ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
Brazilian Journal of Agricultural and Environmental Engineering
v.30, n.2, e295422, 2026
Campina Grande, PB – http://www.agriambi.com.br – http://www.scielo.br/rbeaa

Original Article

Doses of insecticides used in pollination-dependent crops harm *Apis mellifera* in laboratory conditions[1]

Doses de inseticidas usadas em culturas dependentes de polinização prejudicam *Apis mellifera* em condições de laboratório

Diego de A. Coêlho[2], Ana P. S. Macário[2], Carlos H. P. de Barros[3], Tiago A. L. Cardoso[3], Victor H. M. Rocha[3], Anderson B. A. de Andrade[2*], Elton L. Araujo[4] & Ewerton M. da Costa[3]

[1] Research developed at Universidade Federal de Campina Grande, Centro de Ciências e Tecnologia Agroalimentar, Pombal, Paraiba, Brazil
[2] Universidade Federal de Campina Grande/Programa de Pós-Graduação em Horticultura Tropical, Pombal, PB, Brazil
[3] Universidade Federal de Campina Grande/Unidade Acadêmica de Ciências Agrárias, Pombal, PB, Brazil
[4] Universidade Federal Rural do Semi-Arido/ Departamento de Ciências Agronômicas e Florestais Mossoró, RN, Brazil

HIGHLIGHTS:

Flupyradifurone exhibited highly toxicity to Apis mellifera *when applied via direct spray, regardless of the dose.*
Thiacloprid presented low toxicity through ingestion, but caused moderate mortality when sprayed directly.
All insecticides impaired motor functions, independent of the dose or exposure route.

ABSTRACT: Understanding the toxicity of insecticides on the bee *Apis mellifera* (Hymenoptera: Apidae) is crucial for the conservation of this vital pollinator in agricultural systems. From this perspective, the objective in this study was to evaluate the toxicity of the insecticides flupyradifurone (Butenolide) and thiacloprid (Neonicotinoid) to *A. mellifera* under laboratory conditions. Three commercial doses commonly used in Brazilian crops were tested for flupyradifurone (0.1, 0.15, and 0.2 g a.i. L^{-1}) and thiacloprid (0.072, 0.12, and 0.168 g a.i. L^{-1}), along with two controls: absolute (distilled water) and positive (thiamethoxam insecticide). The insecticides were administered via direct spray application and ingestion through a contaminated diet, and their effects on mortality, motor function disorders, and flight ability of surviving bees was evaluated. Flupyradifurone caused 100% mortality at all doses via direct spraying, with a LT_{50} of 2.15 hours at 0.2 g a.i. L^{-1}, and resulted in 16.44% mortality via contaminated diet, with a LT_{50} of 304.56 hours. thiacloprid led to 46.33% mortality via direct spraying (LT_{50} = 70.28 hours), and 33.92% mortality via ingestion, with a LT_{50} of 304.56 hours at 0.072 g a.i. L^{-1} and 143.05 hours at higher doses. Both insecticides, regardless of dose and exposure route, impaired the flight ability of *A. mellifera*, causing motor dysfunction. Flupyradifurone was highly toxic to adult worker bees via direct spraying and mildly toxic via ingestion, whereas thiacloprid was slightly toxic through ingestion and caused moderate mortality when sprayed directly.

Key words: honey bee, mortality, flight ability, direct toxicity, pollinator conservation

RESUMO: Compreender a toxicidade de inseticidas sobre *Apis mellifera* (Hymenoptera: Apidae) é crucial para a conservação desse importante polinizador em ambientes agrícolas. Nessa perspectiva, o objetivo deste estudo foi avaliar a toxicidade dos inseticidas Flupiradifurona (Butenolida) e Tiaclopride (Neonicotinoide) sobre *A. mellifera* em condições de laboratório. Três doses comerciais utilizadas em cultivos no Brasil de Flupiradifurona (0,1; 0,15 e 0,2 g i.a. L^{-1}) e Tiaclopride (0,072; 0,12 e 0,168 g i.a. L^{-1}) foram testadas, juntamente com dois controles: absoluto (água destilada) e positivo (Tiametoxam). Os inseticidas foram expostos por meio de pulverização direta e ingestão de dieta contaminada, e foram avaliados seus efeitos sobre a mortalidade, distúrbios motores e capacidade de voo das abelhas sobreviventes. A Flupiradifurona causou 100% de mortalidade em todas as doses via pulverização direta, com uma TL_{50} de 2,15 horas a 0,2 g i.a. L^{-1}, e via dieta contaminada resultou em 16,44% de mortalidade, com uma TL_{50} de 304,56 horas. O Tiaclopride levou a 46,33% de mortalidade via pulverização direta, com uma TL_{50} de 70,28 horas, e 33,92% de mortalidade via dieta contaminada, com uma TL_{50} de 304,56 horas a 0,072 g i.a. L^{-1} e 143,05 horas nas doses mais altas. Ambos os inseticidas, independentemente da dose e do método de exposição, prejudicaram a capacidade de voo de *A. mellifera*, causando distúrbios nas funções motoras. A Flupiradifurona foi altamente tóxica para abelhas operárias adultas via pulverização direta e levemente tóxica via ingestão, enquanto o Tiaclopride foi levemente tóxico via ingestão e causou mortalidade moderada via pulverização direta.

Palavras-chave: abelha melífera, mortalidade, capacidade de voo, toxicidade direta, conservação de polinizadores

• Ref. 295422 – Received 26 Jan, 2025
* Corresponding author - E-mail: bdeandrade3@gmail.com
• Accepted 09 Sept, 2025 • Published 18 Sept, 2025
Editors: Toshik Iarley da Silva & Hans Raj Gheyi

Introduction

In agricultural landscapes, pollination by bees is essential for the productivity of most economically important crops (Potts et al., 2016; Reilly et al., 2020; Khalifa et al., 2021), with the species *Apis mellifera* Linnaeus (Hymenoptera: Apidae) playing a crucial role as one of the most efficient pollinators (Cridland et al., 2017; Klein et al., 2020). *A. mellifera* is widely employed in crop pollination due to its ease of management, large colony size, and superior efficiency and speed compared to other pollinator species (Pires et al., 2016; Klein et al., 2020).

However, bee populations have experienced a global decline over recent decades, driven by multiple factors such as climate change, habitat fragmentation, monoculture practices, pest infestations, reduced flower resources, and the indiscriminate use of pesticides (Kearns & Inouye, 1997; Rahimi et al., 2022; Ferreira et al., 2022; Rahimi & Jung, 2024). Among these, pesticide application is considered a leading cause of pollinator decline (Devine & Furlong, 2007). In Brazil, Castilhos et al. (2019) reported that approximately 50% of bee colonies are lost annually across various regions, resulting in an estimated 1 billion dead bees, with pesticide exposure identified as one of the primary contributors to this phenomenon.

Bees may come in contact with pesticides through direct exposure to spray droplets, contact with residues on plant surfaces, or by collecting and ingesting contaminated nectar or pollen (Taning et al., 2019; Varikou et al., 2019). In addition to causing mortality, pesticide exposure can lead to sublethal effects, including impaired foraging behaviour, reduced lifespan, diminished learning and memory capacity, and behavioral disturbances such as motor dysfunction (Wu-Smart & Spivak, 2016; Shi et al., 2020; Rükün et al., 2025; Zhang et al., 2025). Therefore, understanding the effects of insecticides on bees is essential to reconcile chemical pest management with pollinator conservation in agricultural settings (Pecenka et al., 2023).

The effects of insecticides such as thiacloprid and flupyradifurone on bees may vary depending on the dose applied and the route of exposure. For instance, Fischer et al. (2014) found that *A. mellifera* bees orally exposed to 1.25 µg per bee of thiacloprid in field conditions showed no significant changes in flight or foraging behaviour. In contrast, Tison et al. (2016) reported significant alterations in foraging activity, navigation, colony return success, and social communication following chronic oral exposure to 4.5 ng μL^{-1} of thiacloprid. Similarly, Hesselbach & Scheiner (2018) demonstrated that acute exposure to 1.2 µg a.i. of flupyradifurone per bee negatively affected cognitive functions, while Tong et al. (2019) observed motor impairments during foraging following flupyradifurone exposure.

Nevertheless, limited data are available regarding the toxicity of thiacloprid and flupyradifurone at doses registered for agricultural use in Brazil, underscoring the need for further research to support honey bee conservation in crop production systems. In ths context, the present study aimed to evaluate the toxicity of thiacloprid and flupyradifurone to *A. mellifera* under controlled laboratory conditions.

Material and Methods

The experiment was conducted at the Entomology Laboratory of the Center for Sciences and Agri-food Technology (CCTA) of the Federal University of Campina Grande (UFCG), Pombal Campus, Paraíba, Brazil. Adult *A. mellifera* workers were collected from three hives installed in Langstroth wooden boxes located at the CCTA/UFCG apiary.

The insecticides evaluated were flupyradifurone (Sivanto Prime® 200 SL), a butenolide-class compound recommended for pest control in several economically important crops in Brazil, such as coffee (*Coffea arabica* L.), melon (*Cucumis melo* L.), and orange (*Citrus sinensis* L.); and thiacloprid (Calypso®), a neonicotinoid-class insecticide indicated for use in crops including melon, tomato (*Solanum lycopersicum* Mill.), and soybean (*Glycine max* L.), all crops of which rely on pollination by *A. mellifera*.

Three commercial doses were tested for each insecticide: 0.072, 0.12, and 0.168 g a.i. L^{-1} for thiacloprid, and 0.1, 0.15, and 0.2 g a.i. L^{-1} of flupyradifurone, based on manufacturer recommendations. Distilled water served as the absolute control, while the positive control consisted of thiamethoxam (Actara® 250 WG) at the maximum recommended dose for melon (0.3 g a.i. L^{-1}).

The lethal effect of flupyradifurone and thiacloprid on *A. mellifera* was evaluated using two bioassays, corresponding to two exposure routes: direct spraying on the bees and oral ingestion of insecticide-contaminated diet, following the methodology described by Costa et al. (2014).

In both bioassays, bees were confined in plastic containers (15 cm in diameter × 15 cm in height), partially covered at the top with anti-aphid mesh. The container sides had 0.1 cm-wide openings to ensure adequate ventilation. Inside each arena, an artificial diet composed of refined sugar and honey (1:1) was provided in 28 mm-wide plastic containers, along with a cotton pad soaked in distilled water, which was replaced at each evaluation interval. To facilitate handling, bees were anesthetized by exposure to a refrigerated environment (± 4 °C for 90 seconds).

The bioassays followed a completely randomized design with eight treatments: 1 – absolute control (distilled water); 2 – positive control (thiamethoxam 0.3 g a.i. L^{-1}); 3 to 5 – flupyradifurone (0.1, 0.15, and 0.2 g a.i. L^{-1}); 6 to 8 – thiacloprid (0.072, 0.12, and 0.168 g a.i. L^{-1}). Each treatment included 20 replications (arenas), with 10 adult *A. mellifera* bees per replicate. All bioassays were conducted in a controlled environment (25 ± 2 °C, 60 ± 10% relative humidity, and 12-hour photoperiod).

After distributed into the arenas, bees were sprayed directly according to each treatment using a manual sprayer. Artificial diet and water were provided, and the evaluations were performed at predetermined time intervals.

In the oral exposure bioassay, bees were fasted for 2 hours prior to the experiment. The artificial diet was prepared, and sprayed with the insecticides using a manual sprayer. Once distributed into the arenas, bees received the contaminated diet and distilled water. Continuous observation was maintained until ingestion was confirmed, and the effects were monitored over a 48-hour period.

Flight ability was evaluated using a flight tower, following the methodology of Gomes et al. (2020). The tower consisted of a wooden structure (35 × 35 × 115 cm) open inside, with a fluorescent lamp on the top. All sides were covered with transparent plastic to allow unobstructed observation. Evaluations were conducted in a dark room (25 ± 2 °C, 60 ± 10% RH), with the lamp serving as the sole light source to stumulate flight via positive phototropism.

Due to the number of bees used, a maximum of 100 individuals per treatment was established for flight assessment. Where fewer than 100 bees survived, all survivors were tested. Each bee was individually placed at the base on the tower (0 cm height), and given 60 seconds to complete its flight. The final height reached was recorded. The tower was divided in five height levels: 1 – base (0 cm); 2 – 1–30 cm; 3 – 31–60 cm; 4 – 61–90 cm; 5 – 91–115 cm (lamp location).

Mortality and motor disorders were evaluated at 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, and 48 hours after exposure. Bees unresponsive to mechanical stimuli (e.g., body touches) were recorded as dead. Flight ability was assessed in surviving bees at 48 hours of following Gomes et al. (2020).

Mortality rates were corrected using Abbott's (1925) equation. Data were analyzed using the Kruskal-Wallis (1952) non-parametric test, followed by Wilcoxon's test, both at a 5% significance level. Survival data were processed using the survival package (Therneau & Lumley, 2020) for R software (R Core Team, 2022) and fitted to a Weibull distribution. Treatments with similar toxicity and mortality profiles were grouped by contrast analysis. Median lethal time (LT_{50}) was calculated for each group. Survival percentage was determined using the Eq. 1.

$$f(x) = e^{\left(-\mu^{-\alpha}\right)\left(t^{\alpha}\right)} \quad (1)$$

where:

μ - lethal time;

α - 1/score of the chosen model;

t - time (hours); and,

f(x) - survival (%).

The effect of treatments on the flight ability was analyzed using permutational analysis of variance (PERMANOVA), followed by Wilcoxon's test for each exposure method. P-values for multiple concentrations were adjusted using the Bonferroni correction, minimizing the risk of type I errors. Comparisons were made only within each height class.

Results and Discussion

The insecticide flupyradifurone caused 100% mortality of *Apis mellifera* workers at all three commercial doses tested, showing no statistical difference from the positive control, thiamethoxam. Within the first hour of observation, from 50 to 70% of the bees exhibited symptoms such as tremors, prostration, and paralysis, prior to death when exposed to 0.1 and 0.2 g a.i. L^{-1} respectively.

Thiacloprid induced moderate mortality at the evaluated doses. However, following the direct spraying, aproximately 40% of the bees displayed transient paralysis, but subsequently regained motor function (Kruskal-Wallis $\chi^2 = 145.55$; g.l. = 7; p-value = 3.49e-28; Table 1).

In the oral exposure bioassay, flupyradifurone caused low mortality, which was statistically different from the positive control (thiamethoxam), responsible for 97.50% mortality. Thiacloprid also differed statistically from thiamethoxam, causing 8.06, 25.43, and 33.92% mortality at doses of 0.072, 0.12, and 0.168 g a.i. L^{-1}, respectively (Kruskal-Wallis $\chi^2 = 84.52$; g.l. = 7; p-valor = 1.63e-15; Table 1).

Direct exposure of bees to spray droplets of flupyradifurone and thiacloprid significantly compromised their survival. Flupyradifurone induced 100% mortality across all tested doses, with mortality rates similar to those of the positive control, thiamethoxam—a compound widely recognized for its toxicity to honey bees (Costa et al., 2014; Pizzaia et al., 2021). Following exposure, bees exhibited neuromotor symptoms such as tremors, prostration, and paralysis, mirroring the effects observed with thiamethoxam (Costa et al., 2014).

The elevated mortalit associated with flupyradifurone is attributed to its agonistic action on nicotinic acetylcholine receptors (nAChRs). This compound induces sustained ion currents leading to neuronal hyperexcitation. Unlike acetylcholine flupyradifurone is not degraded by acetylcholinesterase, resulting in persistent stimulation, neuromuscular dysfunction, and eventual death (Jeschke et al., 2015).

In contrast, thiacloprid demonstrated lower toxicity, with reduced mortality rates and slower onset of lethal effects compared to thiamethoxam. Although data on direct contact effects of thiacloprid are limited, the faster metabolic degradation relative to thiamethoxam may explain the observed differences in toxicity (Alptekin et al., 2016).

The survival analysis revealed marked differences in the Median Lethal Times (LT_{50}) among the insecticides.

Table 1. Mortality (%) of *Apis mellifera* bees exposed via direct spraying and oral ingestion to different doses of use in crops of the insecticides flupyradifurone and thiacloprid

Treatments	Dose (g a.i. L^{-1})	Mortality in each mode of exposure (%)	
		Direct	Oral
Absolute control (distilled water)	-	0 c*	0 e
Positive control (Thiamethoxam)	0.3	100.00 a	97.50 ± 1.2 a
Flupyradifurone	0.1	100.00 a	15.61 ± 3.7 cd
Flupyradifurone	0.15	100.00 a	15.97 ± 3.7 cd
Flupyradifurone	0.2	100.00 a	16.44 ± 3.5 cd
Thiacloprid	0.072	35.30 ± 6.8 b	8.06 ± 2.7 d
Thiacloprid	0.12	45.07 ± 5.7 b	25.43 ± 6.0 bc
Thiacloprid	0.168	46.33 ± 6.9 b	33.92 ± 6.1 b

*Means followed by different letters represent significant differences according to the Wilcoxon test at a 5% significance level

Flupyradifurone, regardless of dose, drastically shortened bee survival, with a LT_{50} values below 4 hours. The dose 0.2 g. a.i.L^{-1} yielded LT_{50} of 2.15 hours, closely approximating the positive control (LT_{50} = 1 hour) (Figure 1A). In contrast, the highest LT_{50} values were recorded for thiacloprid, with the 0.072 g.a.i. L^{-1} dose reaching 70.28 hours—second only to the absolute control (Figure 1B).

All doses of flupyradifurone, as well as the lowest dose of thiacloprid (0.072 g a.i. L^{-1}) were less toxic when administered via ingestion, exhibiting the longest median lethal time (LT_{50} = 304.56 hours) among all insecticides and concentrations tested, with all causing under 20% mortality, indicating low acute oral toxicity. Conversely, thiacloprid at doses 0.12 and 0.168 g a.i. L^{-1} showed higher toxicity, with LT_{50} values of 143.05 hours and mortality rates of 25.43 and 33.92%, respectively (Figures 2A and B).

Bees that survived direct exposure to thiacloprid exhibited motor dysfunctions, including impaired locomotion and flight capacity, indicative of neurotoxic effects resulting from insecticide intoxication. Similar findings were reported by Shi et al. (2018), who identified significant alterations in 115 metabolites following direct exposure to thiacloprid, compared to control bees. These metabolites are associated with oxidative stress and detoxification pathways, suggesting activation of the bees' detoxification system. Among the metabolic changes, a reduction in serotonin levels was observed, indicating a potential impact of thiacloprid on neural processes to learning and cognition.

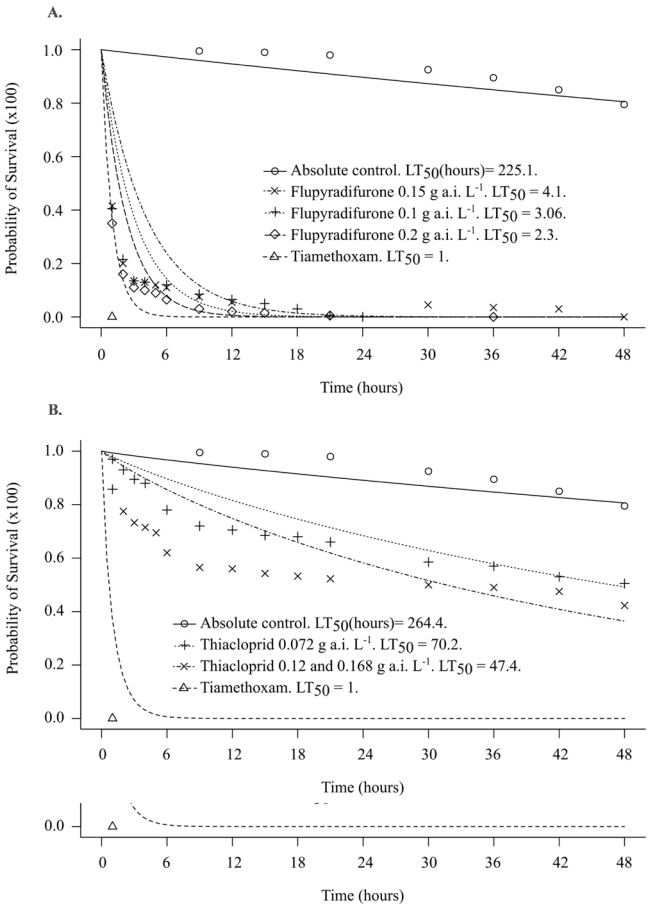


Figure 1. Survival rate (%) of adult worker bees of *Apis mellifera* after direct spray with different doses of use in crops of the insecticides flupyradifurone (A) and thiacloprid (B)

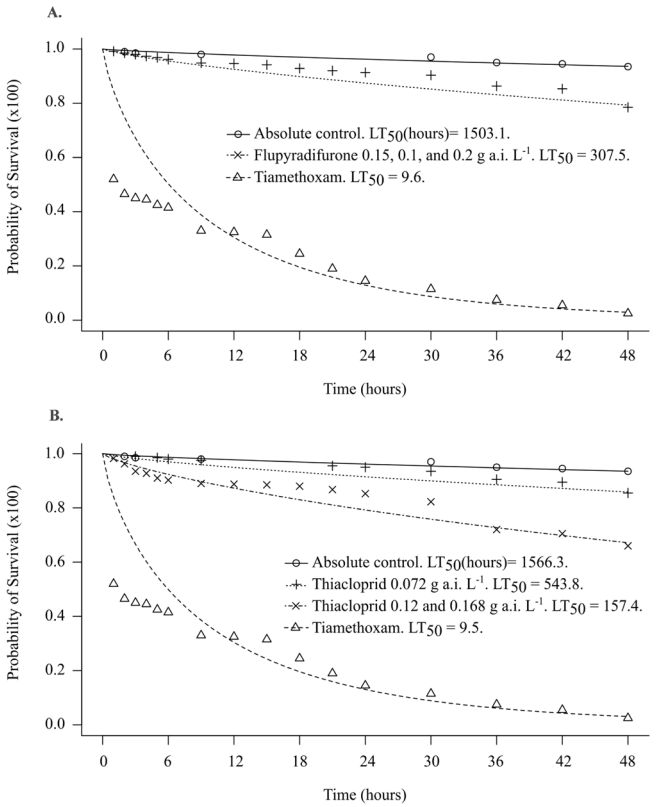


Figure 2. Survival rate (%) of adult worker bees of *Apis mellifera* after ingestion of contaminated diet with different doses of use in crops of the insecticides flupyradifurone (A) and thiacloprid (B)

According to Manjon et al. (2018), cytochrome P450 enzymes play a key role in the detoxification of neonicotinoids, efficiently metabolizing compounds like thiaclopride into less toxic derivatives. The susceptibility of *A. mellifera* to neonicotinoids from the cyano and nitro functional groups is influenced not only by the affinity of these molecules for nicotinic acetylcholine receptors, but also by the efficiency of P450-mediated metabolism. Notably, this metabolic process is less effective for thiamethoxam, contributing to its higher toxicity.

Under oral exposure, flupyradifurone demonstrated lower toxicity than thiacloprid. This reduced toxicity may be partially explained by a repellent effect, as bees exposed to flupyradifurone showed reduced feeding behavior compared to those exposed to thiacloprid, potentially limiting ingestion and subsequent mortality.

The repellent properties of flupyradifurone on *A. mellifera* have been documented by Wu et al. (2021) and other authors. According to Nauen et al. (2015), flupyradifurone exhibits oral toxicity (LD_{50} 1.2 ng a.i. per bee), but low contact toxicity (LD_{50} 0.1 ng a.i. per bee), indicating that ingestion of residues is the primary route of contamination. Despite its toxicity, the compound shows strong repellent activity. Tong et al. (2019), evaluating the effect of flupyradifurone administrated via contaminated diet (4 ppm, daily dose = 241 ± 4 ng per bee) under conditions of nutritional stress (low sugar concentration), reported reduced survival, impaired foraging behavior, compromised thermoregulation, and decline in flight performance in adult worker bees.

Hesselbach & Scheiner (2018) found that flupyradifurone significantly affected gustatory responsiveness of *A. mellifera* workers treated with 8.3×10^{-4} mol L^{-1}, resulting in a marked reduction in the proboscis extension response. Treated bees showed significantly diminished foraging behavior compared to the control group. Additionally, a decreased food-seeking activity was observed, suggesting that flupyradifurone exerts a strong repellent effect on *A. mellifera*.

Al Naggar & Baer (2019) reported that larval exposure to flupyradifurone via contaminated diet (0.025 µg) led to a survival reduction of 7 ± 2%, a pattern also observed in adult workers exposed to 0.645 µg. Moreover, the presence of fungal pathogen *Nosema ceranae* enhanced toxic effects of flupyradifurone in both developmental stages.

Tosi et al. (2021), investigating the lethal and sublethal impacts of flupyradifurone administrated via contaminated sucrose solution (444, 1333, 4000, 12000, and 36000 µg kg^{-1}), observed a significant decline in survival, along with motor coordination deficits and hyperactivity, as well as reductions in foraging and flight performance.

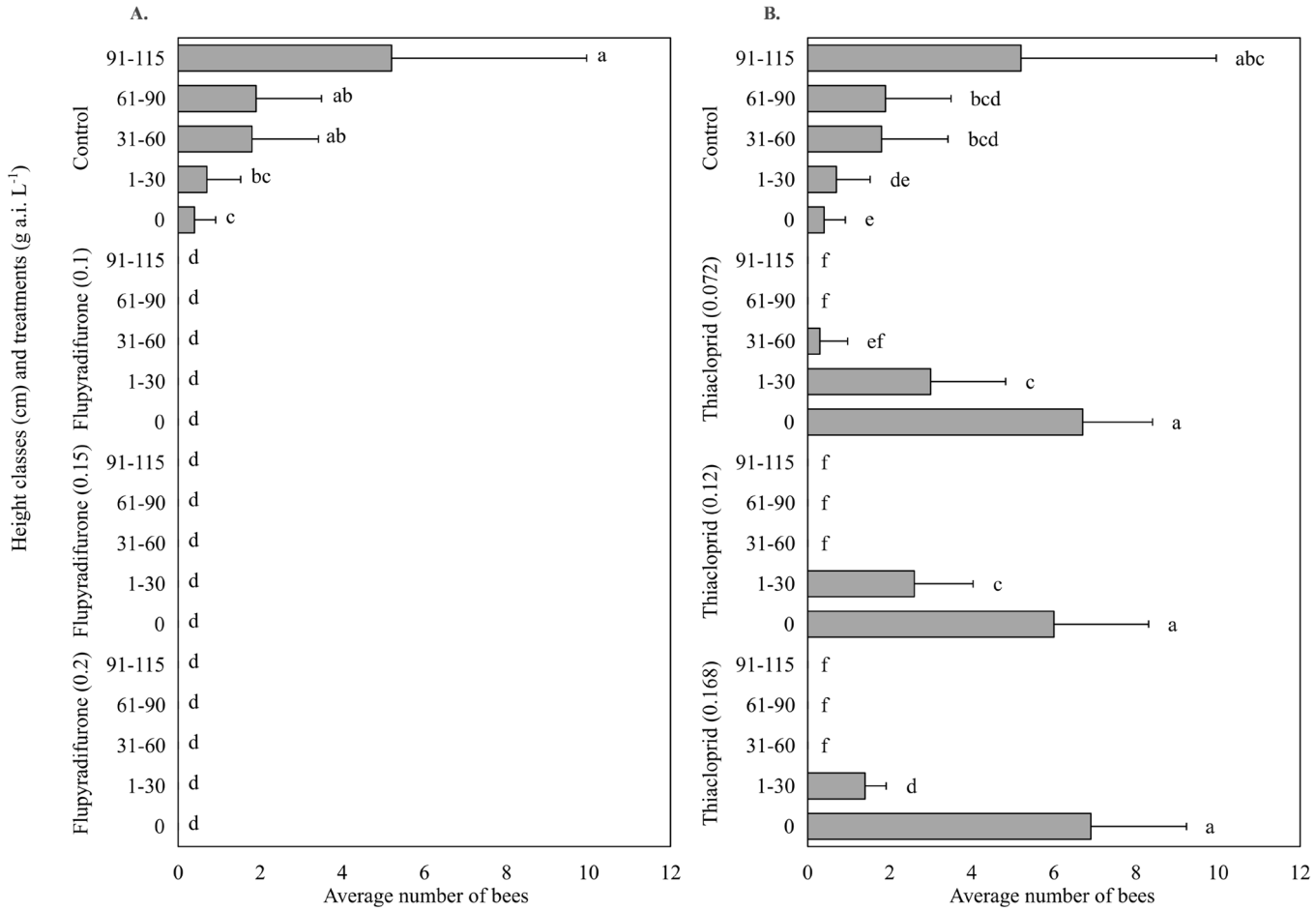
The absence of a repellent effect likely contributed to a greater oral toxicity of thiacloprid compared to flupyradifurone. Bommuraj et al. (2020), evaluating the average daily consumption of diets contaminated with various insectcides, found no significant difference in the intake of thiacloprid-contaminated diet relative to the control, indicating that thiacloprid does not repel bees. This finding supports the higher mortality rates observed in bees exposed to thiacloprid via ingestion.

Jacob et al. (2019) found that oral exposure of *A. mellifera* to acetamiprid, imidacloprid, and thiacloprid resulted in reduced survival and impaired locomotion. Thiacloprid exhibited intermediate toxicity, causing slower movement, extended resting periods, and tremors.

Brandt et al. (2017) assessed the effects of thiacloprid and clothianidin on queens and workers of *A. mellifera* via ingestion of contaminated diet (100 or 200 µg L^{-1} of thiacloprid and 10, 50 or 200 µg L^{-1} of clothianidin). Although thiacloprid was not lethal, it led to a reduction in total hemocytes count, melanization, and antimicrobial activity. These results differ from those of the present study, possibly due to variations in solution preparation and insecticide concentrations.

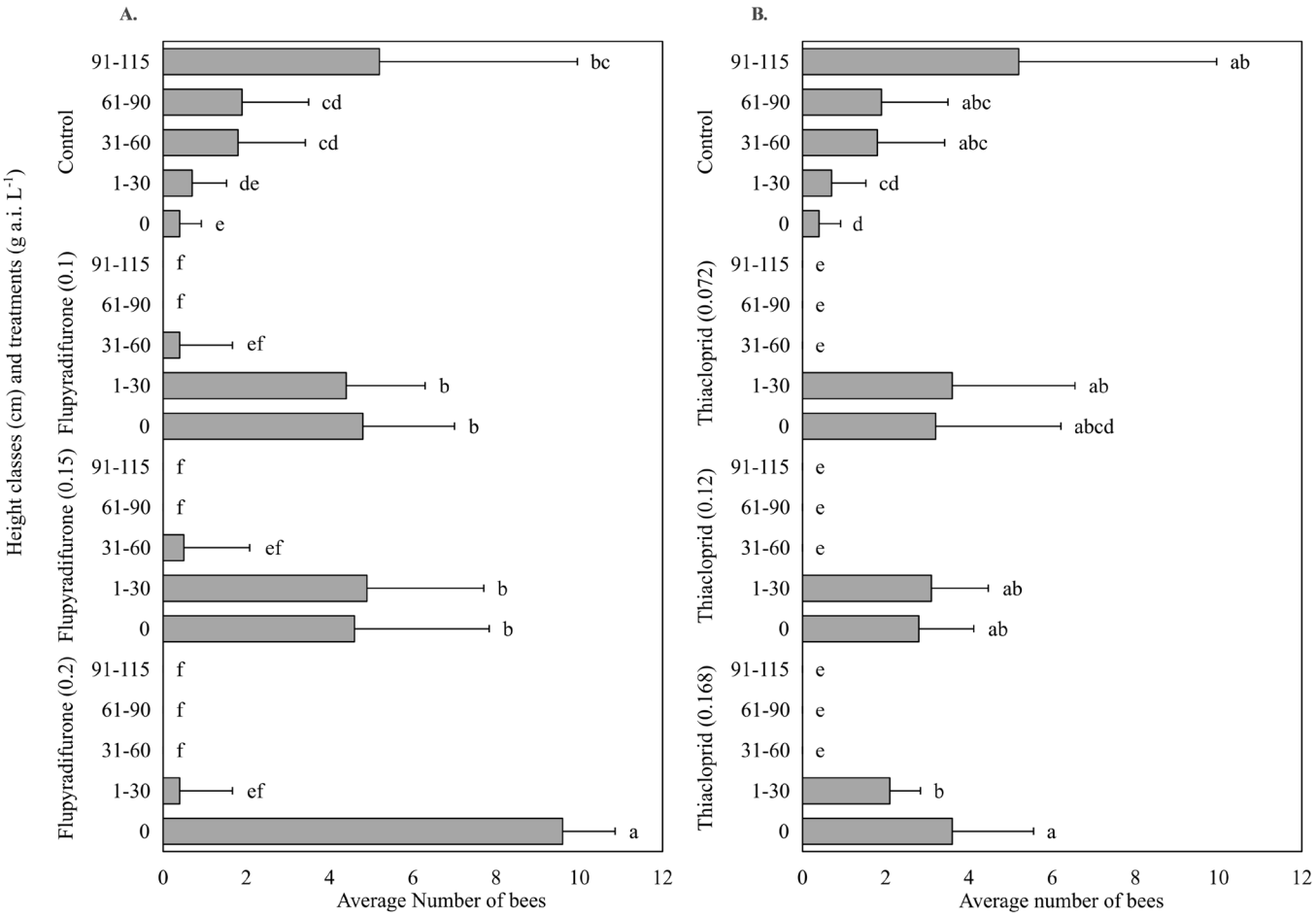
In the direct exposure bioassay, only bees from the absolute control and those exposed to the three flupyradifurone doses were evaluated, as no survivors remained in the other treatments (Figure 3A). In the oral exposure bioassay, bees from the absolute control and those that survived exposure to thiacloprid and flupyradifurone were assessed (Figures 4A and B).

In both exposure modalities, only bees from the absolute control group were able to reach the top of the flight tower (115 cm; Figures 3 and 4). Among the bees exposed to insecticides, the maximum flight height recorded was 60 cm, corresponding



*Means followed by different letters represent significant differences according to the Wilcoxon test at a 5% significance level

Figure 3. Flight ability (cm) of adult worker bees of *Apis mellifera* after direct spray with different doses of use in crops of the insecticides flupyradifurone (A) and thiacloprid (B)



*Different letters represent significant differences according to the Wilcoxon test at a 5% significance level

Figure 4. Flight ability (cm) of adult worker bees of *Apis mellifera* after ingestion of contaminated diet with different doses of use in crops of the insecticides flupyradifurone (A) and thiacloprid (B)

to level 3 of the tower, and only a small number of individuals reached this level. Specifically, three exposed to 0.072 g a.i. L^{-1} of thiacloprid via direct spraying (Figure 3B); four exposed to flupyradifurone at the dose of 0.1 g a.i. L^{-1} via contaminated diet; and five to flupyradifurone at 0.15 g a.i. L^{-1} also via contaminated diet (Figure 4A).

Most bees exposed to insecticides—regardless of the dose or mode of exposure—remained at the bottom of the flight tower (0 cm), accounting for 72.86% of individuals exposed via direct spraying and 59.58% of those exposed through contaminated diet. Additionally, a significant proportion of bees reached only level 2 of the tower: 26.02% for direct spraying and 38.54% for dietary exposure.

Both flupyradifurone and thiacloprid, irrespective of concentration and exposure route, negatively affected the flight ability of *A. mellifera*, disrupting motor functions. Although several studies have emphasized the importance of assessing how insecticide impair bee flight (Fernandez et al., 2012; Henry et al., 2012; Oliveira et al., 2014; Gomes et al., 2020), data specificaly involving flupyradifurone and thiacloprid remain limited.

Among the tested compounds, flupyradifurone was the most detrimental to flight performance. The reduced impact observed in of oral exposure may be attributed to the insecticide's repellent properties, which likely led the bees to consume less contaminated food and, thereby lowering their effective dose. Wu et al. (2021), investigating whether nectar (sucrose solution) containing 4 ppm of flupyradifurone altered foraging and recruitment dance in *A. mellifera*, found that bees were repelled by the compound—visiting feeders less frequently and spending less time imbibing the solution. Moreover, flupyradifurone significantly altered the expression of 116 genes, many of which are associated with olfactory learning deficits, and was shown to impair motor coordination, reducing flight ability.

Although less harmful thiacloprid also compromised flight performance, as no bee—regardless of the dose or exposure route—reached the top of flight tower. Fischer et al. (2014) reported that under field conditions, bees orally exposed to thiacloprid maintained foraging activity but exhibited reduced flight speed and lower return success to the colony. Jacob et al. (2019), evaluating the effects of three neonicotinoid (acetamiprid, imidacloprid, and thiacloprid) on the survival and locomotion of *A. mellifera* and the stingless bee *Scaptotrigona postica*, observed greater susceptibility in *A. mellifera*, with thiacloprid causing slower movement, extended resting periods, and tremors.

The findings of this study contribute to a deeper understanding of how flupyradifurone and thiacloprid interfere with bee flight—an essential behavior for survival and pollination. Foraging is directly linked to the maintenance, organization, and survival of the colony, as it encompasses

both pollination and transport of food resources to the hive. Furthermore, successful foraging depends on cognitive integrity, particularly memory, which enables bees to navigate using previously acquired environmental cues to locate resources and return to the colony (Balbuena et al., 2015).

These results offer valuable insights for the conservation of *A. mellifera* in high-pollination agricultural zones, especially considering that flupyradifurone and thiacloprid are frequently applied during the melon crop cycle. This practice poses risks of mortality, motor dysfunction, and reduced flight capacity. It is therefore recommended to avoid insecticide application during flowering, minimizing direct exposure and contact with contaminated nectar. The findings underscore the need to revise application schedules and promote Integrated Pest Management (IPM) strategies as sustainable alternative. Additionaly, they lay the groundwork for future research aimed at safeguarding *A. mellifera* in agricultural ecosystems.

Conclusions

1. Flupyradifurone was highly toxic to adult worker bees of *A. mellifera* when applied through direct spraying, regardless of the dose used, but exhibited low toxicity when ingested through a contaminated diet.

2. Thiacloprid demonstrated minimal toxicity to adult workers of bees when administrated through a contaminated diet, but caused moderate mortality when applied via direct spraying.

3. The flight ability of *A. mellifera* was impaired by both insecticides, with flupyradifurone causing the most pronounced effects, independent of dose and exposure route.

Contribution of authors: Diego de Albuquerque Coêlho, Ana Paula S. Macário, Victor Hugo M. Rocha, and Carlos Henrique P. de Barros worked on conceptualization, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualization, writing – original draft. Tiago Augusto L. Cardoso, Anderson Bruno A. de Andrade, and Elton L. Araujo worked on conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review & editing. Ewerton M. da Costa worked on conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing.

Data availability statement: The authors declare that there are no data underlying the text.

Conflict of interest: The authors declare no competing interests.

Financing statement: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Literature Cited

Abbott, W. S. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, v.18, p.265-267, 1925. https://doi.org/10.1093/jee/18.2.265a

Al Naggar, Y.; Baer, B. Consequences of a short time exposure to a sublethal dose of flupyradifurone (Sivanto) pesticide early in life on survival and immunity in the honeybee (*Apis mellifera*). Scientific Reports, v.9, p.1-11, 2019. https://doi.org/10.1038/s41598-019-56224-1

Alptekin, S.; Bass, C.; Nicholls, C.; Paine, M. J.; Clark, S. J.; Field, L.; Moores, G. D. Induced thiacloprid insensitivity in honeybees (*Apis mellifera* L.) is associated with up-regulation of detoxification genes. Insect Molecular Biology, v.25, p.171-180, 2016. https://doi.org/10.1111/imb.12211

Balbuena, M. S.; Tison, L.; Hahn, M. L.; Greggers, U.; Menzel, R.; Farina, W. M. Effects of sublethal doses of glyphosate on honeybee navigation. Journal of Experimental Biology, v.218, p.2799-2805, 2015. https://doi.org/10.1242/jeb.117291

Bommuraj, V.; Chen, Y.; Birenboim, M.; Barel, S.; Shimshoni, J. A. Concentration and time dependent toxicity of commonly encountered pesticides and pesticide mixtures to honeybees (*Apis mellifera* L.). Chemosphere, v.266, p.43-54, 2020. https://doi.org/10.1016/j.chemosphere.2020.128974

Brandt, A.; Grikscheit, K.; Siede, R.; Grosse, R.; Meixner, M. D.; Buchler, R. Immunosuppression in honeybee queens by the neonicotinoids thiacloprid and clothianidin. Scientific Reports, v.7, p.1-12, 2017. https://doi.org/10.1038/s41598-017-04734-1

Castilhos, D.; Bergamo, G. C.; Gramacho, K. P.; Gonçalves, L. S. Bee colony losses in Brazil: a 5-year online survey. Apidologie, v.50, p.263-272, 2019. https://doi.org/10.1007/s13592-019-00642-7

Costa, E. M.; Araujo, E. L.; Maia, A. V. P.; Silva, F. E. L.; Bezerra, C. E. S.; Silva, J. G. Toxicity of insecticides used in the Brazilian melon crop to the honeybee *Apis mellifera* under laboratory conditions. Apidologie, v.45, p.34-44, 2014. https://doi.org/10.1007/s13592-013-0226-5

Cridland, J. M.; Tsutsui, N. D.; Ramírez, S. R. The complex demographic history and evolutionary origin of the western honeybees, *Apis mellifera*. Genome Biology and Evolution, v.9, e457, 2017. https://doi.org/10.1093/gbe/evx009

Devine, G. J.; Furlong, M. J. Insecticide use: contexts and ecological consequences. Agriculture and Human Values, v.24, p.281-306, 2007. https://doi.org/10.1007/s10460-007-9067-z

Fernandez, F. C.; Cruz-Landim, C.; Malaspina, O. Influence of the insecticide pyriproxyfen on the flight muscle differentiation of *Apis mellifera* (Hymenoptera, Apidae). Microscopy Research and Technology, v.75, p.844-848, 2012. https://doi.org/10.1002/jemt.22003

Ferreira, J. V. A.; Storck-Tonon, D.; Ramos, A. W. P.; Costa, H. C.; Nogueira, D. S.; Mahlmann, T.; Oliveira, M. L.; Pereira, M. J. B.; Silva, D. J.; Peres, C. A. Critical role of native forest and savannah habitats in retaining neotropical pollinator diversity in highly mechanized agricultural landscapes. Agriculture, Ecosystems & Environment, v.338, e108084, 2022. https://doi.org/10.1016/j.agee.2022.108084

Fischer, J.; Müller, T.; Spatz, A. K.; Greggers, U.; Grünewald, B.; Menzel, R. Neonicotinoids interfere with specific components of navigation in honeybees. PLoS One, v.9, p.1-10, 2014. https://doi.org/10.1371/journal.pone.0091364

Gomes, I. N.; Vieira, K. I. C.; Gontijo, L. M.; Resende, H. C. Honeybee survival and flight capacity are compromised by insecticides used for controlling melon pests in Brazil. Ecotoxicology, v.29, p.97-107, 2020. https://doi.org/10.1007/s10646-019-02145-8

Henry, M.; Béguin, M.; Requier, F.; Rollin, O.; Odoux, J. F.; Aupinel, P.; Aptel, J.; Tchamitchian, S.; Decourtye, A. A common pesticide decreases foraging success and survival in honey bees. Science, v.336, p.348-350, 2012. https://doi.org/10.1126/science.1215039

Hesselbach, H.; Scheiner, R. Effects of the novel pesticide flupyradifurone (Sivanto) on the cognition and flavour of honeybees. Scientific Reports, v.8, p.49-54, 2018. https://doi.org/10.1038/s41598-018-23200-0

Jacob, C. R. O.; Malaquias, J. B.; Zanardi, O. Z.; Silva, C. A. S.; Jacob, J. F. O.; Yamamoto, P. T. Oral acute toxicity and impact of neonicotinoids on *Apis mellifera* L. and *Scaptotrigona postica* Latreille (Hymenoptera: Apidae). Ecotoxicology, v.28, p.744-753, 2019. https://doi.org/10.1007/s10646-019-02070-w

Jeschke, P.; Nauen, R.; Gutbrod, O.; Beck, M. E.; Matthiesen, S.; Haas, M.; Velten, R. Flupyradifurone (SivantoTM) and its novel butenolide pharmacophore: structural considerations. Pesticide Biochemistry and Physiology, v.121, p.31-38, 2015. https://doi.org/10.1016/j.pestbp.2014.10.011

Kearns, C. A.; Inouye, D. W. Pollinators, flowering plants and conservation biology. BioScience, v.47, p.297-307, 1997. https://doi.org/10.2307/1313191

Khalifa, S. A.; Elshafiey, E. H.; Shetaia, A. A.; El-Wahed, A. A. A.; Algethami, A. F.; Musharraf, S. G.; AlAjmi, M. F.; Zhao, C.; Masry, S. H. D.; Abdel-Daim, M. M.; Halabi, M. F.; Kai, G.; Al Naggar, Y.; Bishr, M.; El-Seedi, H. R. Overview of bee pollination and its economic value for crop production. Insects, v.12, e688, 2021. https://doi.org/10.3390/insects12080688

Klein, A. M.; Freitas, B. M.; Bomfim, G. A.; Boreux, V.; Fornoff, F.; Oliveira, M. O. A. Polinização agrícola por insetos no Brasil. Freiburg: University Freiburg, 2020. 162p. https://doi.org/10.6094/UNIFR/151237

Kruskal, W. H.; Wallis, W. A. Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association, v.47, p.583-621, 1952.

Maciel, F. A. O. Recognition of seasonal patterns in *Apis mellifera* bee colonies via clustering. Revista Brasileira de Computação Aplicada, v.10, p.74-88, 2018. https://doi.org/10.5335/rbca.v10i3.8788

Manjon, C.; Troczka, B. J.; Zaworra, M.; Beadle, K.; Randall, E.; Hertlein, G.; Singh, K. S.; Zimmer, C. T.; Homem, R. A.; Lueke, B.; Reid, R.; Kor, L.; Kohler, M.; Benting, J.; Williamson, M. S.; Davies, T. G. E.; Field, L. M.; Bass, C.; Nauen, R. Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. Current Biology, v.27, p.1137-1143, 2018. https://doi.org/10.1016/j.cub.2018.02.045

Nauen, R.; Jeschke, P.; Velten, R.; Beck, M. E.; Ebbinghaus-Kintscher, U.; Thielert, W.; Wölfel, K.; Haas, M.; Kunz, K.; Raupach, G. Flupyradifurone: A brief profile of a new butenolide insecticide. Pest Management Science, v.71, p.850-862, 2015. https://doi.org/10.1002/ps.3932

Oliveira, M. O. Population decline of crop bee pollinators. Acta Apicola Brasilica, v.3, p.1-6, 2015. http://dx.doi.org/10.18378/aab.v3i2.3623

Oliveira, R. A.; Roat, T. C.; Carvalho, S. M.; Malaspina, O. Side-effects of thiamethoxam on the brain and midgut of the Africanized honeybee *Apis mellifera* (Hymenoptera: Apidae). Environmental Toxicology, v.29, p.1122-1133, 2014. https://doi.org/10.1002/tox.21842

Pecenka, J. R.; Ingwell, L. L.; Krupke, C. H.; Kaplan, I. Implementing IPM in crop management simultaneously improves the health of managed bees and enhances the diversity of wild pollinator communities. Scientific Reports, v.13, e11033, 2023. https://doi.org/10.1038/s41598-023-38053-5

Pires, C. S. S.; Pereira, F. M.; Lopes, M. T. R.; Nocelli, R. C. F.; Malaspina, O.; Pettis, J. S.; Teixeira, E. W. Enfraquecimento e perda de colônias de abelhas no Brasil: há casos de CCD?. Pesquisa Agropecuária Brasileira. v.51, p.422-442, 2016. https://doi.org/10.1590/S0100-204X2016000500003

Pizzaia, W. C. S.; Pereira, N. C.; Diniz, T. O.; Toledo, V. A. A.; Ruvolo-Takasusuki, M. C. C. *Apis mellifera* Africanized queens tolerant to the neonicotinoid thiamethoxam. Scientific Electronic Archives, v.14, p.35-42, 2021. https://doi.org/10.36560/14220211292

Potts, S. G.; Roberts, S. P. M.; Dean, R.; Marris, G.; Brown, M. A.; Jones, R.; Neumann, P.; Settele, J. Declines of managed honeybees and beekeepers in Europe. Journal of Apicultural Research, v.49, p.15-22, 2016. https://doi.org/10.3896/IBRA.1.49.1.02

R Core Team. R: uma linguagem e ambiente para computação estatística. Viena: R Foundation for Statistical Computing. 2022.

Rahimi, E.; Barghjelveh, S.; Dong, P. Amount, distance-dependent and structural effects of forest patches on bees in agricultural landscapes. Agriculture & Food Security, v.11, e10, 2022. https://doi.org/10.1186/s40066-022-00360-x

Rahimi, E.; Jung, C. Global trends in climate suitability of bees: Ups and downs in a warming world. Insects, v.15, e127, 2024. https://doi.org/10.3390/insects15020127

Reilly, J. R.; Artz, D. R.; Biddinger, D.; Bobiwash, K.; Boyle, N. K.; Brittain, C.; Brokaw, J.; Campbell, J. W.; Daniels, J.; Elle, E.; Ellis, J. D.; Fleischer, S. J.; Gibbs, J.; Gillespie, R. L.; Gundersen, K. B.; Gut, L.; Hoffman, G.; Joshi, N.; Lundin, O.; Mason, K.; McGrady, C. M.; Peterson, S. S.; Pitts-Singer, T. L.; Rao, S.; Rothwell, N.; Rowe, L.; Ward, K. L.; Williams, N. M.; Wilson, J. K.; Isaacs, R.; Winfree, R. Crop production in the USA is frequently limited by a lack of pollinators. Proceedings of the Royal Society B, v.287, e20200922, 2020. https://doi.org/10.1098/rspb.2020.0922

Rükün, T.; Ercan, N.; Canko, E.; Avşar, B.; Dyer, A. G.; Garcia, J. E.; Çakmak, İ.; Mayack, C. Sub-lethal pesticide exposure interferes with honey bee memory of learnt colours. Science of the Total Environment, v.962, e178460, 2025. https://doi.org/10.1016/j.scitotenv.2025.178460

Shi, J.; Yang, H.; Yu, L.; Liao, C.; Liu, Y.; Jin, M.; Yan, W.; Wu, X. B. Sublethal acetamiprid doses negatively affect the lifespans and foraging behaviors of honey bee (*Apis mellifera* L.) workers. Science of the Total Environment, v.738, e139924, 2020. https://doi.org/10.1016/j.scitotenv.2020.139924

Shi, T.; Burton, S.; Wang, Y.; Xu, S.; Zhang, W.; YU, L. Metabolomic analysis of honey bee, *Apis mellifera* L. response to thiacloprid. Pesticide Biochemistry and Physiology, v.152, p.17-23, 2018. https://doi.org/10.1016/j.pestbp.2018.08.003

Taning, C. N. T.; Vanommeslaeghe, A.; Smagghe, G. With or without foraging for food, field-realistic concentrations of sulfoxaflor are equally toxic to bumblebees (*Bombus terrestris*). Entomologia Generalis, v.39, p.151-155, 2019. https://doi.org/10.1127/entomologia/2019/0784

Therneau, T.; Lumley, T. Survival: Survival analysis, including penalised likelihood. R package version, 2020. Available on: <http://CRAN.Rproject.org/package=survival>. Accessed on: Abr. 2020.

Tison, L.; Hahn, M. L.; Holtz, S.; Rössner, A.; Greggers, U.; Bischoff, G.; Menzel, R. Honey bees behavior is impaired by chronic exposure to the neonicotinoid thiacloprid in the field. Environmental Science & Technology, v.50, p.7218–7227, 2016. https://doi.org/10.1021/acs.est.6b02658

Tong, L.; Nieh, J. C.; Tosi, S. Combined nutritional stress and a new systemic pesticide (flupyradifurone, Sivanto®) reduce bee survival, food consumption, flight success, and thermoregulation. Chemosphere, v.237, p.114-120, 2019. https://doi.org/10.1016/j.chemosphere.2019.124408

Tosi, S.; Nieh, J. C.; Brandt, A.; Colli, M.; Fourrier, J.; Giffard, H.; Hernández-López, J.; Malagnini, V.; Williams, G. R.; Simon-Delso, N. Long-term field-realistic exposure to a next-generation pesticide, flupyradifurone, impairs honey bee behaviour and survival. Communications Biology, v.4, e805, 2021. https://doi.org/10.1038/s42003-021-02336-2

Van Engelsdorp, D.; Evans, J. D.; Saegerman, C.; Mullin, C.; Haubruge, E.; Nguyen, B. K.; Frazier, M.; Frazier, J.; Cox-Foster, D.; Chen, Y.; Underwood, R.; Tarpy, D. R.; Pettis, J. S. Colony collapse disorder: a descriptive study. PLoS One, v.4, e6481, 2009. https://doi.org/10.1371/journal.pone.0006481

Varikou, K.; Garantonakis, N.; Birouraki, A. Exposure of *Bombus terrestris* L. to three different active ingredients and two application methods for olive pest control. Entomologia Generalis, v.39, p.53-60, 2019. https://doi.org/10.1127/entomologia/2019/0706.

Wu, Y. Y.; Pasberg, P.; Diao, Q. Y.; Nieh, J. C. Flupyradifurone reduces nectar consumption and foraging but does not alter honey bee recruitment dancing. Ecotoxicology and Environmental Safety, v.207, e111268, 2021. https://doi.org/10.1016/j.ecoenv.2020.111268

Wu-Smart, J.; Spivak, M. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. Scientific Reports, v.6, e32108, 2016. https://doi.org/10.1038/srep32108

Zhang, W.; Jiang, Z.; Ding, M.; Wang, X.; Huang, A.; Qiu, L.; Qi, S. Novel neonicotinoid insecticide cycloxaprid exhibits sublethal toxicity to honeybee (*Apis mellifera* L.) workers by disturbing olfactory sensitivity and energy metabolism. Journal of Hazardous Materials, v.485, e136923, 2025. https://doi.org/10.1016/j.jhazmat.2024.136923