



RESEARCH ARTICLE - BEES

Honey Bee Survival and Flight Capacity After Exposure to Sulfoxaflor Residues

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Article History

Edited by

Evandro Nascimento Silva, UEFS, Brazil

Received 24 April 2024

Initial acceptance 10 November 2024

Final acceptance 20 November 2024

Publication date 03 December 2024

Keywords

Africanized bee, *Cucumis melo* L.,
Insecticide, Toxicity.

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Abstract

Knowing the toxicity of insecticides used for pest control in melon on the honey bee *Apis mellifera* is essential for the conservation of this species in production areas, thus enabling the conciliation of insecticide applications with the sustainable use of pollinators in the field. From this perspective, this study aimed to evaluate the survival and flight ability of *A. mellifera* after exposure to residues of the insecticide Sulfoxaflor on melon leaves. The bioassay was conducted under laboratory conditions in a completely randomized design set up in a 3 x 6 factorial arrangement, represented by two doses of the insecticide Sulfoxaflor (0.048 and 0.192 g i.a./L), one control (distilled water), and six exposure times after application of the product on melon leaves: immediately after spraying and 1h, 2h, 3h, 24h, and 48h after spraying. The insecticide Sulfoxaflor caused 100% mortality in the bees exposed to the dose of 0.048 g i.a./L immediately after spraying and 1h, 2h, and 3h after spraying. The dose of 0.192 g i.a./L, regardless of the exposure time after spraying, resulted in 100% mortality. The median lethal time (TL₅₀) provided by the Sulfoxaflor doses was significantly lower than the control, regardless of the time after foliar spraying. Sulfoxaflor negatively affected the ability of surviving bees to fly. Despite the dose and exposure time after spraying, Sulfoxaflor was highly toxic due to residues in *A. mellifera* under laboratory conditions.

Introduction

Melon (*Cucumis melo* L.) is one of the main cucurbits produced and exported by Brazil (Ibge, 2023), counting on several management strategies used to ensure the production potential of this crop, including chemical pest control and the installation of beehives with the honey bee *Apis mellifera* L. (Hymenoptera: Apidae) around production areas (Guimarães et al., 2008; Sousa et al., 2009; Klein et al., 2020). However, given the need to apply insecticides and the presence of bees in the fields, there may be negative impacts on pollinators.

Castilhos et al. (2019) reported that about 50% of beehives are annually lost in different regions of Brazil,

with insecticide application in crops representing one of the main causes of this population decline. Bees can be exposed to insecticides mainly through contact with spray droplets, collecting and ingesting contaminated food, and contact with insecticide residues on plant parts (e.g., leaves) (Heard et al., 2017). Bee contact with insecticides can be lethal or result in sublethal effects, e.g., tremors, paralysis, and loss of flight ability (Gomes et al., 2020; Tosi et al., 2022).

The insecticide Sulfoxaflor, belonging to the chemical group of Sulfoxamines, is very efficient in the control of several melon pests, e.g., the silverleaf whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) and the melon aphid *Aphis gossypii* (Hemiptera: Aphididae) (Agrofit, 2024). However,



despite its efficiency in pest control, some researchers have been warning about the adverse effects of Sulfoxaflor on bees. Capela (2022), after providing a diet contaminated with three sublethal doses of sulfoxaflor [720, 1200, and 2600 ppb ($\mu\text{g/l}$)] for *A. mellifera* bees, connecting them to a chip, and releasing them in the environment at a distance of 1 km from the hive, observed that all three doses tested caused adverse effects in the bees' ability to return to the hives, with only 28% of them successfully managing to return. In experiments in which the bee *A. mellifera* was exposed via ingestion to Sulfoxaflor, it was observed that exposure significantly reduced bee survival and caused negative effects on their intestinal microbiota (Naggar et al., 2022). Another study revealed that continuous exposure to high doses of Sulfoxaflor (0.5 and 2 mg/L) and decreasing survival rates also affected food consumption and the body weight of *A. mellifera* bees (Cheng et al., 2023).

However, despite the results presented above, there is still no information about the residual effect of the Sulfoxaflor levels recommended for melon on *A. mellifera*. Therefore, evaluating the toxicity of this insecticide on *A. mellifera* is essential for the sustainable management and conservation of this bee in production areas. From this perspective, this study aimed to evaluate the survival and flight ability of *A. mellifera* after contact with residues of the insecticide Sulfoxaflor on melon leaves under laboratory conditions.

Material and Methods

The experiment was conducted at the Laboratory of Entomology of the Center of Sciences and Agri-food Technology (CCTA) of the Federal University of Campina Grande (UFCG), Campus Pombal, Paraíba, Brazil. The research employed adult *A. mellifera* workers from three colonies maintained at the apiary of CCTA/UFCG, which receives constant management to maintain the health and vitality of the swarms.

The insecticide evaluated was Sulfoxaflor (commercial name Verter® SC), at the minimum (100 mL/ha: 0.048 g i.a./L) and maximum doses (400 mL/ha: 0.192 g i.a./L) registered for control of the melon aphid *A. gossypii* and the silverleaf whitefly *B. tabaci*, in melon (Agrofit, 2024). According to the manufacturer's recommendations, an average mixture volume of 500 L/ha was used to calculate the dilutions. Distilled water was used as a control.

The bioassay was performed according to the methodology proposed by Costa et al. (2014) in a completely randomized design set up in a 3 X 6 factorial arrangement, corresponding to two doses of the insecticide Sulfoxaflor (0.048 g i.a./L and 0.192 g i.a./L), one control (distilled water), and six exposure times after spraying (immediately and after 1h, 2h, 3h, 24 h, and 48h) the product on leaves, with ten replications. Ten adult bees formed each experimental unit.

Yellow melon plants of the cultivar Iracema (SAKATA®) were used to evaluate the residual toxicity of the insecticide. The plants were cultivated in a plant nursery covered with an anti-aphid screen. The plants were produced and maintained

in pots (with a capacity of 1 kg) containing soil + organic matter as substrate (2:1 proportion) and irrigated twice daily. Ten plants were selected for each treatment when the plants reached the minimum of six mature leaves.

The selected plants were separated into two groups: Group 1 – Plants that would receive the dose of 0.048 g i.a./L and Group 2 – Plants that would receive 0.192 g i.a./L. Within each of these two groups of plants, six groups were created for spraying with the insecticide Sulfoxaflor, namely: Group 1 – freshly sprayed, Group 2 – 1 hour of drying after spraying, Group 3 – 2 hours of drying after spraying, Group 4 – 3 hours of drying after spraying, Group 5 – 24 hours of drying after spraying, and Group 6 – 48 hours of drying after spraying. Spraying was performed using a manual sprayer and by simulating the application of the product in the field while ensuring an even leaf coverage with the mixture.

For the proper drying of the sprayed product, the plants were transferred to an airy and shaded place, where they remained during the abovementioned hours. The leaves of each plant group were cut at the height of the petiole and then placed in arenas (plastic containers 15 X 15 cm (diameter x height) and partially covered with an anti-aphid screen to allow proper air circulation in the environment) along with a cotton pad soaked in water and an artificial diet (bee candy). After this procedure, adult *A. mellifera* workers were released into the arenas for contact with the residues of the product. After this, at each time after leaf spraying, the mortality and motor disorders (prostration, tremors, paralysis) of the insects were evaluated after 1, 2, 3, 4, 5, 6, 12, and 24 hours of exposure.

The bees that survived after 24 hours of exposure to the residues of Sulfoxaflor at each drying time were evaluated for their flight ability using a flight tower, according to the methodology proposed by Gomes et al. (2020). The flight tower was built from wood, measuring 35 x 35 x 115 cm, and covered with resistant, transparent plastic. A metric tape was attached to the sides and a fluorescent lamp at the top so that, when lit, it stimulated positive phototropism in the bees.

For flight analysis, the surviving bees were released one by one inside the tower, and 60 seconds was counted for flight conclusion. This time interval was used to assess how far the bees managed to climb the tower, either flying or walking. The flight tower had five height levels: 1 (base of the tower), 2 (from 1 cm to 30 cm in height), 3 (from 31 cm to 60 cm in height), 4 (from 61 cm to 90 cm in height), and 5 (from 91 cm to 115 cm, top of the tower). The evaluations were conducted in a dark room at an average room temperature of 26 ± 2 °C and a relative humidity (R.H.) of $65 \pm 10\%$, with the only light source corresponding to the lamp installed at the top of the tower.

Data Analysis

The mortality rate was calculated for each treatment and corrected using Abbott equation (1925), followed by permutational analysis of variance (Permanova) at 5%

significance, and then by Wilcoxon test. The data on adult survival were analyzed using the statistical package Survival (Therneau & Lumley, 2010) of the software R and subjected to Weibull distribution analysis. Contrast clustered treatments with similar effects (toxicity and mortality speed). The median lethal time (TL_{50}) was also calculated for each group. Permanova was applied at 5% significance for the flight ability, followed by the Wilcoxon test. All analyses were performed using the software R (R development core team, 2022).

Results and Discussion

The two doses of Sulfoxaflor (0.048 g i.a./L and 0.192 g i.a./L) showed high toxicity to *A. mellifera* in all six exposure times, significantly differing from the control. A mortality of 100% was observed in the bees exposed to the dose of 0.048 g i.a./L after contact with the freshly sprayed leaves and 1, 2, and 3 hours after spraying. The mortality of the bees exposed to the residues of Sulfoxaflor after 24h and 48h of spraying was 77.9% and 76.8%, respectively. Regarding the dose of 0.192 g i.a./L, 100% mortality was observed regardless of the exposure time after spraying (Fig 1).

In the treatments with the insecticide Sulfoxaflor, especially at the highest dose (0.192 g i.a./L) and in contact with freshly sprayed leaves, the insects showed peculiar behaviors likely caused by stress, e.g., scraping the leaves and attempting to escape, crowding at the top of the arenas. Furthermore, the bees quickly showed tremors followed by paralysis and prostration before death. The lower the interval between applying the product on plants and bee exposure to insecticide residues, the faster they manifested adverse effects in their motor abilities before death. The bees that managed

to resist were visibly weakened and did not stay for long under these conditions. Gomes et al. (2020) reported that after contact with insecticides, bees may die or manifest sublethal effects that alter their natural behavior, affecting the colony long-term.

The high mortality observed, as well as the adverse effects on motor ability, are probably related to the mechanism of action of the insecticide, which causes hyperexcitation of the insects' nervous system, leading to irreversible effects such as tremors and prostration prior to death (Marques, 2022). The product acts similarly to neonicotinoids, which can change the flight ability of bees and the functioning of the colony (Tosi et al., 2017). For example, the neonicotinoid Thiamethoxam, when evaluated on *A. mellifera* at the dose of 0.15 g i.a./L and with an exposure time of 1 hour after spraying, caused 100% bee mortality (Costa et al., 2014).

The survival analysis, which presents the bee mortality rate, showed that the median lethal time (TL_{50}) provided by the doses of the insecticide Sulfoxaflor was significantly lower compared to the control, regardless of the time after spraying. The TL_{50} of 24h and 48h after spraying with the dose of 0.048 g i.a./L was 18.6 hours, the highest TL_{50} recorded for the insecticide. These treatments differed significantly between 2 and 3 hours after spraying the same dose, which provided a TL_{50} of 13 hours. The lowest TL_{50} recorded was at the maximum dose of 0.192 g i.a./L, at the time immediately after exposure to spraying, with only 3 hours (Fig 2).

Despite the lack of more information on the residual effect, the high mortality rate observed in the present study is possibly due to Sulfoxaflor acting similarly to a neonicotinoid on bees, affecting the central nervous system of these insects. Kim (2022) also observed high and fast mortality caused by Sulfoxaflor in *A. mellifera* larvae after exposure via ingestion.

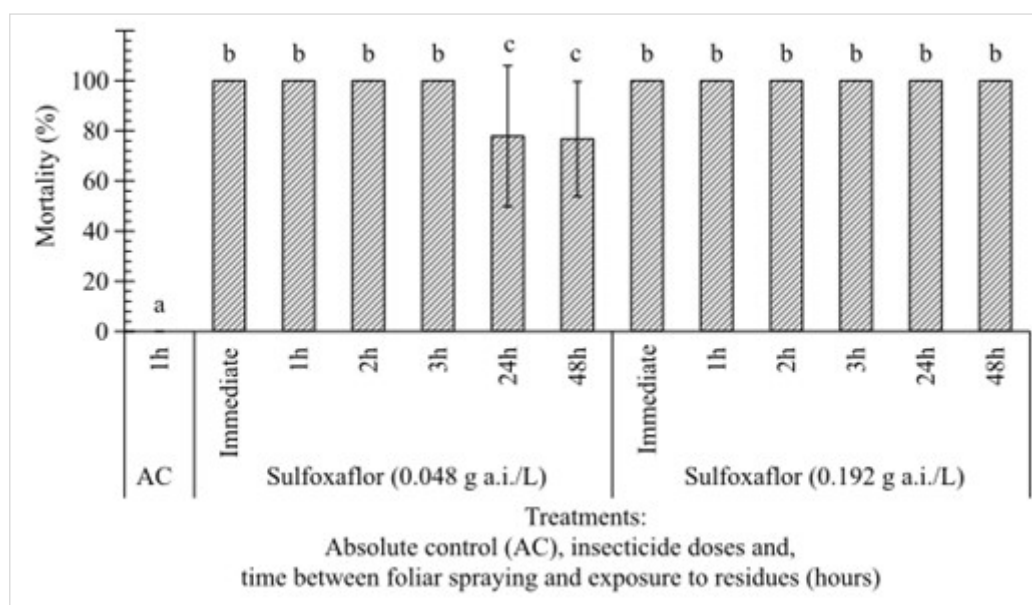


Fig 1. Mortality (%) of *Apis mellifera* after exposure to residues of the insecticide Sulfoxaflor on melon leaves (*C. melo* L.).

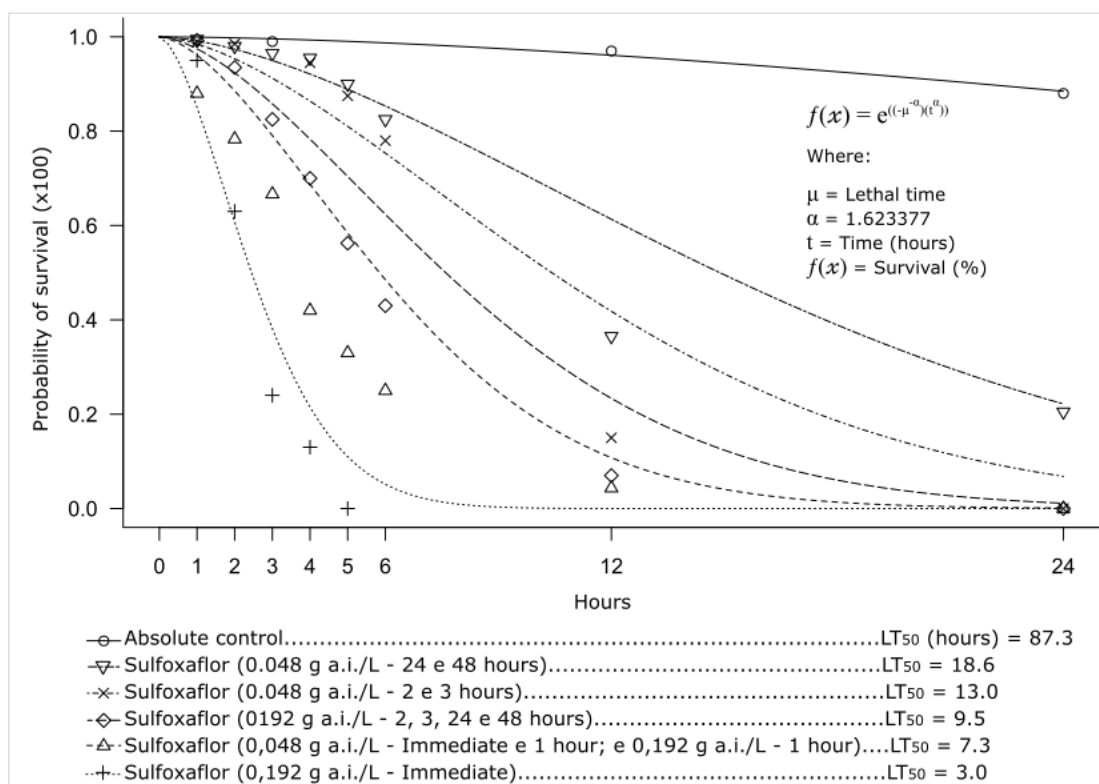


Fig 2. Survival (%) of *Apis mellifera* workers after contact with melon leaves (*C. melo* L.) sprayed with Sulfoxaflor, with median lethal times (TL₅₀) in hours.

The product acts by causing changes in bee metabolism, accelerating the insect's death (Shengang, 2023). Sulfoxaflor-based products have recently been reported to cause high mortality among pollinating insects and are directly related to losses in *A. mellifera* swarms (Tooge, 2019; Fontes, 2021). This fact can affect the yield of several crops, including melon, and compromise ecosystem services.

Concerning flight ability, only the bees from the treatments referring to 24h and 48h after spraying the dose of

0.048 g i.a./L and the bees of the absolute control were used. There was a significant difference between the control bees and those exposed to Sulfoxaflor residues since virtually 100% of the bees only exposed to water usually managed to walk or fly. On the other hand, the bees exposed to Sulfoxaflor were less able to walk or fly regardless of the contact happening 24 or 48 hours after the product was applied to melon leaves (Fig 3). It should be noted that the bees exposed to the insecticide moved with great difficulty in the flight tower.

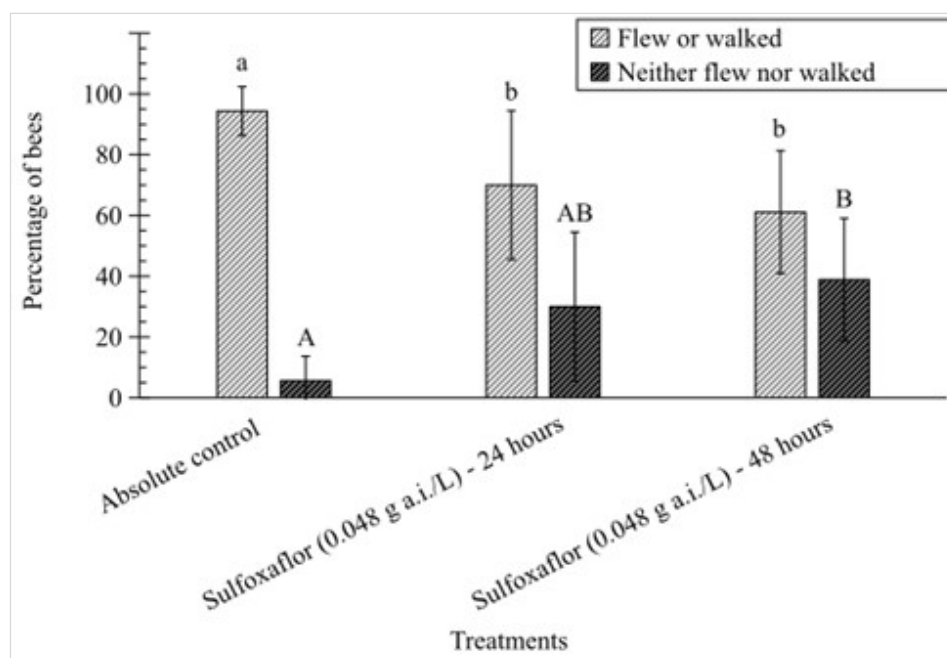


Fig 3. Percentage of bees that flew or walked x neither flew nor walked after exposure to Sulfoxaflor residues on melon leaves.

Concerning the height levels, the bees exposed only to distilled water (control) managed to reach the top of the flight tower in higher numbers than those exposed to Sulfoxaflor, which, due to being visibly weakened, often did not reach the higher levels of the tower (Fig 4). The adverse effect of Sulfoxaflor is more intense in the three top height levels than in the two lower heights (0 and 1-30 cm).

After coming into contact with chemical products, in addition to mortality, bees can manifest adverse effects that change their normal behavior, e.g., flight ability, with the potential to compromise the entire colony (Gomes et al., 2020). It is known that direct contact with Sulfoxaflor compromises the ability of bees to return to the hive, representing a great risk to the colony, which may face irreversible impacts (Capela et al., 2022). Furthermore, it was possible to observe, in the present study, that contact with insecticide residues also affects the flight ability of insects.

Adverse effects on the natural behavior of bees and their motor functions could directly impact the colony since basic functions will not be carried out as chemical products will affect workers (Pacífico-Da-Silva; Melo & Blanco, 2016). In melon cultivation, healthy bees are crucial because, without these organisms, there is no pollination, consequently affecting fruit

production (Kiill et al., 2011). Bees must be healthy to function in the environment, which involves pollination and propagation of species and ensures that the ecosystem can function perfectly and that crops of economic importance are produced.

These are the first results on the residual toxicity of the insecticide Sulfoxaflor on *A. mellifera* at the doses registered for use in melon and at different exposure times after spraying. The results obtained will assist in managing and conserving bees in melon production areas and subsidize new research, especially under field conditions. It should also be noted that the purpose of this study is not to either recommend or not recommend the use of Sulfoxaflor for pest control but rather to alert that it is indispensable to avoid using this product during flowering, the moment of highest foraging activity of *A. mellifera* in the crop.

Conclusion

The insecticide Sulfoxaflor, regardless of the dose evaluated and the exposure time after spraying, was highly toxic to *A. mellifera* bees via residual contact on melon leaves, also harming the flight ability of bees that survived after exposure to the product's residues.

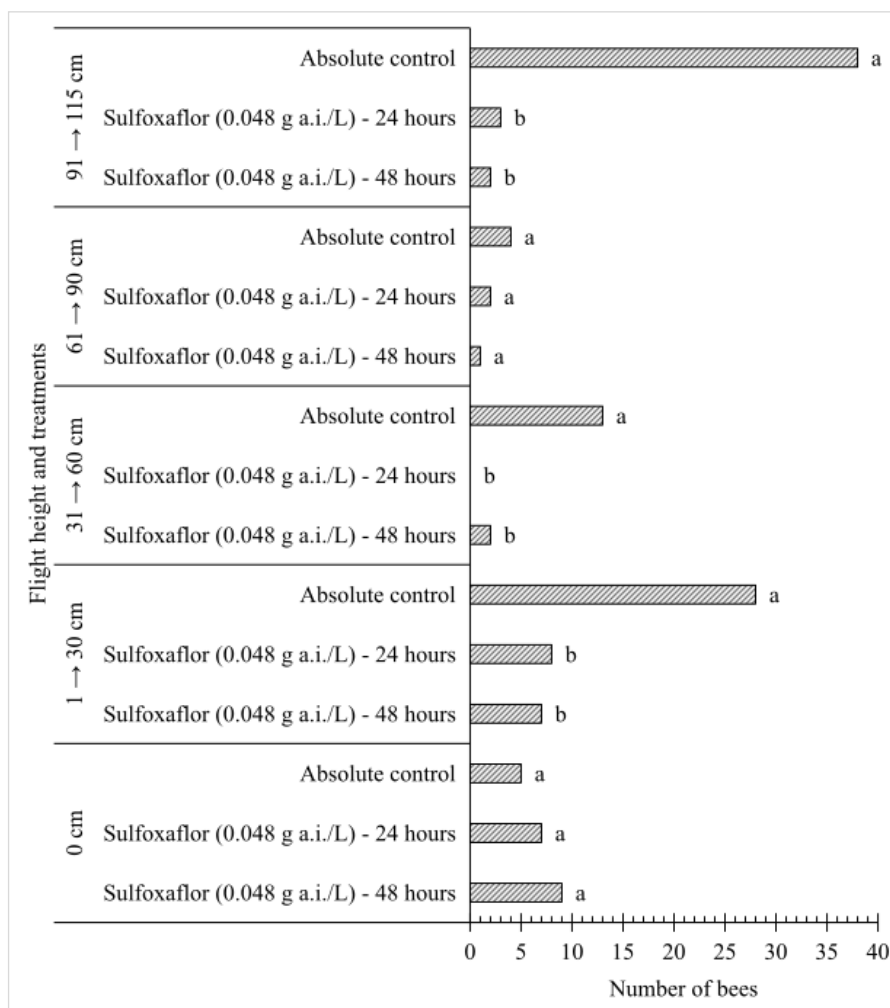


Fig 4. Flight height reached by bees after exposure to the insecticide Sulfoxaflor.

Author's Contribution

EMC: Conceptualization, methodology, formal analysis, writing-review & editing.

LPA: Investigation, writing-original draft.

EKSS: Writing-original draft, writing-review & editing.

VHMR: Investigation, writing-original draft.

TALC: Formal analysis, figure design, writing-review & editing.

ELA: Conceptualization, writing-review & editing.

FAA: Writing-review & editing.

References

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

Agrofit. (2024). Sistema de Agrotóxicos Fitossanitários. <https://agrofit.agricultura.gov.br/>. (data de acesso: 26 de fevereiro de 2024).

Capela, N., Sarmiento, A., Simões, S., Azevedo-Pereira, H. & Sousa, J. (2022). Sub-lethal doses of sulfoxaflor impair honeybee homing ability. *Science of the Total Environment*, 837:155-710. <https://doi.org/10.1016/j.scitotenv.2022.155710>

Castilhos, D., Bergamo, G.C. Gramacho, K.P & Gonçalves, L.S. (2019). Bee colony losses in Brazil: a 5-year onlinesurvey. *Apidologie*, 50: 263-272. <https://doi.org/10.1007/s13592-019-00642-7>

Cheng, S., Dai, P., Li, R., Chen, Z., Liang, P., Xie, X., Zhen, C. & Gao, X. (2023). The sulfoximine insecticide sulfoxaflor exposure reduces the survival status and disrupts the intestinal metabolism of the honeybee *Apis mellifera*. *Journal of Hazardous Materials*, 442: 130-109. <https://doi.org/10.1016/j.jhazmat.2022.130109>

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

Fontes, Y. M. (2021). Considerações sobre a importação de abelhas rainhas *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) originadas do Mercosul. *Revista Brasileira de Gestão Ambiental e Sustentabilidade*, 7: 1011-1019. [https://doi.org/10.21438/rbgas\(2020\)071634](https://doi.org/10.21438/rbgas(2020)071634)

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

Guimarães, J. A., Sobrinho, R.B, Azevedo, F.R., Araújo, E.L., Terão, D. & Mesquita, A.L.M. (2008). Manejo integrado de pragas do meloeiro. Em Braga Sobrinho, R., Guimarães, J.A., Freitas, J.A.D. & Terão, D. (Eds.), *Produção Integrada de Melão* (pp. 183-199). Embrapa Agroindústria Tropical.

Heard, M.S., Baas, J., Dorne, J.L., Lahive, E., Robinson, A.G., Rortais, A., Spurgeon, D. J., Svendsen, C. & Hesketh, H. (2017). Comparative toxicity of pesticides and environmental contaminants in bees: Are honey bees a useful proxy for wild bee species? *Science of the Total Environment*, 578: 357-365. <https://doi.org/10.1016/j.scitotenv.2016.10.180>

IBGE. Instituto Brasileiro de Geografia e Estatística. Lavoura temporária (2022). <https://cidades.ibge.gov.br/brasil/pesquisa/14/10193>. (data de acesso: 05 de janeiro de 2023).

Kiill, L.H.P., Coelho, M. S., Siqueira, K.M.M. & Costa, N.D. (2011). Avaliação do padrão de visitação de *Apis mellifera* em três cultivares de meloeiro, em Petrolina -PE, Brasil. *Revista Brasileira de Fruticultura*, 33: 455-460. <https://doi.org/10.1590/S0100-29452011000500060>

Kim, J. (2022). Assessment of acute and chronic toxicity of cyantraniliprole and sulfoxaflor on honeybee (*Apis mellifera*) larvae. *Pest Management Science*, 78: 5402-5412. <https://doi.org/10.1002/ps.7162>

Klein, A.M., Freitas, B.M., Bomfim, G.A., Boreux, V., Fornoff, F. & Oliveira, M.O.A. (2020). Polinização agrícola por insetos no Brasil. Maranguape, Unifreiburg.

Marques, L. (2022) Mecanismo de ação dos inseticidas neonicotinóides, organofosforados e carbamatos. <https://elevagro.com/blog>. (data da acesso: 10 janeiro de 2023).

Naggar, Y.A., Singavarapu, B., Paxton, R.J. & Wubet, T. (2022). Bees under interactive stressors: the novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honeybees and increase opportunistic bacterial pathogens. *Science of the Total Environment*, 849: 157-941. <https://doi.org/10.1016/j.scitotenv.2022.157941>

Pacífico-Da-Silva, I.P.D, Melo, M.M. & Blanco, B.S. (2016). Efeitos tóxicos dos praguicidas para abelhas. *Revista brasileira de higiene e sanidade animal*, 10: 142-157. <http://doi.org/10.5935/1981-2965.20160013>

R Development Core Team. R: A language and environment for statistical computing. (2011). <http://www.R-project.org/>. (Accessed on: 14 February 2022).

Shenhang, C., Pingli, D., Ren, L., Zhibin, C., Pingzhuo, L., Xiaoping, X., Congai, Z. & Xiwu, G. (2023). The sulfoximine insecticide sulfoxaflor exposure reduces the survival status and disrupts the intestinal metabolism of the honeybee *Apis mellifera*. *Journal of Hazardous Materials*, 442: 130-109. <https://doi.org/10.1016/j.jhazmat.2022.130109>

Sousa, R.M., Aguiar, O.S., Freitas, B.M., Neto, A.A.S. & Pereira, T.F.C. (2009). Requerimentos de polinização do meloeiro (*Cucumis melo* L.) no município de Acaraú-CE – Brasil. *Revista Caatinga*, 22: 238-242.

Therneau, T. & Lumley, T. (2010). Survival analysis, including penalized likelihood. R package version, 2: 36-2.

Tooge, R. (2019). Governo autoriza mais 63 agrotóxicos sendo 7 novos; total de registros em 2019 chega a 325. <https://g1.globo.com/economia/agronegocios/noticia/2019/09/17>. (Accessed on: 27 March 2020).

Tosi, S., Burgio, G. & Nieh, J.C. (2017). A common neonicotinoid pesticide, thiamethoxam, impairs honeybee flight ability. *Scientific reports*, 7: 1-8. <https://doi.org/10.1038/s41598-017-01361-8>

Tosi, S., Sfeir, C., Carnesecchi, E., Vanengelsdorp, D. & Chauzat, M. P. (2022). Lethal, sublethal, and combined effects of pesticides on bees: A meta-analysis and new risk assessment tools. *Science of the Total Environment*, 844: 1-12. <https://doi.org/10.1016/j.scitotenv.2022.156857>

