



APIMONDIA

48TH INTERNATIONAL CHILE
APICULTURAL CONGRESS 2023

September 4th - 8th, 2023

Sustainable Beekeeping, from the south of the world

ABSTRACT BOOK

ORGANIZED BY



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INDEX

Oral Presentations

3-122

Poster Presentations / Presentaciones de Carteles 124-342



PP-274

Effects of individual and combined exposure to sublethal doses to the pesticides Chlorpyrifos, Bifenthrin and Imidacloprid on detoxification and neurotoxicity enzymes in *Apis mellifera* workers

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The honeybee (*Apis mellifera* L.) is an important pollinator and a model for pesticide effects on insect pollinators. Bees can be exposed to multiple pesticides that may interact synergistically, amplifying their side effects. Some combinations show synergistic or antagonistic combined effects that go far beyond what is predicted with current effect models. Up until now, only the combined additive effects of similar acting chemicals have been assessed accurately, whereas the combined effects of dissimilar acting chemicals have been greatly underestimated in many cases. In recent years, the mortality of bees, such as *Apis mellifera*, has become a strange global phenomenon that is having a negative impact given its great importance. The aim of this work was to analyze the impact of exposure to different agrochemicals for agricultural use in sublethal doses and individually and in combination, on detoxification enzymes (Catalase (CAT) and Glutathione-S-transferase (GST)) and neurotoxicity (Acetylcholinesterase (AChE)). Bees up to 3 days old were collected and divided to carry out the different treatments, using 10 bees in each replicate. 10 ul of Bifenthrin (BIF), Chlorpyrifos (CLOR) and Imidacloprid (IMI) were applied topically in concentrations corresponding to LD10 (The range of concentrations used was based on previous studies) as follows: BIF; 2) CLOR; 3) IMI; 4) BIF+CLOR; 5) BIF+IMI; 6) IMI+CLOR; 7) CONTROL. After each treatment, the surviving bees were kept in a freezer at -80°C for their subsequent enzymatic analysis. The results obtained showed that for GST there is a significant inhibition for all treatments with the control ($p < 0.05$), except for the IMI+CLOR combination where there is no significant difference ($p > 0.05$). For the CAT enzyme all treatments were significantly lower than the control ($p < 0.05$). In the case of AChE, the individual treatments of IMI, BIF, and CLOR as well as the IMI+CLOR combination were lower than the control ($p < 0.05$) while the BIF+CLOR combination was not different from the control ($p < 0.05$) and the IMI+BIF combination was significantly superior. These results report the synergistic (IMI+BIF for the AChE enzyme) and antagonistic (IMI+CLOR for GST and AChE) effects caused by simultaneous exposures to pesticide combinations in bees.

PP-275

Towards sustainable and productive apiculture: Selection of resilient honeybees free of zoosanitary products (ECOAPI)

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The beekeeping sector in Spain is the largest in Europe but suffers from large annual honeybee colony losses driven by climate change and pests and parasites, with *Varroa destructor* and associated viruses being a major concern. Varroosis requires recurring chemical treatments and thus the sector is in need of finding more eco-friendly solutions.

Within the ECOAPI project (2022 - 2024), we aim at a long-term sustainable solution against varroosis based on the selection of varroa-resistant lines that could avoid or minimize chemical treatments. Varroa resistance can be achieved by natural, or targeted selection. Within ECOAPI, we study two populations of *Apis mellifera iberiensis*: a putative varroa-resistant population ("MENA population"), that consists of colonies being untreated since 2015 and managed by the beekeeping association MENA; and a breeding population ("ERBEL population") managed by the bee breeding association ERBEL within a genetic improvement program that selects for varroa resistance among other performance traits. In the untreated "MENA population", we study the factors (environment, genome, microbiome) and mechanisms (social immunity, management) that enable Varroa resistance and survival. In the "ERBEL population", we apply and evaluate a new pheromone-based test (UBeeO) for hygienic behaviour specifically against varroa-parasitized brood that promises to be a faster and more efficient measurement to predict colony-level Varroa resistance than commonly used strategies, and it could therefore be implemented in breeding programs. We will correlate it with Varroa infestation levels across the season, social immunity traits and other colony performance parameters collected within the breeding program. Moreover, we aim to identify genetic markers associated with varroa-resistance that could lead to genetic tools employed in breeding of resistant stocks across the country.

In conclusion, the ECOAPI study will evaluate for the first time the traits involved in varroa resistance of Iberian honeybees. The results will provide know-how on the major factors and mechanisms of colony losses through varroosis, enabling adapted low-input management or breeding solutions that will lead the beekeeping sector towards sustainable production without the use of synthetic acaricides.

