leaves,

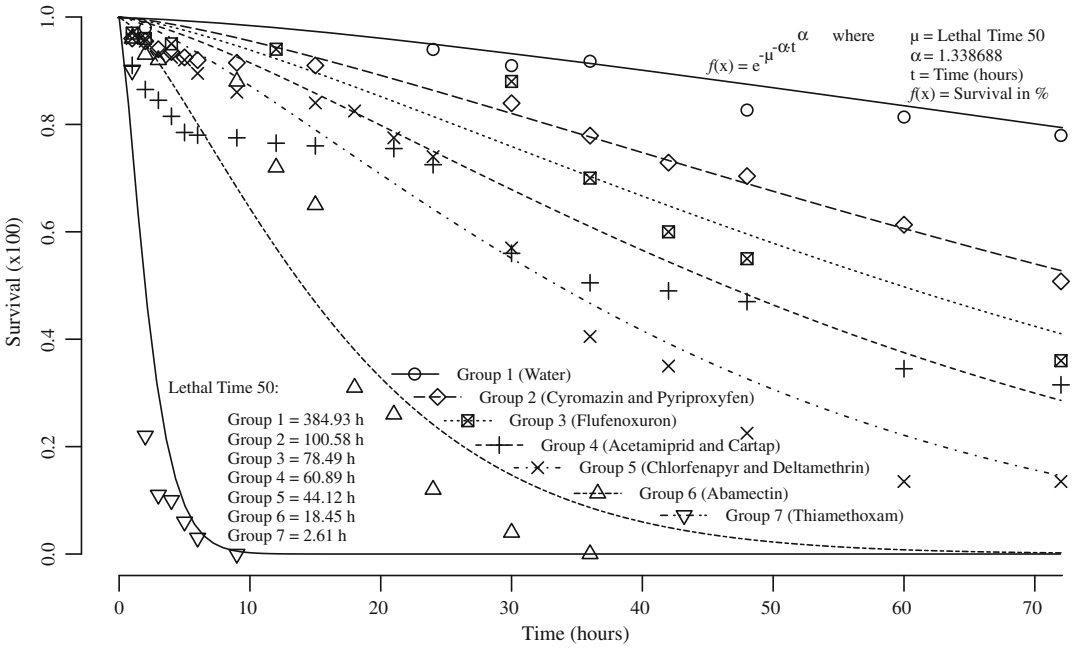

Figure 3. Survivorship (%) of *Apis mellifera* after contact with melon leaves contaminated with insecticide residues and lethal times (LT_{50}) in hours.

6 % of bees rapidly showed paralysis followed by death. The rest of the bees exhibited slow movements and kept dying along the assessment period, and only 28 % remained alive. Acetamiprid caused 7 % of bees to exhibit prostration followed by paralysis and subsequent death right after contact with contaminated leaves, but the remaining bees did not show disturbances, just died along the assessments until the end of 72 h, where only 40 % survived. Insects exposed to residues of cartap chloride showed limited mobility, and the great majority of deaths occurred after the 30th hour after exposure. Insects exposed to flufenoxuron and pyriproxyfen did not show behavioral disorders.

4. DISCUSSION

4.1. Effect of insecticides directly sprayed on *A. mellifera*

Our results showed that, at the dosage recommended for the control of pest insects on the melon crop in Brazil, the insecticides thiamethoxam, deltamethrin, abamectin, acetamiprid, chlorfenapyr, and cartap chloride were highly toxic to honey bees in direct contact assays. However, there were differences in the reaction of honey bees to the various compounds, probably due to the mode of action of each insecticide. The high toxicity of the neonicotinoid thiamethoxam to *A. mellifera* had already been reported in other studies (Iwasa et al. 2004; Rhodes and Scott 2006; Carvalho et al. 2009; Laurino et al. 2011). The symptoms of motor disturbance and prostration caused by thiamethoxam on honey bees are due to the effect of the compound on the synapses of the insect central nervous system (Kagabu 1997). The mortality data obtained for deltamethrin diverged from those found by Carvalho et al. (2009). In that study, authors applied a higher concentration than the one used in this study (0.0125 g a. i./L) and observed a knockdown effect; however, they concluded that deltamethrin was low toxic when sprayed on *A. mellifera*. In contrast, Fletcher and Barnett (2003) reported that pyrethroids such as deltamethrin were involved in the decline of bee populations in the UK. According to Nica et al. (2004), deltamethrin rapidly paralyzes the insect nervous system causing the knockdown

effect and eventually its death. The results obtained in this study regarding abamectin are similar to those reported by Carvalho et al. (2009), who observed a bee mortality of 99 %, 30 h after spraying. The LT_{50} (13.04 h) was lower than the one found in Carvalho et al. (2009), most likely due to the lower concentration of the active compound used by those authors. Two neonicotinoids, thiamethoxam and acetamiprid, even though highly toxic for *A. mellifera*, did not show the same speed of mortality. According to Iwasa et al. (2004), this difference could have been due to the effect of the nitro group of thiamethoxam, which makes its molecule 192 times more toxic to bees than those insecticides that have a cyano group, such as acetamiprid. Besides the direct effects, neonicotinoids can show sublethal effects on honey bees (Desneux et al. 2007; Carvalho et al. 2009; Laurino et al. 2011; Cresswell et al. 2012; Blacquiere et al. 2012; Henry et al. 2012). Our results corroborate previous findings that chlorfenapyr is a compound classified as harmful to bees and has a strong contact action; therefore, its spraying is not recommended during bee foraging activity (Ware and Whitacre 2004; Rhodes and Scott 2006). In the exposure methods tested in this study, cartap chloride caused temporary motor paralysis in the insects that did not die right after exposure. Paralysis is the main symptom of cartap chloride intoxication, which can be intensified and lead to insect death. This compound inhibits sodium ion conductance in the postsynaptic membrane and consequently blocks neural impulse transmission (Marçon 2011).

Pyriproxyfen, cyromazin, and flufenoxuron were of low toxicity to *A. mellifera*. Baptista et al. (2009) associated the low toxicity of pyriproxyfen to *A. mellifera* adult workers to the action mode of pyriproxyfen, which is a juvenile hormone analog. The results of our study regarding the treatment with pyriproxyfen corroborated those obtained by Baptista and colleagues. The low toxicity of cyromazin we report could also be associated to its mode of action. In addition to being a very specific product for dipteran control, cyromazin is more effective in the initial stages of insect development with a low contact effect (Friedel and McDonell 1985; Eto 1990). The results in the present study regarding flufenoxuron were similar to those reported by Carvalho et al. (2009) for lufenuron, both in the benzoylphenylurea group. The low mortality caused by flufenoxuron was possibly related to its mechanism of action. Flufenoxuron acts as a growth regulator inhibiting the synthesis of chitin and is therefore most effective on the juvenile phases (Ware and Whitacre 2004).

4.2. Effect of insecticide contaminated diet on *A. mellifera*

Thiamethoxam, abamectin, and chlorfenapyr were the most toxic of all insecticides tested and were equally as toxic to *A. mellifera* by oral administration as by spraying. According to Thompson (2003) and Desneux et al. (2007), not only does thiamethoxam cause direct damage to *A. mellifera*, but it also causes indirect damage, e.g. reduction of flight activity and olfactory ability in adults, also influencing in foraging and food storing. In the present study, it was noticed that the bees rapidly died soon after the loss of motor coordination, tremors, and prostration. Carvalho et al. (2009), evaluating a higher concentration of thiamethoxam, reported a 99 % mortality for adult workers of the honey bee 24 h after the initial ingestion. Abamectin toxicity in this study was similar to that reported by Carvalho et al. (2009), who classified this compound as extremely toxic to *A. mellifera* when ingested. It is known that chlorfenapyr, both by contact and ingestion, inhibits ATP synthesis by uncoupling active protons (H^+), affecting oxidative phosphorylation in the mitochondria and causing mortality (Ware and Whitacre 2004; Marçon 2011).

One member of the benzoylphenylurea group of insecticides was tested in our study. We found that flufenoxuron was more toxic to *A. mellifera* by ingestion than by contact. The results reported here were similar to those found by Carvalho et al. (2009) for the insecticide lufenuron. Acetamiprid and deltamethrin were of medium toxicity to bees when ingested. The behavior of acetaprimid in this way of exposure differed from the other neonicotinoid tested, thiamethoxam. This result emphasized the conclusions reached by Iwasa et al. (2004) regarding a lower toxicity of acetamiprid

on bees when compared to thiamethoxam. The results for deltamethrin in this study were similar to those reported by Carvalho et al. (2009) who found a LT_{50} of 64.65 h and a mortality rate of 67 % for *A. mellifera* at the end of the experiment at the concentration of 0.0125 g a.i./L of the pyrethroid. Authors also verified that deltamethrin caused reduction in the movements of the remaining bees, impairing locomotion and feeding. According to Ramirez-Romero et al. (2005), in addition to causing mortality, deltamethrin may significantly affect the ability of foraging.

Cartap chloride, cyromazin, and pyriproxyfen were the insecticides least toxic by ingestion tested in our study. The most likely explanation for the low toxicity of cartap chloride in this study is the fact that soon after they started feeding on the diet contaminated with this active compound, the bees rapidly showed temporary total motor paralysis. Approximately 6 h later, the surviving bees recovered their movement and resumed feeding, which induce another temporary total motor paralysis, repeating throughout the experiment. In this way overall ingestion was reduced due to the cyclical rounds of paralysis. The low toxicity of cyromazin in our study, even though it is a compound that shows a high toxicity via ingestion, could be explained by its dipteran specificity; furthermore, cyromazin acts on the initial stages of insect development and causes mortality when ingested by larvae (Friedel and McDonell 1985; Eto 1990). Pyriproxifen was also less toxic to *A. mellifera* in the study reported by Baptista et al. (2009).

4.3. Effect of insecticide contaminated leaves on *A. mellifera*

Leaves contaminated with thiamethoxam, abamectin, chlorfenapyr, and deltamethrin were highly toxic to honey bees. Even being an insecticide readily absorbed by the plant, it was found that the contact with leaves contaminated with thiamethoxam resulted in a similar effect to what was verified in the other two ways of exposure, in which the insecticide was either directly applied on the bees or provided via insecticide contaminated diet. The results in this study regarding the effect of leaves contaminated with thiamethoxam on honey bees were similar to those obtained by Iwasa et al. (2004) and Thomazoni et al. (2009). These authors assessed the residual effect of thiamethoxam using alfalfa and cotton, respectively, and confirmed the high toxicity of contact with residues of the insecticide thiamethoxam to bees. We found abamectin residues to be highly toxic to bees, albeit with a slower effect when compared to the other insecticides tested herein. Carvalho et al. (2009) also detected high toxicity for abamectin residues on citrus leaves to *A. mellifera*, reporting a mortality of 88 % at the end of the evaluation. Chlorfenapyr can cause high mortality rates in insects due to its inhibiting action on ATP synthesis (Marçon 2011) and is considered harmful to bees (Rhodes and Scott 2006). The toxicity of the pyrethroid deltamethrin was also reported by Nica et al. (2004), Rhodes and Scott (2006), and Carvalho et al. (2009).

Acetamiprid and cartap chloride caused mortality throughout the observation period. Contact with the acetamiprid was considered toxic to bees (Iwasa et al. 2004). Acetamiprid can cause hyperexcitation of the insect nervous system and result in the collapse of the central nervous system and subsequent death among other effects (Marçon 2011). Exposure to cartap chloride residues caused a decline of honey bee motor movements, and more bees showed the same decline in movement over the test. Such an effect was probably due to the interaction of cartap chloride with acetylcholine receptors, which results in a modification of receptor conformation. This leads to the inhibition of sodium ion conductance in the post-synaptic membrane and consequent blockage of neural impulse transmission. Neural impulse blockage displays as paralysis and is the main intoxication symptom; and paralysis may eventually lead to death (Marçon 2011). The results in this study regarding cartap chloride were similar to those reported by Thomazoni et al. (2009), who assessed the residual effect of cartap chloride on cotton leaves and verified that it is highly toxic to *A. mellifera*. Unexpectedly, due to its mode of action and contrary to what was verified in the first way of exposure, flufenoxuron caused considerable mortality.

This study shows that some commonly used insecticides in conventional melon crop production

systems have negative impacts on honey bees. Thiamethoxam, abamectin, and chlorfenapyr were highly toxic to adults of *A. mellifera* independent of the exposure method. Acetamiprid, deltamethrin, and cartap showed higher toxicity when directly sprayed on bees. Cyromazin, pyriproxyfen, and flufenoxuron were the least toxic to *A. mellifera* and caused low mortality rates. There was an exception for flufenoxuron when ingested, which caused medium rates of mortality. Thus, our results can be used as guidelines regarding which insecticides and spraying methods may be toxic to these insects. This information may assist during necessary insecticide spraying, such that this may be carried out in a manner incurring the least negative impact to pollinators.

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Toxicité des insecticides utilisés en cultures de melon au Brésil vis-à-vis de l'abeille *Apis mellifera*, en conditions de laboratoire

Conservation / mortalité / organisme non-cible / pollinisateur / traitement phytosanitaire

Toxizität von im brasilianischen Melonenanbau eingesetzten Insektiziden für die Honigbiene *Apis mellifera* unter Laborbedingungen

Schutz / Mortalität / Nicht-Ziel Organismen / Bestäubung / Pflanzengesundheitsbehandlung

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