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Original Article

The Longevity Of Africanized *Apis mellifera L.* Influenced By Synthetic Phytosanitary Products Used In Soybean

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Abstract

Honey bees are the main pollinators, but they are in decline and synthetic phytosanitary products (SSPs) are suspected to be related. Therefore, this study aimed to evaluate the effect of three commercial SPPs, used to control the stink bug in soybean, formulated with Acetamiprid + Alpha-cypermethrin; Imidacloprid + Beta-Cyfluthrin and Fenitrothion + Esfenvalerate, on adult worker Africanized *Apis mellifera*. For this, worker honey bees was exposed to SPPs through three bioassays: 1) sprayed on the bees, 2) sprayed on Petri dishes in which the bees was subsequently placed, and 3) mixed with the Candy paste supplied to the bees. The honey bees in bioassay 3 had their midgut submitted to histopathological analysis. All bioassays was maintained in a climatized room (34 ± 2ºC, RH 60 ± 5%) and the workers longevity was verified at 1 up to 120 hours. In bioassay 1 at the time of 6 hours evaluation, 100% of the bees of all the SPPs was dead. In bioassay 2 all bees was dead after two hours of exposure of the three SPP. In bioassay 3 the SPP1 and SPP2 caused 100% mortality after 60 hours of exposure, to theSPP3 all bees was dead after 96 hours, while for the control, after 96 hours only 45% of the bees were dead. Histological analysis revealed the destruction of the midgut cells of all workers from the three SPPs treatments. The three evaluated SPPs showed no selectivity for *A. mellifera* workers, reducing bee longevity.

Keywords: Honey bee, insecticide, mortality, selectivity.

INTRODUCTION

Honey bees are of great interest, being responsible for the production of honey, royal jelly, propolis, beeswax (Costa-Maia *et al.*, 2010), and especially in agricultural environments, because they are important pollinating organisms, contributing to increase the yield and quality of seeds and grains, increasing the size, weight, quality (acidity, volume juice, sugar content) and number of fruit, and reduce deformities and enable uniform ripening of these (Calderone, 2012; Costa-Maia *et al.*, 2010; Giannini *et al.*, 2015; IPBES, 2016; Potts *et al.,* 2010; Sanford, 2011) the cultivated area (hectares. Thus, these insects ensure the balance and maintenance of agricultural cropping systems, in addition to providing an

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increase in production even in autogamous plants, such as in soybean culture (Glycine max), when pollinated by bees, it presents an increase of up to 37% in productivity of grains compared to unpollinated soybeans (Chiari *et al.,* 2008).

However, with the intensification of agricultural production, especially due to the increased use of SPPs, there were the first reports of a decrease in bee populations (Grixti *et al*., 2009). However, research relating the effects of SPPs on bees was intensified only from the year 2006, due to the first reports of Colony Collapse Disorder (CCD) (Abati *et al*., 2021). CCD is characterized by the disappearance of bees, without the presence of dead bees in the vicinity of colonies (Kaplan, 2012; VanEngelsdorp *et al.*, 2017), and in addition, another recurrent case in recent years is the mortality of bees, where they are found dead containing traces of SPPs (Grigori, 2019; MAP, 2017).

In this context, it has been observed that synthetic chemical insecticides are closely related to CCD and bee mortality, as most of them do not show selectivity to non-target organisms such as pollinators. Insecticides have the potential to cause the immediate death of bees, and can also cause negative effects even in sublethal doses and still interfere with colony functioning (Amaro & Godinho, 2012; Catae, Roat, *et al.*, 2018; Simone Tosi *et al*., 2017; Wolff *et al.*, 2008). In this sense, among the studies carried out related to the exposure of bees to SPPs, between the years 1945 to mid-2020, approximately 58% investigated the possible occurrence of sublethal effects and 27% investigated the lethal effects (Abati *et al.*, 2021). However, most studies are focused on evaluating chemical groups of SPPs in isolation.

In some countries, such as Brazil, chemical insecticides are marketed containing more than one chemical group, in order to increase the spectrum of action and efficiency in pest control. Among the products used in recent years is the compound Acetamiprid + Alpha-cypermethrin, and Imidacloprid + Beta-cyfluthrin the both a neonicotinoid and a pyrethroid that act as a systemic and contact insecticide, used against the neotropical stink bug (*Euschistus heros* Fabr., 1974; Hemiptera: Pentatomidae), and contact and ingestion insecticides belonging to the chemical group of organophosphates and pyrethroids (Fenitrothion + Esfenvalerate) are also used for controlling soybean-stinkbugs (Agrofit, 2022). These insecticides when used alone and in sublethal doses affects the learning of bees, reducing the ability of *A. mellifera* workers to perform activities, especially the activities determined by age group within the colonies and reduces bee longevity (Laurino *et al.*, 2011; Thany *et al.*, 2015). However, the application of mixtures of different chemical groups of SPPs can cause synergistic or antagonistic effects on honey bees, aggravating the negative effects on these pollinators (Wang *et al.*, 2020).

So it becomes necessary of constantly studying the impact of synthetic phytosanitary products released and used in agriculture on non-target organisms. It is also necessary to test these products and solutions on africanized *A. mellifera* due to its importance in pollination and in the beekeeping industry. Therefore, the aim of this study was to evaluate the effects of three SSPs, widely used in annual crops for pest control, simulating in the laboratory the possible presence of Africanized *A. mellifera* worker bees in the field at the time of application (direct spraying), or shortly after (contact and feeding).

MATERIAL AND METHODS

This study was conducted at the Universidade Tecnológica Federal do Paraná (Federal University of Technology – Paraná), Campus Dois Vizinhos (UTFPR-DV), at the Biological Control Laboratory I and II, and Apiculture Education and Research Department (UNEPE-Apicultura).

Apis mellifera specimens

Frames with capped brood chambers of 19-day Africanized *A. mellifera* workers were obtained from the Apiary of the Apiculture Education and Research Department (UNEPE-Apicultura), from four different colonies. The frames were transported to the Biological Control Laboratory I and stored in sealed and punched Kraft paper bags (weight 50), rolled in voile-type fabric, and kept in a chamber climatic (34 ± 2ºC, 60 ± 5% RH) for two days. This procedure was performed so that the emergence of the workers would be uniform for use in the bioassays, simulating the environment of the colony of origin and using bees with 24 to 48 hours. The workers used in the bioassays were Candy paste (50 g of confectioners' sugar + 10 ml of pure honey, forming a homogeneous mass).

Synthetic phytosanitary products

Three commercial SPPs was provided by an agricultural inputs company in Dois Vizinhos - Paraná. All products were prepared, respecting the dosages indicated by the manufacturers used to control the stink bug in soybean (Table 1).

Table 1. Synthetic Phytosanitary Products (SPPs) used, active ingredient, chemical group, indicated crop, target insects, dosage, and syrup.

Product	Active Ingredient/ Concentration	Functional group	Crop	Insects	Dosage	Syrup (SPP+H_2O)
SPP1	Acetamiprid (10% m/v) / 100g/L + Alpha-cypermethrin (20% m/v) / 200g/L	Neonicotinoid + Pyrethroid	Irrigated rice and soy bean	Southern-green-stink-bug Soya bean-brown-stink-bug	300 mL/ha 200L of syrup/ha	0,15 mL of product + 100 mL of deionized sterilized water
SPP2	Imidacloprid (10% m/v) / 100g/L + Beta-Cyfluthrin (1,25% m/v) / 12.5 g/L	Neonicotinoid + Pyrethroid	Cotton, potato, bean, melon, corn, soybean, tomato, and wheat	Whitefly, Boll weevil, Cucurbit beetle, Potato aphid, Fall armyworm, Stink bugs, Soya bean caterpillar (Anticarsiagem-matalis)	750 mL/ha 200L of syrup/ha	0,38 mL of product + 100 mL of deionized sterilized water
SPP3	Fenitrothion (80% m/v) / 800g/L + Esfenvalerate (4% m/v) / 40 g/L	Pyrethroid + organophosphate	Cotton, onion, chrysanthemum, and soybean	Boll weevil,Thrips, and stink bugs	300 mL/ha 200L of syrup/ha	0,15 mL of product + 100 mL of deionized sterilized water

All products are commercial and used in soybean for bed bug control.

Source: Agrofit (2022).

Bioassay 1. Spraying of synthetic phytosanitary products on A. mellifera

The *A. mellifera* workers were anesthetized for 120 seconds with CO_2, put in Petri dishes and then sprayed with the solutions (290 µL in each Petri dish, based on the area of the plaque in which the liquid was sprayed) using a Pneumatic Sagyma® airbrush coupled to a Fanem® pump at a constant pressure of 1.2 kgf/cm. Then, ten worker bees was transferred to plastic containers (100mm X 120mm), each container counted as a replication, totaling six replications per treatment. The containers was sealed with voile-type fabric and supplied with candy paste and a piece of cotton soaked in distilled water. The spraying of sterilized deionized water was the control and each treatment consisted of one of the SPPs prepared according to dosages indicated by the manufacturers. The experiments were kept in a chamber climatic (34 ± 2 ºC, RH 60 ± 5%) and the bees mortality was evaluated at 1, 2, 3, 4, 5, 6, 12; 15; 18; 21; 24; 30; 36; 42; 48; 60; 72; and 96 hours after spraying the products (adapted from Baptista *et al.*, 2009; Colombo *et al*., 2020; Libardoni *et al*., 2021).

Bioassay 2. Contact of A. mellifera with surface sprayed with synthetic phytosanitary products

We used glass Petri dishes, 15 cm in diameter × 1,5 cm in height, sprayed with 290 µL of the SPPs using a Pneumatic Sagyma® airbrush coupled to a Fanem® pump at a constant pressure of 1.2 kgf/cm. The volume to be sprayed was determined based on the total area of the dish. Subsequently, these Petri dishes were arranged in a horizontal laminar flow chamber for the complete evaporation of liquids.

The honey bees' workers were anesthetized with CO_2 for 120 seconds, distributed in groups of 10, and placed in previously sprayed Petri dishes. The dishes were mounted so that the mouths almost fitted, but preventing the workers from passing through and allowing air flow (methodology adapted from Carvalho *et al.* (2009); Potrich *et al*. (2020)). The bees remained for two hours exposed to the products and were then transferred to plastic containers and supplied with fondant and a cotton pad soaked in water.

Each plaque counted as one replication, totaling six replications per treatment. Sterilized deionized water was sprayed over the control. The experimental conditions and the parameters evaluated were the same as in Bioassay 1.

Bioassay 3. Candy paste containing synthetic phytosanitary products

We anesthetized the honey bees' workers with CO_2 for 120 seconds, then individually packed them in a flat glass bottom tube (2.5 x 8.5 cm), sealed with voile-type tissue and supplied with a piece of cotton soaked in distilled water. We added to the Candy paste a SPP according to each treatment. Each group of ten tubes counted as one replication, totaling six replications per treatment. The control was composed of pure Candy paste (metodology adapted from Colombo *et al*. (2020); Potrich *et al*. (2020)). The other experimental conditions, the evaluated parameters, and the analysis of the data were the same described in Bioassay 1.

Randomly, we selected eight dead workers in each treatment for histological analysis of the mesenteric (worker deaths occurred due to exposure to SPPs and the bees were dissected immediately after death to prevent cell autolysis, the control bees were also dissected after death). For this, the mesenteries were dissected from the workers, fixed in Bouin Fixer (250 mL of formaldehyde 40%+ 50 mL glacial acetic acid PA + 750 mL of saturated solution of picric acid 1.4%) for 4 h, washed in alcohol 70% (3 × 15 minutes) and stored in alcohol 70% until the histological procedure.

The samples were dehydrated through baths of progressively more concentrated ethyl alcohol and diaphanized by immersion in Xylol, followed by paraffinization and embedding in Histological Paraffin (Histological Paraffin/ Beeswax 4:1). The embedded material was cut with a Manual Rotating Microtome, in sections of 2 to 7 µm thickness, and stained by the H/E (Hematoxylin/Eosin) method.

We pre-screened the slides containing the sections with a Binocular Biological Microscope, Zeiss Primo Star®, with a digital camera for image capture, using a 40X magnification lens. The tissues of *A. mellifera* workers fed Candy paste containing the SPPs were compared with the workers of *A. mellifera* fed with pure Candy paste.

Statistical analyses

For the longevity data of the *A. mellifera* workers in the three bioassays we realized a survival analysis using the Kaplan-Meier method. The treatments were compared using the log-rank test, 95% confidence interval, and the entire analysis were performed using the package *survival* (Therneau, 2020) from R software (R core Team, 2019).

RESULTS

In bioassay 1, three synthetic phytosanitary products evaluated – SPP 1 - Acetamiprid + Alpha-cypermethrin; SPP 2 - Imidacloprid + Beta-Cyfluthrin; and SPP 3 - Fenitrothion + Esfenvalerate – reduced the longevity of *A. mellifera* workers, in relation to control, in the direct spraying bioassay. Bees from the SPP 1 and SPP 2 treatments died with one hour. The amount of 88% of bees, from SPP 3 treatment, died after 5 hours of exposition. After 6 hours, all the bees were dead, differing from the control bees (at the end of 96 hours 75% of the bees were still alive).

In bioassay 2, bees were exposed for two hours to the products in the Petri dish, and then they would be relocated in plastic containers. However, at the end of two hours, 100% of the bees that had direct contact with the three SPPs were dead. Bees that were exposed to the Petri dish sprayed with sterilized deionized water (control) showed a 93.33% survival at the end of 96 hours.

The three SPPs evaluated, when mixed into candy paste, reduced worker longevity compared to the control ($p < 0.05$) in bioassay 3. At the end of 60 hours of observation, the bees that were fed Candy paste mixed with SPP 1 and SPP 2 presented 100% mortality; and, at the end of 96 hours, the bees that were fed Candy paste mixed with SPP 3 presented 100% mortality (Figure 1). While the control at the end of the 96 hours presented 45% mortality of the bees.

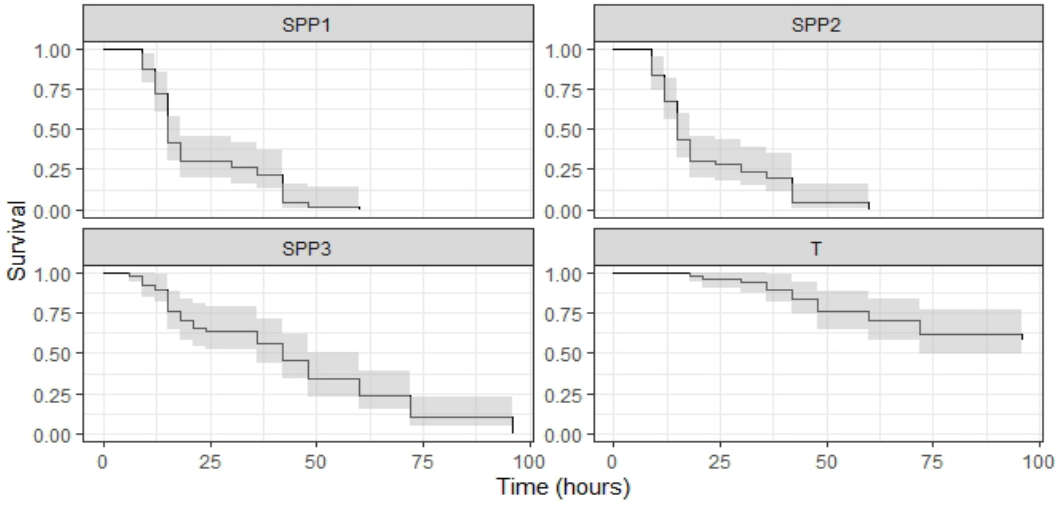

Figure 1. Kaplan-Meier survival after feeding of adult *Apis mellifera* workers with the different synthetic phytosanitary products

Survival plot adjusted to the period (hours). T: Control; SPP 1 - Acetamiprid + Alpha-cypermethrin; SPP 2 - Imidacloprid + Beta-Cyfluthrin; and SPP 3 - Fenitrothion + Esfenvalerate. Temperature (34 ± 2 °C, RH 60 ± 5%). UTFPR, Campus Dois Vizinhos, PR, 2016. SPP: Synthetic phytosanitary product. Different letters indicate significant differences at $p < 0.05$.

The histopathological analysis showed alterations in the tissue organization and structure of the midgut epithelium of the workers of *A. mellifera* that were fed the three SPPs (Figures 2B, C and D) when compared to the tissue integrity of the coating epithelium of the midgut of the bees fed pure candy paste (control - Figure 2A). We found that the SPPs, at the concentrations tested, caused necrosis in the epithelium of the mesentery of *A. mellifera* (compare Figure 2A with the others). Although was not performed specific test for apoptosis, some cells with apoptotic cell characteristics were observed, due to the presence of pyknotic nuclei in the samples of the treated groups.

DISCUSSION

Through bioassays carried out in the laboratory, we can observe how synthetic phytosanitary products behave on bees in a controlled environment. In general, toxicity assessments after acute SPP exposure to bees are standardized and follow OECD protocols, especially through the provision of treatments, individually, orally and also by micropipetting treatments on the thorax of each bee (OECD, 1998)possessing as many as 4 disulfide bonds. The elements of antiparallel β-structure take origin from the hydrophobic core formed by the disulfides. The β-strands adopt the

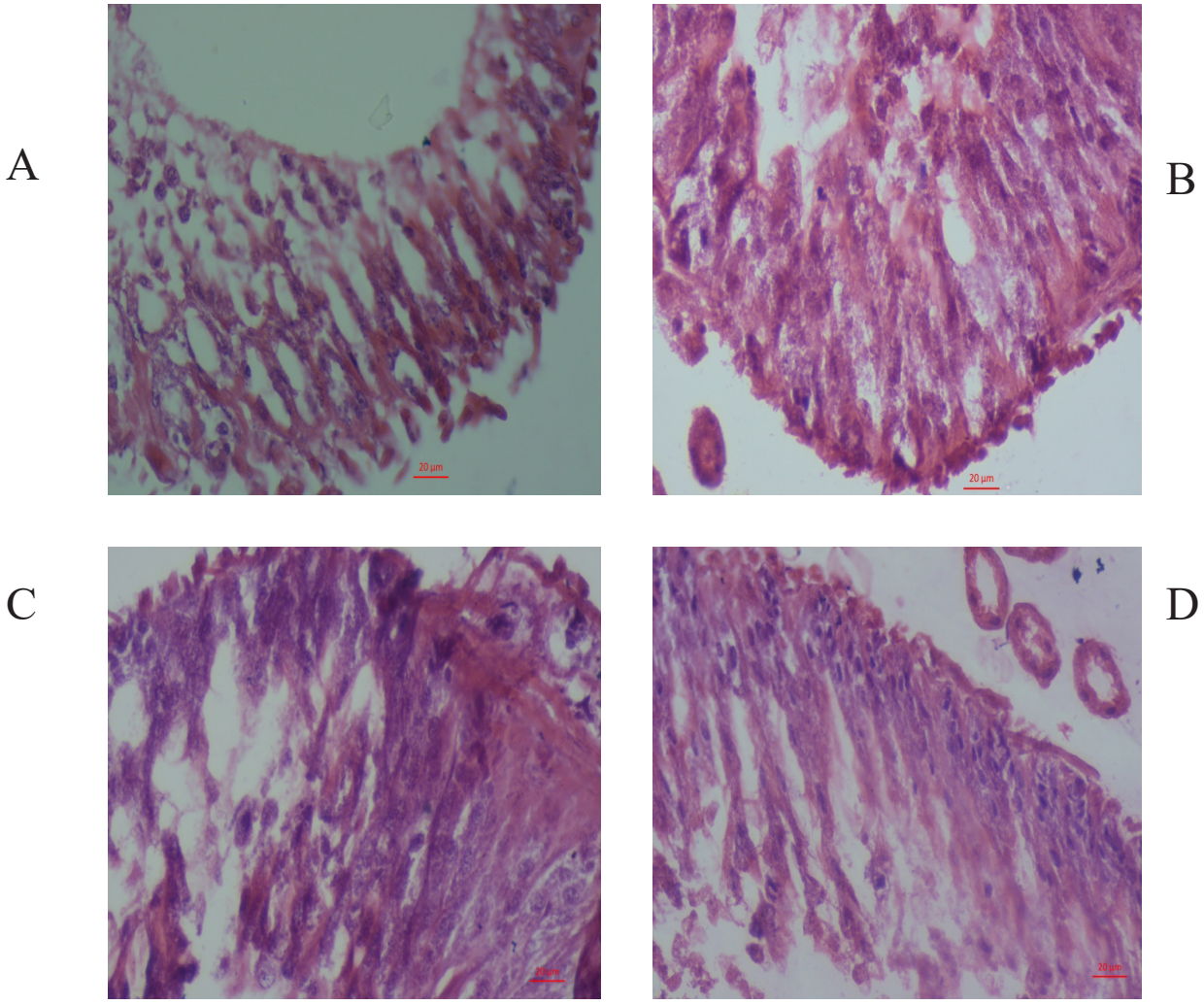

Figure 2. Photomicrographs of the midgut of *Apis mellifera* after feeding with the different synthetic phytosanitary products

(Binocular Biological Microscope, Zeiss Primo Star, with digital camera for image capture using a 40X magnification lens). Worker bees supplied with: A) pure candy paste; B) candy paste + SPP 1: Acetamiprid + Alpha-cypermethrin; C) candy paste + SPP 2: Imidacloprid + Beta-Cyfluthrin; D) candy paste + SPP 3: Fenitrothion + Esfenvalerate. The midgut epithelium of *A. mellifera* supplied with fondant mixed with SPPs showed tissue disorganization and disruption, verified by the presence of necrosis, due to the action of the products (compare with A). In addition, it was also possible to observe cell nuclei with characteristic of picnosis (arrows). e = Epithelium; c = Mesenteric cavity; circulated areas = Necrosis regions; arrows = Pyknotic nuclei.

shape of the three loops, giving the name of the fold. While neurotoxins (NTs. However, in this work we sought to verify in the laboratory the possible ways of exposing bees to synthetic phytosanitary products in the field, simulating their presence in the field at the time of application or soon after (Carvalho *et al*., 2009; Colombo *et al.*, 2020; Potrich *et al*., 2020).

Our results demonstrate that the presence of bees in the field at the time of application (direct spray bioassay), can cause the mortality of 100% of the bees sprayed with SPP 1 and SPP 2, after one hour, both products consisting of neonicotinoid + pyrethroid. As in the contact bioassay, where the three SPPs caused bees mortality at the end of two hours of contact. Insects exposed to neonicotinoids, which bind to the acetylcholine receptors of pos synaptic neurons, show a state of hyperexcitement (Gallo *et al*., 2002; Simon-Delso *et al.*, 2015; Taillebois *et al.*, 2018).

Neonicotinoid products were tested for selectivity on different bees, either by direct spray or contact in Petri dishes. It was found that the neonicotinoid thiamethoxan (150g a.i/100L H_2O) caused 100% mortality in africanized *A. mellifera* workers, 9 hours after sprayed (Carvalho *et al.*, 2009), 56% mortality after 1 hour of contact (Carvalho *et al.*, 2009) and 100% mortality of *A. mellifera* and *A. cerana* after 48 hours of exposure (Stanley *et al*., 2015). This shows that even varying the mode of contact and the species of the bees the SPPs are toxic to them, causing a marked mortality. Besides that, *A. mellifera* workers, after exposure to neonicotinoid thiamethoxan (1.34ng), for 30 and 60 minutes, present difficulty in locomotion and falls, which makes it difficult to perform foraging efficiently (Tosi & Nieh, 2017), this demonstrates that even at lower doses than the present study, this insecticide can cause behavioral changes in honey bees.

The exposure of colonies of *A. mellifera* bees to neonicotinoids can affect the reproductive capacity of the males (drones), thus affecting the development of the colony, since they are the ones that fertilize the queen bee. Thiamethoxam and clothianidin, both neonicotinoids, had no significant effects on body mass and sperm count, but there was a reduction in the lifespan of the drones, as well as a reduction in sperm viability and in the living sperm count by 39% (Straub et al., 2016)their possible impact on male insect reproduction is currently unknown, despite the key role of sex. Here, we show that two neonicotinoids (4.5 ppb thiamethoxam and 1.5 ppb clothianidin. This demonstrates that neonicotinoid insecticides, in addition to direct toxicity, can have sublethal effects, such as having a contraceptive effect on non-target insects.

When evaluating the action of the neonicotinoid imidacloprid incorporated into the diet of worker bees, at sublethal doses corresponding to 0.014651 ng imidacloprid μL^{-1}, after 8 days of exposure, it was possible to verify biochemical changes in bees brains and when comparing the treated bees with the controls, it was possible to verify modifications in synapse regulation and apoptosis and oxidative stress (Catae *et al.*, 2018). Probably, this effects also occur in workers of *A. mellifera* tested in this bioassay.

Carvalho *et al.* (2009) showed that the neonicotinoid thiamethoxam mixed with the feed caused 99% mortality in *A. mellifera* after 24 hours and that the organophosphate methidathion insecticide caused 100% mortality after consumption of a contaminated Candy paste (15 hours after the start of the bioassay). These values differ from those observed in our study, which showed 100% mortality after 60h or 96h of ingestion. Abbo et al. (2017) also found that imidacloprid (neonicotinoid) has a negative effect on the health and survival of *A. mellifera*, like reduction in the titer of vitellogenin, which regulates the honey bee development and behavior and could be affect the energy homeostasis in bees.

In many cases, bee survival or mortality are not directly affected by exposure to SPPs. Sublethal effects may occur, such as in the case of exposure of *A. mellifera* larvae to thiamethoxam (neonicotinoid - 0.001 ng/mL) which, according to Friol *et al.* (2017), does not influence the larval and pupal survival or the emergence percentage of adult bees. However, this exposure causes ultra-structural changes in target and non-target organs of newly emerged bees. The digestive cells and mitochondria were compromised indicating tissue degradation, thus confirming the toxicity of the SPPs used and suggesting harm to the cellular function of midgut cells and, consequently, to the longevity of the bees and of the whole colony when exposed to this product.

The analysis of the tissues of bees that were exposed to chemicals is a tool to evaluate the toxicity and the effect of these products on this insect. In this sense, Gregorc and Ellis (2011) verified that larvae of *A. mellifera* bees treated with imidacloprid (neonicotinoid) presented high cellular death by apoptosis in the midgut, salivary glands, and ovaries. Although it was not quantified, apoptosis was observed in tissues of the midgut of workers that were fed with Candy paste mixed with SPPs in the bioassay. Oliveira et al. (2014) analyzed Africanized *A. mellifera* exposed to sublethal doses of thiamethoxam (neonicotinoid) [1/10 and 1/100 of LC_{50} (4.28 ng of active ingredient/µL of feed)] and they presented morphological and histochemical changes in the digestive and regenerative cells of the midgut, such as vacuolization of the cytoplasm, increase in apocrine secretion, and in cellular elimination, contributing to reduce their survival. Aljedani (2017) also observed this when he performed histological analysis of the midgut of *A. mellifera jemenatica* bees fed with sublethal doses of deltamethrin (pyrethroid) and verified digestive disorders and epithelial tissue with morphological alterations.

Pyrethroids affect the sodium channels of the central and peripheral nervous system of the insects, causing them to be constantly open, and the subsequent influx of sodium triggers membrane depolarization and the opening of the calcium channels, also leading to hyperexcitementin insects (Gallo *et al.*, 2002; Li *et al.*, 2015).

When studying the main products that cause risks to *A. mellifera*, it was possible to observe, by contact, the molecule Esfenvalerate, presented moderate risks, while

Beta-cyfluthrin was low risk. Furthermore, when evaluating the risk of ingestion, it was verified that Beta-cyflutrin is also low risk (Sanchez-Bayo & Goka, 2014). The opposite result was observed in the present study, when these were associated with another molecule, however, it should be considered that these products are not used alone in the field.

The pyrethroid fluvalinate was found in the wax of colonies of *A. mellifera* exposed to it, influencing the accumulation of residues in workers' tissues over time. It is not clear whether the presence of this SPP at the detected levels may induce physiological or behavioral effects on these bees, but the buildup of residues in the tissues may be cumulative due to a deficiency in the detoxification enzymes encoded in the bee genome (Boyle & Sheppard, 2017). Analysis of the presence of SPPs in honeycomb wax from honey bee colonies in Belgium has shown the presence of residues of almost 300 organochlorine and organophosphorus compounds and 18 SPPs used in local agriculture, including the presence of products banned in Europe (Ravoet *et al.*, 2015). In addition, when performed locomotion test with the pyrethroids can be said that they are as toxic as a neonicotinoid for bees and, compromising the locomotion and location of bees in laboratory tests (Charreton *et al.*, 2015)in particular phenylpyrazoles and neonicotinoids, given that they are widely used and highly toxic for insects. Along with their ability to kill insects at lethal doses, they can compromise survival at sublethal doses by producing subtle deleterious effects. In this study, we compared the bee's locomotor ability, which is crucial for many tasks within the hive (e.g. cleaning brood cells, feeding larvae. . ..

The organophosphates can act by contact or ingestion, inhibiting the enzyme acetylcholinesterase and causing an accumulation of acetylcholine in the synapse, thus generating a hyperexcitement of the nervous system that leads to a cholinergic syndrome (Gallo *et al.*, 2002). In this sense, studies indicate that the immune system of the bees, as well as the metabolic system can suffer harmfull alterations when bees (*A. mellifera carnica*) are exposed to organophosphates chlorpyrifos (Christen & Fent, 2017).

In the tests performed by Al Naggar *et al.* (2015) to identify and quantify the organophosphorus molecules present in honey, pollen and worker bees during spring and summer, higher concentrations of pesticides of this class were observed in pollen, ranging from 0.2 to 26.4 ng/g of pollen, although this number is worrying, the concentrations found in the study in question were not considered toxic to the colonies.

Forage worker bees, when exposed to SPP while foraging, transporting them into the colonies, which can lead to their disappearance in more extreme situations (Gill *et al.*, 2012) so it is important to understand and mitigate the causes of current declines in bee populations. Recent studies have implicated the role of pesticides in these declines, as exposure to these chemicals has been associated with changes in bee behaviour and reductions in colony queen production. However, the key link between changes in individual behaviour and the consequent impact at the colony level has not been shown. Social bee colonies depend on the collective performance of many individual workers. Thus, although field-level pesticide concentrations can have subtle or sublethal effects at the individual level, it is not known whether bee societies can buffer such effects or whether it results in a severe cumulative effect at the colony level. Furthermore, widespread agricultural intensification means that bees are exposed to numerous pesticides when foraging, yet the possible combinatorial effects of pesticide exposure have rarely been investigated. Here we show that chronic exposure of bumblebees to two pesticides (neonicotinoid and pyrethroid. Bees are believed to bring with them pollen and nectar containing the products that end up contaminating the entire colony when they return from foraging (Sanchez-Bayo & Goka, 2014).

The effect of insecticides on bees and other insects usually occurs through the paralysis of some physiological or biochemical process, and the main target of insecticides being the nervous system to ensure the rapid efficiency and effectiveness in controlling insect pests. These mechanisms of action, especially when combined, cause rapid mortality of Africanized *A. mellifera* workers, as observed in the comparison between the workers in the treatments SPP 1, SPP 2, and SPP 3 and the workers in the control treatment.

It is also worth noting that when more than one SPP is added, the mortality of the non-target insects increases, due to the greater toxicity. Zhu *et al.* (2017) found that when a neonicotinoid was sprayed on *A. mellifera*, it caused less than 20% of mortality and with the use of an organophosphate the mortality reached almost 40%. The two products combined, however, caused 60% mortality.

Although at the time of application in the field there are intrinsic and extrinsic factors that can add to or intensify the effects of synthetic phytosanitary products on bees, these laboratory tests allow us to understand in a simple way the possible damages of these products in the survival of workers of *A. mellifera* africanized. It is highlighted here the importance of always considering the times at the end of the day to use these products, in order to reduce the chance of the presence of pollinators in the field, as well as to increase the interval of possible contact. Considering the toxicity of SPPs on *A. mellifera* verified in the laboratory, it would be important to develop field studies on the issue, including also other breeds and the breeding process, in order to analyze the effects. In addition, it is essential to evaluate the selectivity of these products in the field, in order to assess the risks to the safety of Africanized *A. mellifera*, an insect with outstanding environmental, social, and economic importance.

Finally, it is noteworthy that the three synthetic phytosanitary products analyzed formulated with – Acetamiprid + Alpha-cypermethrin; Imidacloprid + Beta-Cyfluthrin; and Fenitrothion + Esfenvalerate – reduced the longevity of Africanized *A. mellifera* workers in all bioassays, in addition to causing tissue damage in the mesentery. These products, in the tested concentrations, did not present selectivity for Africanized *A. mellifera* workers.

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CREDIT AUTHOR STATEMENT

GL, MP: Conceptualization, Methodology. **GL, RA, FCC, RMAM**: Data curation, Writing- Original draft preparation. **GL, RA, FCC, RMAM**: Visualization, Investigation. **FMCM, ERL, MP**: Supervision. **FMCM, ERL, FCC, MP**: Writing-Reviewing and Editing.

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