HONEY BEE (*Apis mellifera*) COGNITION UNDER EXPOSURE TO FIELD-REALISTIC DOSES OF DELTAMETHRIN AND IMIDACLOPRID: INDIVIDUAL AND COMBINED EFFECTS

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1. INTRODUCTION

Bees are one of the main pollinators worldwide, being essential for the maintenance of ecosystems and agricultural productivity (KLEIN et al., 2007). Despite their ecological importance, bee populations face significant declines due to a variety of factors (POTTS et al., 2010), including the use of pesticides, particularly insecticides (BRITTAIN et al., 2010; VANENGELSDORP; MEIXNER, 2010).

Agricultural insecticides can lead to the death of bees or cause sublethal effects, such as disorientation, morphological alterations, behavioral changes, and impairment of learning and memory capacities (DI NOI et al., 2021). Although lethal effects are frequently studied, sublethal impacts and interactions between different substances are still undervalued (TOSI et al., 2022). Thus, our study aims to analyze, through classical conditioning of the Proboscis Extension Reflex (PER) method (MATSUMOTO, 2012), how the insecticides Imidacloprid (neonicotinoid) and Deltamethrin (pyrethroid), alone and in combination, affect the learning and memory of bees. We hypothesize that contamination with insecticides will cause reduced learning and memory. Furthermore, the interaction between insecticides will potentiate the individual effects of insecticides.

2. METHODOLOGY

Honey bees *Apis mellifera* (L., 1758) were captured at the Palma Agricultural Center, Capão do Leão municipality, Rio Grande do Sul State, between June and August of 2024. After the capture, bees were immediately transported to the laboratory at UFPel, cold-anesthetized, and mounted in cut pipette tips, which only allowed free movement of the antennae and mouthparts (SCHEINER et al., 2013). Bees were randomly separated into four groups and offered oral treatments of Imidacloprid (Nortox 480 SC): 0.0005 μg.bee^{-1}, Deltamethrin (Decis 25 EC®): 0.002 μg.bee^{-1} and the combination of Imidacloprid+Deltamethrin: 0.0005+0.002 μg.bee-1. Bees were kept for one hour in a dark chamber (24° C) to adapt to the restriction of movements and metabolization of the insecticides.

After this, bees were submitted at five acquisition trials of classical conditioning of PER (MATSUMOTO et al., 2012) consisting of: 1- adaptation to the test setup for 10s; 2- Presentation of geranium scent (Conditioned Stimulus, CS) for 4s; 3- Contact of the antennae with a sugar solution (0,5 g.g^{-1}) (Unconditioned Stimulus, EI) to provoke PER (Unconditioned Response, UR) and feeding with the solution for 3s; 4- Rest for 10s before being returned to the group. Inter-trial intervals were conducted between 15 to 20 minutes. After each trial, proboscis extension observed during the first 3s of presentation of the CS was counted as a conditioned PER (cPER). After the acquisition trials, the bees were returned to the dark chamber. The memory tests were performed after one and 24 hours and consisted of the presentation of the CS and count of cPER.

Statistical analysis was conducted in the Rstudio environment (R Core Team, 2024). Using the package glmmTMB (MOLLIE et al., 2017), a generalized linear mixed model was estimated with cPER as the response variable, the interaction of insecticide groups and trials as fixed factors, training blocks, and bee individual identification as random factors. Models were validated by visual inspection of residual dispersion using DHARMa package (HARTIG, 2022). An ANOVA was conducted to compare mortality rates across different groups.

3. RESULTS AND DISCUSSION

Both isolates insecticides, and the combination reduced bee learning performance ($p < 0.001$; Figure 1). 124 bees were trained for the learning tests, 31 for each group. Control bees were conditioned with 90.3% success. Bees treated with deltamethrin (0.0025 µg a.i /bee) had a 48.4% success, a reduction in learning of 41.9%. For the Imidacloprid group (0.0005 µg a.i /bee), 38.7% of bees were conditioned, and for the Combination group (0.0025 +0,0005 µg a.i /bee), 48.4% of success, a reduction of learning of 51.6% and 38.7%, respectively. This reduction in learning corroborates results found by Tosi et al. (2022), although the interaction of the two insecticides had not yet been described in the literature. For the Combination group, we expected the lowest performance between the groups due to the sum of insecticide doses. Still, the effect was similar to isolated insecticides, suggesting that the nature of the interaction is antagonistic.

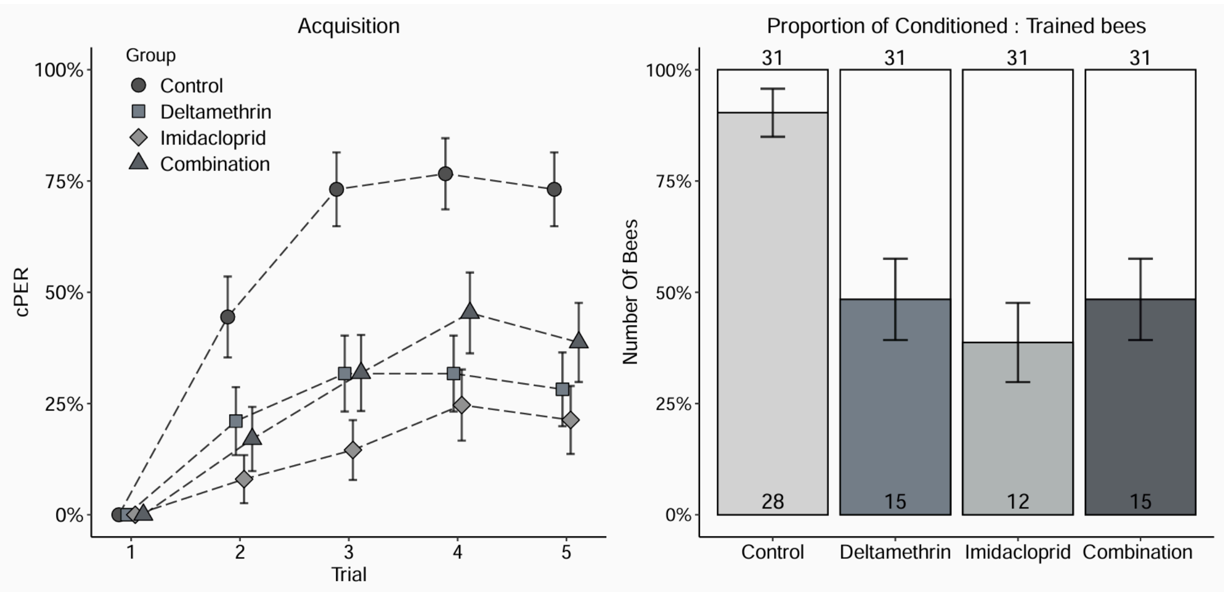

Figure 1 - Acquisition curves and cPER probability for each group, and bars for the number of conditioned bees with standard error represented.

Insecticide contamination did not affect short-term (one-hour) memory retention (Figure 2, Left). Memory retention after 24 hours was lower for the Imidacloprid group ($p = 0.048$) and the Combination group ($p = 0.010$), resulting in a retention reduction of 26% and 41%, respectively, compared to the Control group. There was no difference between Deltamethrin and the Control (p = 0.18493).

Reduction of memory retention capacity caused by contamination with imidacloprid is in line with the results found by Tosi et al. (2022). Deltamethrin alone did not produce significant effects on 24-hour memory tests; however, when combined with imidacloprid, the effect on memory is potentialized, which characterizes this interaction as synergistic.

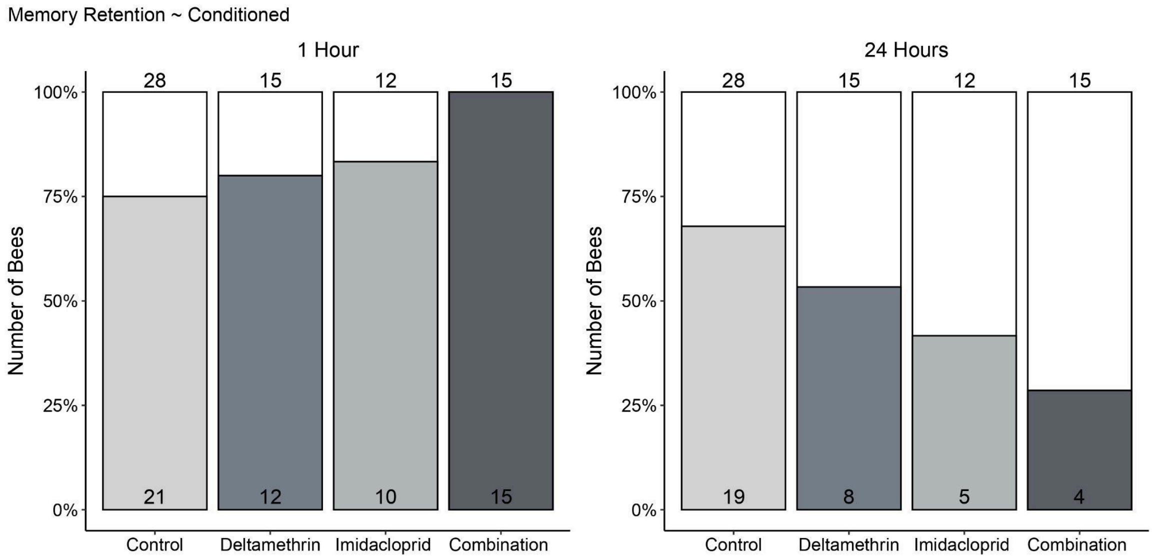

Figure 2 - Memory retention of bees in the distinct groups after one hour (Left) and 24 hours (Right).

There was no significant difference in mortality between the groups (p = 0.801). However, the cognitive changes caused by the insecticides Imidacloprid and Deltamethrin can have a major impact on bee populations. Bees rely on their ability to learn and memorize to identify favorable floral resources, improve foraging routes and use this information during next forages. This optimizes the use of energy resources and may be a key point of success or failure of a colony.

This work also brings attention to the interaction of substances that can commonly occur and affect bees within a short time, which is the case of these two agricultural insecticides, Imidacloprid and Deltamethrin. The harmful effects of individual substances can be potentialized, as shown in the 24-hour memory tests, but are little considered in assessment risks. Also, can be masked by antagonistic interactions, as shown in learning tests, if the assessment is superficial and does not consider the complexity of bee behavior.

4. CONCLUSIONS

Based on the results obtained, we concluded that contamination of bees with field-realistic doses of Imidacloprid reduces their learning and memory retention capacity. Contamination with Deltamethrin does cause a significant reduction in learning but does not affect memory retention. The combination of insecticides causes a decrease in learning. However, due to the antagonistic interaction, the effect is no greater than the single substance effect. Conversely, to memory retention (24h) the effect of combination was superior to the sum of each insecticide, showing a synergistic effect of the substances.

Our work brings attention to the necessity of including poorly known interactions of substances in toxicological surveys of bees. Furthermore, to avoid masking effects, the integration of different tests are needed to better understand the effect of these substances on bees' life.

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