Sociobiology
An international journal on social insects

RESEARCH ARTICLE - BEES

Survival and Flight Capacity of *Apis mellifera* after Contact with Residues of Spiromesifen on Melon Leaves

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Article History

Edited by
Evandro Nascimento Silva, UEFS, Brazil
Received 29 April 2024
Initial acceptance 30 August 2024
Final acceptance 10 November 2024
Publication date 25 November 2024

Keywords
Bee, Acaricide, Insecticide, Pollinators, Toxicity.

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Abstract

Balancing the application of insecticides with the preservation of bees in agricultural areas poses a significant challenge to farmers. Therefore, this study aims to assess the survival and flight capacity of *Apis mellifera* (L.) bees after contact with residues of the insecticide/acaricide Spiromesifen on melon leaves. The experiment occurred under laboratory conditions, in a completely randomized design and a 4x3 factorial scheme, with two doses of Spiromesifen (0.24 and 0.288 g a.i./L), a positive control (Thiamethoxam), and an absolute control (distilled water), at three exposure times: 1, 2, and 3 hours after treatment application. Spiromesifen caused mortality of 15.33% and 13.80% after 1 hour of spraying, 20.05% and 17.72% after 2 hours of spraying, and 21.52% and 14.33% after 3 hours of spraying, for lower and higher doses, respectively. Spiromesifen provided a median lethal time of 112.9 hours, regardless of dose and exposure time. After exposure to the lower dose of Spiromesifen at 1, 2, and 3 hours after spraying, 28.41%, 8.0%, and 17.0% of the bees were able to fly, respectively, while at the higher dose, 33.55% and 8.11% were able to fly after 1 and 2 hours of spraying. Bees exposed after 3 hours of spraying at the higher dose showed a flight activity similar to the absolute control. Contact with Spiromesifen residues on melon leaves was slightly lethal to the honey bee *A. mellifera*, but it reduced the flight capacity of surviving bees after exposure.

Introduction

The cultivation of melon *Cucumis melo* (L.) is one of the most important segments of agribusiness in the Northeast region of Brazil. To ensure successful melon production, farmers rely on fundamental management practices such as chemical pest control and the installation of beehives of the honey bee *Apis mellifera* (L.) (Hymenoptera: Apidae) in the surrounding areas (Guimarães et al., 2008; Klein et al., 2020).

However, researchers have identified the use of insecticides in crops as one of the main factors responsible for the worldwide decline in bee populations (Pires et al., 2016). Castilhos et al. (2019) reported that pesticide exposure is the leading cause of colony losses in Brazil, and products such as Imidacloprid, Thiamethoxam, and Clothianidin are highly toxic to bees. Among the ways bees are exposed to pesticides, contact with residues on plants and ingesting contaminated food are the most common, where the contaminants absorbed along with the food may induce histological alterations in the midgut of these organisms (Serra et al., 2021).

Espiromesifen is a non-systemic contact insecticide/acaricide, a growth regulator, efficient in controlling mites *Tetranychus urticae*, *Eriophyes guerreronis*, *Brevipalpus phoenicis*, and whiteflies *Bemisia tabaci* in various crops,

including melon (Agrofit, 2022). The effects of Spiromesifen on *A. mellifera* were evaluated by Ratnakar et al. (2017), who assessed residual toxicity using half the manufacturer's recommended dose (0.25 L/ha) through the dry film method by spraying the product into a container and allowing it to dry to form a thin layer. According to the authors, 4.76%, 13.03%, and 22.21% mortality rates were observed after 2, 6, and 24 hours of exposure, respectively.

However, there is still no information about the toxicity of Spiromesifen on *A. mellifera* at the doses used in pest control in melon crops, especially regarding contact with residues of the product on leaves. Understanding the effects of the products on these insects is critical to reconciling insecticide applications in crops with the preservation of bees in the field. The lack of information on the adverse effects of pesticides on bees is one of the main obstacles to the sustainable use of pollinating agents in the field (Pinheiro & Freitas, 2010).

Therefore, this study aimed to evaluate the survival and flight capacity of the bee *A. mellifera* after contact with residues of the insecticide/acaricide Spiromesifen on melon leaves under laboratory conditions. We hypothesize that the commercial product, application dose, and time to bee exposure affect bee flight activity and survival.

Material and Methods

The experiment took place at the Entomology Laboratory of the Academic Unit of Agricultural Sciences (UAGRA), part of the Center for Agri-Food Sciences and Technology (CCTA) at the Federal University of Campina Grande (UFCG), Campus Pombal, Paraíba, Brazil. Adult workers of *A. mellifera* from three colonies kept in the UAGRA/CCTA/UFCG apiary were used for the study. The insecticide/acaricide evaluated was Spiromesifen (Oberon®), at the minimum (0.24 g a.i./L) and maximum (0.288 g a.i./L) doses registered for pest control in melon crops (Agrofit, 2022).

The experiment was conducted in a completely randomized design, in a 4x3 factorial scheme, with two doses of the insecticide/acaricide Spiromesifen (0.24 g a.i./L and 0.288 g a.i./L), a positive control [Thiamethoxam (0.3 g a.i./L)], and a negative control (distilled water), considering three exposure times: 1, 2, and 3 hours after spraying the treatments on melon leaves. There were ten repetitions per treatment, with each experimental unit comprising ten adult bees.

Yellow melon seedlings were produced in the greenhouse of CCTA/UFCG to evaluate the residual toxicity of Spiromesifen. The seedlings were grown in 1kg pots containing a soil substrate + organic matter (2:1) and irrigated twice a day. Ten plants were selected for each treatment when the plants reached a minimum of six definitive leaves. The selected plants were sprayed with the respective treatments using a manual sprayer. All plants received uniform coverage of Spiromesifen on the leaf surface.

The plants were transferred to a well-ventilated and shaded area for proper drying of the sprayed products, where they remained for three periods: 1, 2, and 3 hours after spraying. After drying, the leaves were cut at the petiole height and placed in the arenas. The arena consisted of a plastic container (15 cm in diameter X 15 cm in height), whose end was partially covered with anti-aphid mesh and had side openings of approximately 2 mm to allow air circulation in the environment.

A cotton wad soaked in water and an artificial diet (Candi paste) were inserted in each arena. After the complete preparation of the arenas, adult workers of *A. mellifera* were released inside the containers to come into contact with the product residues on the leaves. The bees were previously anesthetized using cold (± 4 °C for 120 seconds) to facilitate handling. The bioassay was conducted in a climate-controlled room at 25 ± 2 °C, 50 ± 10% RH, and a 12-hour photoperiod.

After the application of treatments, mortality and possible motor disturbances (prostration, tremors, paralysis, etc.) of the bees were evaluated at 1, 2, 3, 4, 5, 6, 12, 24, and 48 hours after the start of exposure to the residues of the product at each time after spraying. The bees were considered dead when they did not respond to mechanical stimuli at each evaluation time.

The flight capacity was assessed for all bees that survived after 48 hours of exposure to Spiromesifen residues. Flight towers were used following the methodology proposed by Gomes et al. (2020). The flight tower was made of wood (35 x 35 x 115 cm), with sides covered in transparent plastic and a fluorescent lamp at the top. On each side of the tower, a measuring tape was fixed, establishing five levels of height: 1 (base of the tower: 0 cm), 2 (from 1 cm to 30 cm in height), 3 (from 31 cm to 60 cm), 4 (from 61 cm to 90 cm), and 5 (from 91 cm to 115 cm, where the lamp was located).

Flight capacity was evaluated in a completely dark environment, where the only light source was the lamp installed at the top of the flight tower to stimulate the movement of the bees. Each surviving bee was released individually inside the tower, with a period of 60 seconds allowed for the completion of the flight, recording the bees that could fly or not, as well as the height reached. Bees that eventually climbed the flight tower by walking and showed no apparent locomotor difficulties were also recorded.

The survival data of adults were analyzed using the Survival package (Therneau & Lumley, 2010) and subjected to Weibull distribution analysis. Treatments with similar effects (toxicity and mortality rate) were grouped through contrasts. The median lethal time (LT_{50}) was also calculated for each group. The percentage of mortality was calculated for each treatment and corrected using the Abbott (1925) equation, followed by an Analysis of Variance with Permutation (Permanova) and the Wilcoxon test. Permanovas investigated the data regarding flight capacity with two and three factors at a significance level of 5%, followed by the Wilcoxon test. All analyses were conducted using the R software (R development core team, 2011).

Results and Discussion

The mortality caused by the insecticide/acaricide Spiromesifen differed statistically from the absolute and positive controls. Spiromesifen, at doses of 0.24 and 0.288 g a.i./L resulted, respectively, in mortality rates of 15.33% and 13.80% after 1 hour of spraying, 20.05% and 17.72% after 2 hours of spraying, and 21.52% and 14.33% after 3 hours of spraying, being significantly less lethal than the positive control (Tiametoxam) (Fig 1).

The low mortality observed after contact with Spiromesifen residues, regardless of the dose and exposure time after spraying, is likely associated with its mechanism of action, which regulates growth by inhibiting the biosynthesis of acetyl-CoA carboxylase (Bielza et al., 2018; Irac, 2024). In other words, it exerts its lethal effect, especially during the early stage of insects. Although there are few results on the effect of Spiromesifen on *A. mellifera*, some researchers have also reported low lethality through the evaluation of another dose and methodology. Ratnakar et al. (2017) assessed the

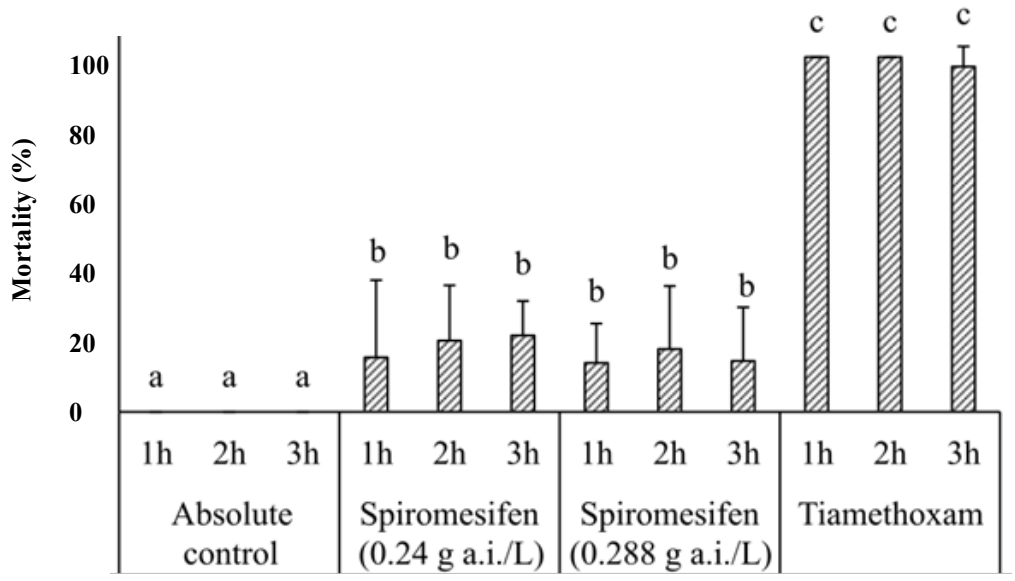

Fig 1. Mortality of *Apis mellifera* when exposed to insecticide residues at different times after spraying, Pombal-PB, 2022.

residual toxicity of Spiromesifen with half the manufacturer's recommended dose (0.25 L/ha) using the dry film method. They observed mortality rates of 4.76%, 13.03%, and 22.21% after 2, 6, and 24 hours of exposure, respectively, demonstrating low mortality when bees come into contact with insecticide residues.

Regarding the behavior of the bees, it was observed that there were clusters in the center, on the sides, and mainly at the top of the arenas, starting from 1 hour after the beginning of exposure, suggesting that the bees were trying to avoid contact with the residues on the leaves. Additionally, it was noted that some bees were scraping the melon leaves, which could be a possible sign of stress. The behaviors mentioned above were not observed in the absolute control. At sufficiently high doses, insecticides can cause the death of bees. However, doses that do not cause immediate death often have other deleterious effects and can interfere with bees' cognitive abilities and behavior (Silva et al., 2016).

The survival analysis revealed that the insecticide/acaricide Spiromesifen had a median lethal time (LT_{50}) lower than the absolute control but higher than Thiamethoxam, regardless of the exposure time after spraying. Spiromesifen showed a slower mortality rate than the positive control, with an LT_{50} of 112.9 hours for both doses evaluated at different times after spraying. The positive control had an LT_{50} of 2.966 hours at 1h and 5.36 hours at 2h and 3h after spraying, meaning it caused fast mortality in bees (Fig 2).

The assessment of flight capacity was conducted only for bees from the absolute control (distilled water) and those exposed to Spiromesifen, as the positive control (Tiametoxam) resulted in 100% mortality. The number of bees that managed to fly or walk after contact with Spiromesifen residues was lower than the absolute control. The percentage of bees that were able to fly was 28.41%, 8.0%, and 17% when exposed within 1, 2, and 3 hours after spraying Spiromesifen at a dose of 0.24 g a.i./L, and 33.55% and 8.11% at 1 and 2 hours after spraying the product at a dose of 0.288 g a.i./L. Only bees exposed after 3 hours of spraying on the leaves at a dose of 0.288 g a.i./L could fly in a similar quantity to the absolute control, 45.25%. (Fig 3).

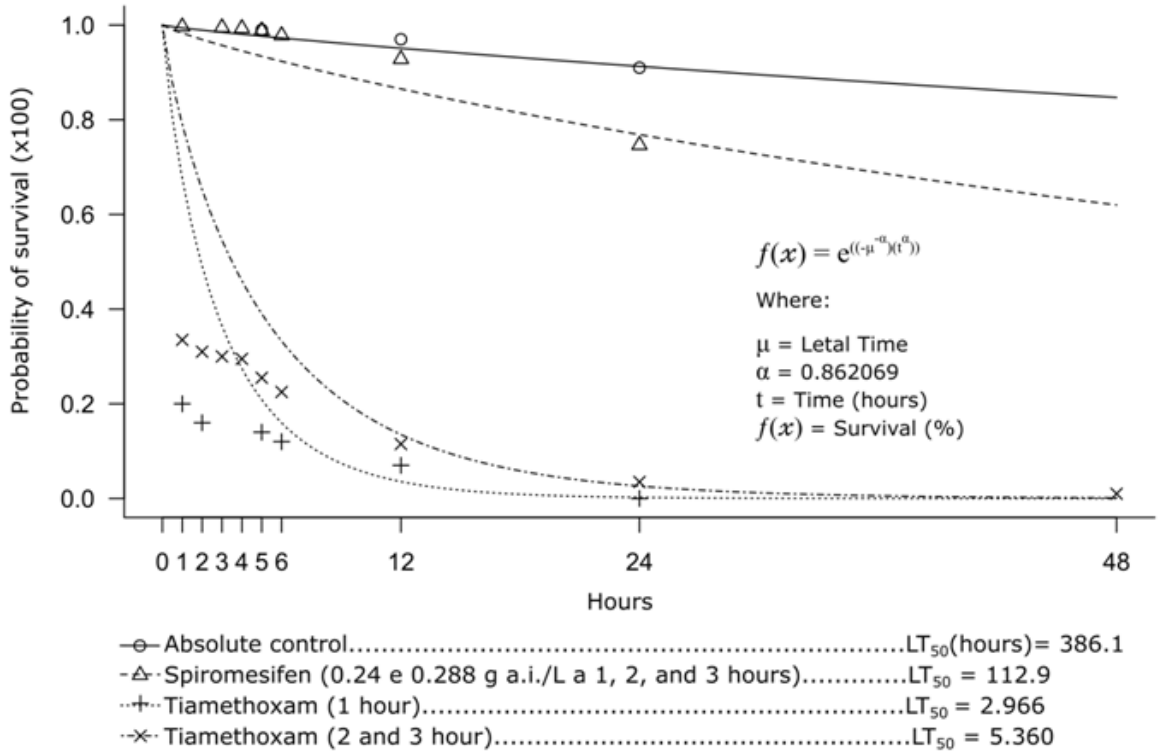

Fig 2. Survival (%) of *Apis mellifera* after contact with melon leaves sprayed with insecticides, median lethal times (TL_{50}) in hours, Pombal-PB, 2022.

There was no significant difference in the percentage of bees that did not fly/walk among the treatments, which may be caused by the high variability of these data. Flight capacity is essential during bee foraging activity, and any alteration that impairs this activity can pose a risk to survival. Since the colony requires this service from foragers, any change in this behavior caused by exposure to insecticides may decrease bee performance in the field, affecting foraging activity and, consequently, food supply in the colony, thereby interfering with its entire dynamics and harming its maintenance and survival (Colin et al., 2004). During foraging, bees require the integrity of their cognitive abilities related to memory, which allows them to navigate through acquired environmental information, recognize resources such as food, and later return to the colony (Balbuena et al., 2015).

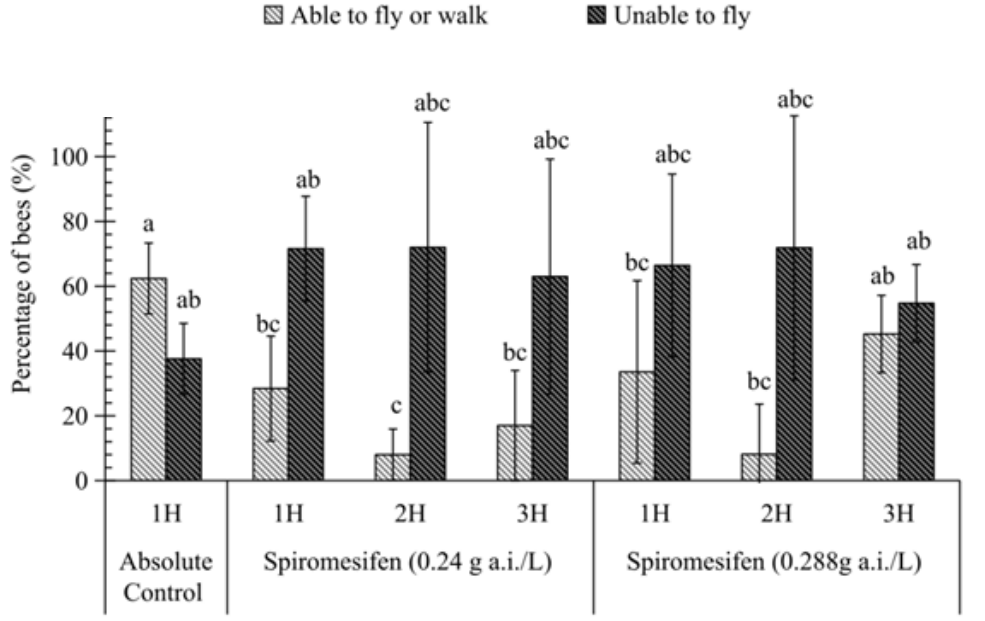

Fig 3. Flight activity (%) flew x did not fly for *Apis mellifera* after contact with Spiromesifen, Pombal-PB, 2022.

However, despite the reduction in flight or walking capacity, some bees could fly or walk normally regardless of the dose and exposure time after spraying on the leaves. Probably, under field conditions, the bees would have fled after contact with Spiromesifen residues, and the adverse effect on flight capacity could have been attenuated. However, only field studies can test this hypothesis. In general, insecticides in the ketoenol group present a favorable ecotoxicological profile and are less harmful to beneficial agents such as pollinators (Bielza et al., 2005).

These are the first results regarding the residual effect of the insecticide Spiromesifen on melon leaves on *A. mellifera*. Given the low percentage of mortality observed under laboratory conditions, the insecticide/acaricide Spiromesifen appears to be less lethal to bees, making it a less harmful alternative for field use compared to other insecticides and acaricides already reported as lethal to *A. mellifera*. The findings will support further research, especially under field conditions, to clarify the effects of the insecticide/acaricide Spiromesifen on *A. mellifera*.

Conclusion

The contact with the insecticide Spiromesifen residues on melon leaves was slightly lethal to the honey bee *A. mellifera.* However, it resulted in a reduction in the flight capacity of surviving bees after the exposure period.

Author's Contribution

AAS: Conducted the experiment, investigation, writing-original draft.
EKSS: Writing-original draft, writing-review and editing.
EMC: Conceptualization, methodology, data analysis, writing-review and editing.
TALC: Data analysis, figure design and writing-review.
JAMAC: Conceptualization, writing-review and editing.
DMTS: Conducted the experiment, investigation, writing-original draft.
AROG: Writing-review and editing.

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