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Residual toxicity of thiacloprid in melon leaf on *Apis mellifera* (Hymenoptera: Apidae)

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ABSTRACT

Sustainable use of *Apis mellifera* in melon production requires knowledge of the toxicity of insecticides applied for pest control. This study evaluated the residual effect of thiacloprid in melon leaves on *A. mellifera* at different intervals after spraying. The bioassay was conducted under laboratory conditions in a completely randomized design with a 4 × 3 factorial scheme, consisting of two commercial doses of thiacloprid (0.144 g a.i. L^{-1} and 0.192 g a.i. L^{-1}), an absolute control (distilled water), and a positive control (thiamethoxam, 0.3 g a.i. L^{-1}). Exposure times were 1h, 2h, and 3h after spraying. At 0.144 g a.i. L^{-1}, mortality rates of 54%, 57%, and 44% were observed at 1h, 2h, and 3h, respectively. At 0.192 g a.i. L^{-1}, mortality was 45%, 42%, and 27% at the same intervals. For the 0.144 g a.i. L^{-1} dose, the median lethal time (TL_{50}) was 38.36h, similar to the 0.192 g a.i. L^{-1} dose after 1h and 2h of spraying. At 0.192 g a.i. L^{-1} and 3h after spraying, the TL_{50} was 63.69h. Thiacloprid, at the evaluated doses, was moderately toxic to *A. mellifera* regardless of post-spray interval and interfered with bee flight capacity.

Keywords: Bees, Insecticides, Neonicotinoids, Mortality.

Toxicidade residual de tiacloprido em folha de meloeiro sobre *Apis mellifera* (Hymenoptera: Apidae)

RESUMO

Para viabilizar o uso sustentável de *Apis mellifera* nas áreas de produção de melão é imprescindível conhecer a toxicidade dos inseticidas utilizados para o controle de pragas sobre a referida abelha. Portanto, objetivou-se avaliar o efeito residual do tiacloprido em folhas de meloeiro sobre *A. mellifera* em função de diferentes tempos após a pulverização. O bioensaio foi realizado sob condições de laboratório, distribuído em delineamento inteiramente casualizado e esquema fatorial 4 x 3, sendo constituído por duas doses comerciais do inseticida tiacloprido (0,144 g i.a. L^{-1} e 0,192 g i.a. L^{-1}), testemunha absoluta (água destilada) e testemunha positiva (tiametoxam 0,3 g i.a. L^{-1}) em função de 3 tempos de exposição após aplicação do produto nas folhas de meloeiro: 1h, 2h e 3h após a pulverização. Para dose 0,144 g i.a. L^{-1} foi observada mortalidade de 54%, 57%, e 44% em 1h, 2h e 3h após a pulverização e para dose 0,192 g i.a. L^{-1} em 1h, 2h e 3h após a pulverização a mortalidade foi de 45%, 42% e 27%, respectivamente. Na dose 0,144g i.a. L^{-1}, independente do tempo de exposição, o Tempo Letal Mediano (TL_{50}) foi de 38,36 horas, sendo igual a dose 0,192 g i.a. L^{-1} após 1h e 2h da pulverização. Para a dose 0,192 g i.a. L^{-1} e após 3h da pulverização, o TL_{50} foi de 63,69 horas. O tiacloprido nas doses avaliadas foi moderadamente tóxico via residual para *A. mellifera*, independente do tempo após a pulverização nas folhas e interferiu na capacidade de voo da abelha.

Palavras-chave: Abelhas, Inseticidas, Neonicotinoide, Mortalidade.

1. Introduction

Bees are key pollinators of great ecological, environmental, and economic importance (Barbosa et al., 2017; Maciel et al., 2018). In agricultural landscapes, bee-mediated pollination is essential for fruit and seed production, with *Apis mellifera* L. (Hymenoptera: Apidae) standing out as one of the most widely used and effective pollinators (Sousa et al., 2016). In melon crops (*Cucumis melo* L.), the presence of *A. mellifera* during flowering is indispensable for effective pollination and, consequently, for fruit set (Kiill et al., 2015).

A wide range of insecticides is employed to control pests during melon cultivation, which poses the challenge of reconciling chemical applications with pollinator conservation in the field, since pesticide use is one of the main drivers of pollinator population decline (Castilhos et al., 2019; Nchang et al., 2023). Exposure to insecticides can cause bee mortality as well as sublethal effects that impair motor functions, jeopardizing bee survival in the field and disrupting essential colony activities (Simom-Delso et al., 2015).

The neonicotinoid insecticide thiacloprid is registered for the control of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) and the aphid *Aphis gossypii* (Hemiptera: Aphididae) in melon crops (Agrofit, 2024), and is an effective product for reducing populations of these pests. However, little information is available regarding the effects of field-registered thiacloprid doses for melon on *A. mellifera*, particularly through exposure to insecticide residues on plants after spraying. Suh et al. (2020) assessed the residual effect of thiacloprid on *A. mellifera* after aerial spraying in pine forests and reported neither mortality nor abnormal behavior following exposure. Under other exposure routes, such as ingestion of contaminated diets, thiacloprid has been shown to reduce hemocyte density and impair immune functions in *A. mellifera*, in addition to altering mitochondrial function and energy allocation, ultimately compromising foraging capacity and social behavior (Brandt et al., 2017; Fent et al., 2020). High levels of mortality and oxidative stress have also been reported in honeybees following ingestion of thiacloprid-contaminated diets (Laurino et al., 2011; Orčić et al., 2022).

Given that thiacloprid is widely used for pest control in melon crops, and considering the scarcity of information regarding the residual effects of commercial doses on *A. mellifera*, research addressing this issue is essential to harmonize chemical control with bee presence in the field. Understanding the effects of insecticides on bees enables the selection of less harmful products and helps secure pollination services, which are vital for biodiversity conservation and food production (Klein et al., 2020).

Therefore, the objective of this study was to evaluate the residual effect of thiacloprid on melon leaves on *A. mellifera* at different intervals after spraying, under laboratory conditions.

2. Material and Methods

The experiment was conducted at the Entomology Laboratory of the Academic Unit of Agricultural Sciences (UAGRA), Center for Science and Agro-Food Technology (CCTA), Federal University of Campina Grande (UFCG), Pombal Campus, Paraíba, Brazil. Adult worker bees of *A. mellifera* were collected from honey supers between 06h and 07h from three colonies maintained at the UAGRA/CCTA/UFCG apiary. Colonies received periodic management as needed to ensure productivity and colony health.

The insecticide evaluated was thiacloprid (commercial name Calypso®), belonging to the neonicotinoid chemical group. Thiacloprid was tested at the minimum (0.144 g a.i. L^{-1}) and maximum (0.192 g a.i. L^{-1}) doses recommended by the manufacturer for pest control in melon crops, based on an average spray volume of 500 L ha^{-1}. Distilled water was used as the negative control, while thiamethoxam (Actara®) at the maximum recommended dose of 600 g ha^{-1} (0.3 g a.i. L^{-1}) was used as the positive control for melon pest management.

The bioassay followed a completely randomized design in a 4 × 3 factorial scheme, consisting of four treatments: 1 - negative control (distilled water); 2 - thiacloprid [150 mL ha^{-1} (0.144 g a.i. L^{-1})]; 3 - thiacloprid [200 mL ha^{-1} (0.192 g a.i. L^{-1})]; and 4 - positive control, thiamethoxam [600 g ha^{-1} (0.3 g a.i. L^{-1})], evaluated at three post-spray intervals: 1h, 2h, and 3h. Each treatment consisted of 10 replicates, and each experimental unit comprised 10 adult bees, following the methodology of Costa et al. (2014).

For residual toxicity assessment, yellow melon plants were grown in the CCTA/UAGRA/UFCG greenhouse in 1-kg pots containing a soil + organic matter substrate (2:1). Once plants reached a minimum of six true leaves, 10 plants were selected for each treatment according to the post-spray interval. Plants were separated into four groups: Group 1 - distilled water; Group 2 - thiacloprid at 0.144 g a.i. L^{-1}; Group 3 - thiacloprid at 0.192 g a.i. L^{-1}; and Group 4 - thiamethoxam at 0.3 g a.i. L^{-1}. Each group was subdivided into three subgroups based on leaf drying times after spraying: 1h, 2h, and 3h. Applications were performed with a calibrated hand sprayer producing fine droplets to ensure uniform foliar coverage. Sprays were conducted in a wind-protected environment to minimize drift, and visual inspections confirmed complete leaf coverage.

After drying, leaves were cut at the petiole and placed into arenas. Arenas consisted of plastic containers (15 cm diameter × 15 cm height) with the upper end partially covered with anti-aphid mesh and lateral openings of ~2 mm to allow adequate air circulation. Each arena contained a cotton pad moistened with water (rewetted hourly) and artificial diet (Candi paste). Adult worker bees of *A. mellifera* were introduced into the arenas for contact with insecticide residues. For handling, bees were anesthetized by chilling (±4 °C for ~90 seconds). The bioassay was maintained in a climate-controlled room at 25 ± 2 °C, 60 ± 10% RH, and a 12h photophase.

Bee mortality and motor disorders (prostration, tremors, paralysis) were assessed at 1, 2, 3, 4, 5, 6, 12, 24, and 48 h after exposure to residues. Bees were considered dead if they did not respond to mechanical stimuli (touch) at each evaluation (Costa et al., 2014; Araújo et al., 2017).

Surviving bees after 48h of exposure were subjected to flight capacity tests using a flight tower, according to Gomes et al. (2020). The tower was a wooden structure (35 × 35 × 115 cm), lined with transparent resistant plastic, marked with a measuring tape on the sides, and fitted with a fluorescent lamp at the top.

For flight assessment, bees were released individually at the base of the tower and observed for 60 seconds. Their ability to ascend by walking or flying was recorded. The tower had five height levels: 1 (base), 2 (1–30 cm), 3 (31–60 cm), 4 (61–90 cm), and 5 (91–115 cm, top). Evaluations were conducted in a dark room at 26 ± 2 °C and 65 ± 10% RH, with the lamp at the top as the only light source.

Mortality percentages were corrected using Abbott's formula (Abbott, 1925) and subjected to permutational analysis of variance (PERMANOVA) (Anderson, 2001) at a 5% significance level, followed by Wilcoxon's test. Adult survival data were analyzed using the Survival package (Therneau and Lumley, 2010) in R software and fitted to Weibull distribution. Treatments with similar effects (toxicity and mortality rate) were grouped by contrasts. Median lethal time (LT_{50}) was also calculated for each group. For flight capacity, PERMANOVA was applied at a 5% significance level, followed by the Scott–Knott test. All analyses were performed using R software (R Development Core Team, 2022).

3. Results and Discussion

The evaluated doses of the insecticide thiacloprid differed significantly from both the negative and positive controls. At the 0.144 g a.i. L^{-1} dose, regardless of exposure time after spraying, no significant differences were observed in mortality rates, with 54%, 57%, and 44% mortality of *A. mellifera* recorded at 1h, 2h, and 3h post-application, respectively (Figure 1).

At the 0.192 g a.i. L^{-1} dose, no differences were detected between 1h and 2h after spraying, with 45% and 42% mortality, respectively. The lowest mortality rate (27.0%) was observed 3h after spraying. Thus, thiacloprid proved less lethal than the positive control, which caused 100% mortality of bees by the end of the evaluation period, irrespective of exposure time. Information on the residual effects of thiacloprid on *A. mellifera* remains scarce; however, Suh et al. (2020) evaluated mortality and behavior of *A. mellifera* in pine forests receiving monthly thiacloprid spraying and reported no significant mortality or behavioral alterations. Based on the present results, thiacloprid was less lethal than other insecticides previously tested at registered doses for melon crops via residual exposure, such as thiamethoxam, abamectin, and chlorfenapyr (Costa et al., 2014), representing a less harmful alternative to these products.

More information is available for other exposure routes and doses of thiacloprid in *A. mellifera*. Oral exposure through contaminated diets has been associated with reduced hemocyte density and impaired immune function in queens (Brandt et al., 2017), as well as negative impacts on foraging activity and social behavior (Fent et al., 2020). Laurino et al. (2011), in laboratory assays evaluating the toxicity of neonicotinoids, reported behavioral alterations and high mortality caused by thiacloprid only under oral exposure, when honey bees were deprived of food for two hours prior to testing. Orčić et al. (2022) further demonstrated that sublethal thiacloprid concentrations induced oxidative stress and compromised immune defenses in *A. mellifera*, attributed to reduced profenoloxidase and phenoloxidase activity, which may explain the substance's high toxicity to bees.

With respect to behavior, bees exposed to thiacloprid residues were more agitated compared to those in the negative control, particularly during the first two evaluation periods. In addition, some bees scraped melon leaves, a behavior not observed on leaves treated with distilled water alone. According to Pashalidou et al. (2020), bees scrape leaves to accelerate flowering when resources are scarce, as they rely on floral pollen for survival. Since no notable behavioral alterations occurred in the negative control, it is evident that thiacloprid residues induced discomfort, reflected in heightened agitation and stress.

Survival analysis showed that thiacloprid exhibited a median lethal time (LT_{50}) lower than the negative control but higher than thiamethoxam, regardless of exposure time. At 0.144 g a.i. L^{-1}, LT_{50} was 38.36 h across exposure times, and the same was observed for the 0.192 g a.i. L^{-1} dose at 1h and 2h post-spray.

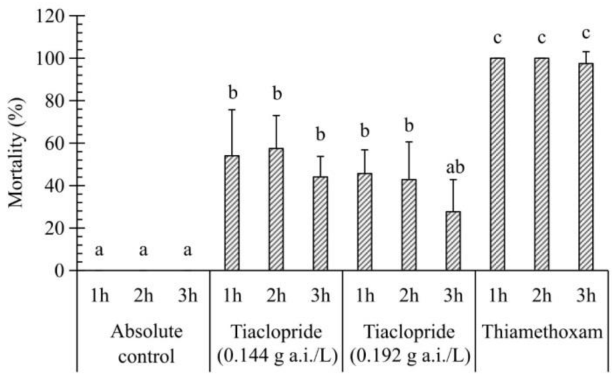

Figure 1. Mortality (%) of *Apis mellifera* after residual exposure to thiamethoxam and thiacloprid at maximum and minimum doses, Pombal-PB, 2023.

At 3h post-spray with 0.192 g a.i. L^{-1}, LT_{50} increased to 63.69 h. The positive control showed LT_{50} values of 3.17 h (1h post-spray) and 5.75 h (2h and 3h post-spray) (Figure 2).

The slower mortality rate of thiacloprid compared with thiamethoxam, both neonicotinoids, can be explained by chemical group differences: thiacloprid belongs to the cyanoamidine subgroup, considered less toxic within the neonicotinoids, whereas thiamethoxam is part of the nitroguanidine subgroup, highly toxic to honey bees (Iwasa et al., 2004). Toxicity levels of active ingredients are also associated with their metabolic degradation rate, and thiacloprid is metabolized more rapidly than other neonicotinoids, such as acetamiprid, thiamethoxam, and imidacloprid (Alptekin et al., 2016).

Flight capacity analysis was performed only with bees from the negative control (distilled water) and those exposed to thiacloprid residues at both doses.

Regardless of dose and post-spray exposure time, significantly fewer bees exposed to thiacloprid were able to fly compared with the negative control, in which 62.5% of bees successfully flew, and even those remaining at the tower base exhibited no apparent motor disorders (Figure 3).

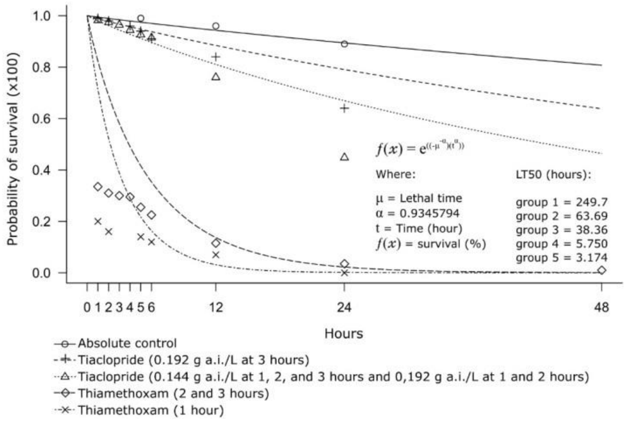

Figure 2. Survival (%) of *Apis mellifera* workers after contact with melon leaves (*C. melo* L.) sprayed with thiacloprid and corresponding median lethal times (LT_{50}), Pombal-PB, 2022.

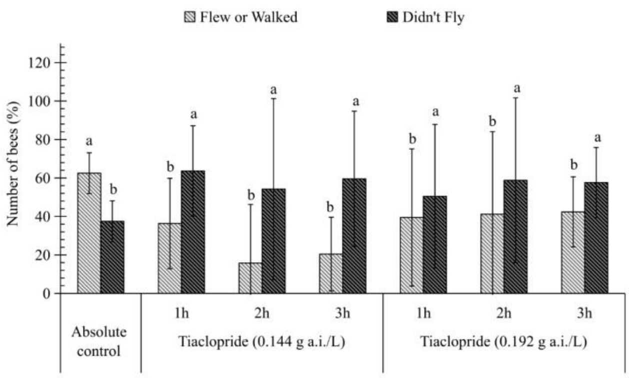

Figure 3. Flight capacity of *Apis mellifera* after residual exposure to thiacloprid, Pombal-PB, 2023. Percentages of bees that flew (gray column) and did not fly (black column) were compared separately. Different letters indicate significant differences according to the Scott–Knott test at 5% significance.

Although significantly different from the negative control, some bees exposed to thiacloprid retained their flight capacity and showed no mobility impairments. Discussions on the adverse effects of thiacloprid on bee flight ability remain inconclusive; however, studies have reported varying impacts depending on the evaluation method and dosage used. According to Tison et al. (2016), thiacloprid can impair flight activity in the field by delaying bee locomotion, interfering with speed, distance traveled, duration and frequency of resting, and continuous mobility, ultimately affecting food collection and return to the hive. Fisher et al. (2014), in field trials, found that oral exposure to thiacloprid did not compromise *A. mellifera* flight performance but did reduce flight speed during foraging.

These are the first results on the minimum and maximum doses of thiacloprid registered for pest management in melon crops, under residual exposure, on *A. mellifera*. Furthermore, this study provides a basis for future research aimed at advancing sustainable pest management strategies to mitigate pollinator decline and disappearance in agricultural landscapes. It is important to emphasize that thiacloprid plays a key role in pest management; however, applications should be avoided during flowering and bee foraging periods.

4. Conclusions

Thiacloprid, at the minimum and maximum doses recommended for melon crops, was moderately toxic to adult worker bees of *A. mellifera* after contact with leaf residues, regardless of the evaluated post-spray intervals. Thiacloprid also interfered with the flight capacity of *A. mellifera* under laboratory conditions.

Authors' Contribution

Diandra Santana Perônica: Investigation, writing-original draft. Emanoely Karoliny Santos da Silva: Writing-original draft, writing-review and editing. Tiago Augusto Lima Cardoso: Formal analysis, figure design, writing-review and editing. Jacquelinne Alves de Medeiros Araújo Costa: Conceptualization, writing-review and editing. Ewerton Marinho da Costa: Conceptualization, methodology, formal analysis, writing-review and editing. Alesia Alves de Sousa: Investigation, writing-original draft.

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