Laboratory Evaluation of Phytochemicals Extracts of *Humulus lupulus* Against *Varroa destructor*

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Abstract

The search for alternative strategies to manage *Varroa destructor* has intensified due to increasing resistance to synthetic miticides and concerns about chemical residues in hive products. In this study, we evaluated five proprietary botanical compounds (A, B, C, D, and E) derived from *Humulus lupulus* for their efficacy against *V. destructor* and their safety across different castes of *Apis mellifera*. Thymol (positive control) and compound A caused high mortality in queens, workers, and drones, whereas the remaining compounds did not adversely affect queens. Worker sensitivity was age-dependent, with newly emerged bees more susceptible to all treatments than nurses or foragers. Notably, two of the five compounds (C and E) achieved significant acaricidal activity (60-80% mite mortality), while remaining safe for queens.

Introduction

In recent years, managed honey bees (*Apis mellifera* L.) have faced severe health challenges, with annual colony losses averaging 30% or more—double the long-term historical average of approximately 15% (CAPA 2025). Among the contributing factors, *Varroa destructor* (Anderson and Trueman) remains the leading cause of colony mortality and is recognized as the most widespread and damaging honey bee pest worldwide. Management options are limited, particularly for large-scale commercial beekeepers, as resistance has reduced the efficacy of several registered Varroacides. This underscores the need to explore new miticidal compounds. The objective of this study was to adapt laboratory bioassay protocols described by Bahreini et al. (2020, 2022) to evaluate both the acaricidal potential and honey bee toxicity of five proprietary natural compounds derived from the hop plant (*Humulus lupulus* L.).

Materials and Methods

Queens and drones were exposed to serial dilutions of five pure active ingredients from *Humulus lupulus* using the Apiarium contact technique. Worker bees—newly emerged, nurses, and foragers—were tested with the Mason jar bioassay method (Bahreini et al. 2020, 2021). Queen survival was monitored for 14 days, while mortality of drones and workers was recorded after 24 h. To assess acaricidal activity, 24 h mite mortality was determined by treating *Varroa*-infested bees with the same dilutions.

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Results

The Kaplan-Meier survival analysis showed differences among treatments with respect to queen mortality. Compounds B, C, D, and E had greater (100%) queen survivorship over 14-day post-treatment comparted to others ($p<$ 0.0001). The survival rate for queens treated with Thymol (50%) or compound A (67%) was lower than others (Fig. 1).

A significant difference in treatment x dilution interaction was found in drone mortality: A higher mortality rate (>20%) occurred in drones that were exposed to a certain dilutions of compounds A, D, E and Thymol. Overall, compounds B and C, and the negative control showed lower drone mortality than others ($p<$ 0.0001) (Fig. 2).

We observed the highest bee mortality in compound A (83% mortality) treatments, followed by Thymol (74% mortality), and compound C (38% mortality) treatments for a 24h post-exposure in a Mason jar test ($p<$ 0.0001). Overall, nurses (35% mortality) survived the compounds better than newly-emerged bees (50% mortality) and foragers (49% mortality) for 24 post-treatment ($p<$ 0.0001) (Fig. 2).

Across all dilutions, greater mite mortality at 24h post-treatment was observed for compounds C (39%), A (30%), and Thymol (32%) compared to the other compounds ($p=$ 0.0166). Higher mite mortality rate (>50%) was recorded for the highest concentrations of compounds A and C ($p=$ 0.001) (Fig. 3). The interaction of the treatment × dilutions indicated significant differences in 24h mite mortality between different dilutions of compounds A, C, and E ($p<0.05$).

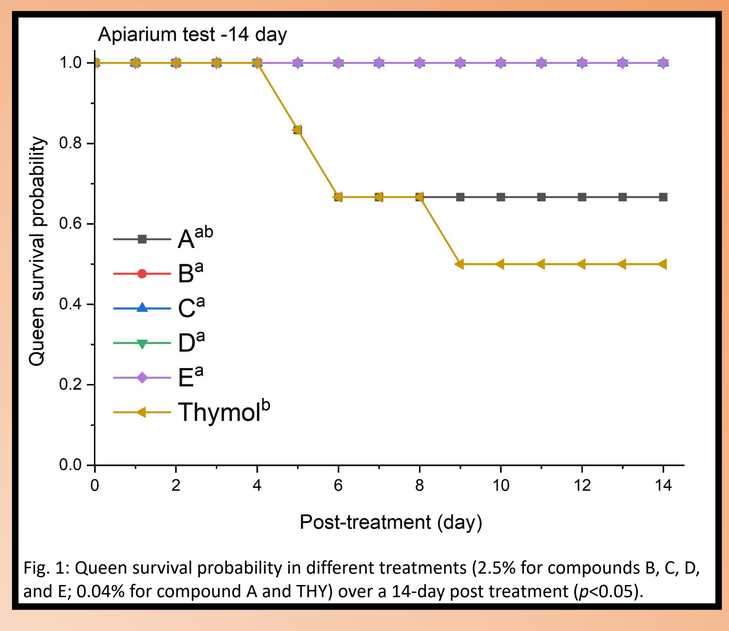

Fig. 1: Queen survival probability in different treatments (2.5% for compounds B, C, D, and E; 0.04% for compound A and THY) over a 14-day post treatment ($p<0.05$).

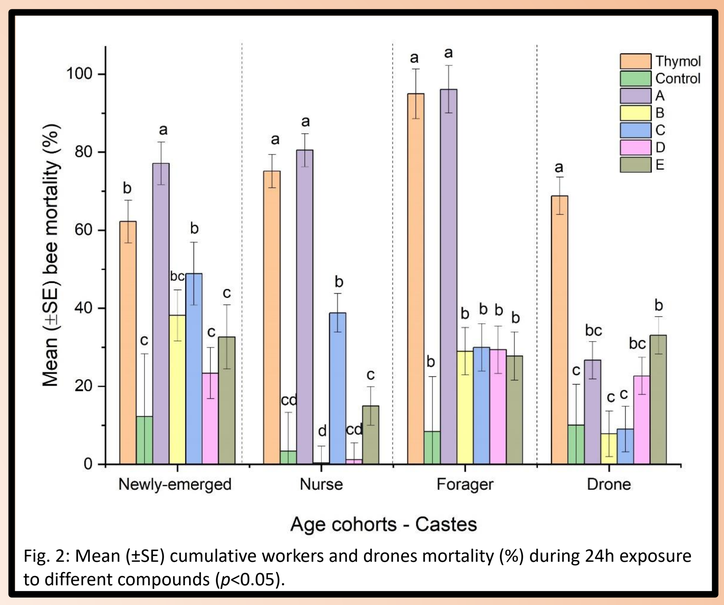

Fig. 2: Mean (±SE) cumulative workers and drones mortality (%) during 24h exposure to different compounds ($p<0.05$).

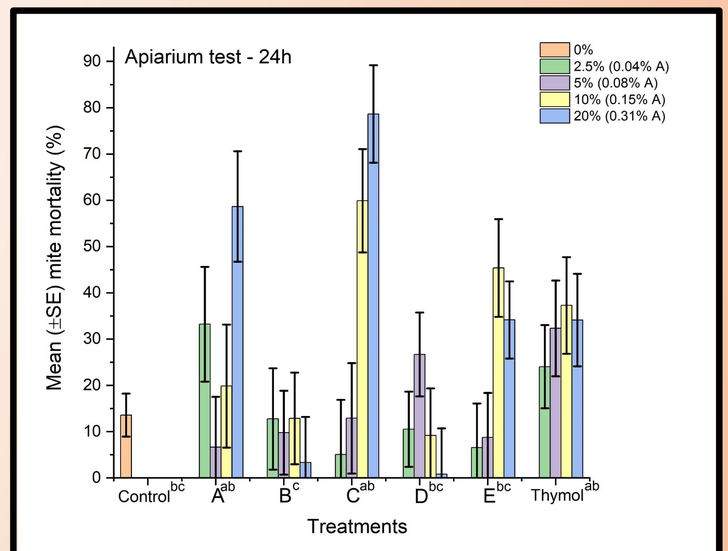

Fig. 3: Mean (±SE) *Varroa* mite mortality (%) during 24h exposure to different dilutions of compounds ($p<0.05$).

Discussion

The search for alternative strategies to control *V. destructor* has intensified in response to resistance against synthetic miticides and concerns over chemical residues in hive products. Several studies have reported promising results with plant-derived compounds (DeGrandi-Hoffman et al. 2012; Sabahi et al. 2022; Benga et al. 2023). In this study, we evaluated five active ingredients extracted from *H. lupulus* for their effects on honey bees and their potential as miticidal agents against *Varroa* mites. Overall, compound A and thymol exhibited high toxicity (>75%) across all honey bee castes. Among the tested groups, newly emerged workers were the most susceptible, particularly when directly exposed to contaminated surfaces in Mason jar assays. Notably, compounds C and E demonstrated strong acaricidal activity against *Varroa,* while showing lower adverse effects on honey bees in comparison to thymol. To our knowledge, this is the first evaluation of the acute lethal effects of compounds B, D, and E on both *V. destructor* and *A. mellifera*. Collectively, our findings highlight the potential of hop-derived active ingredients as promising alternatives for *Varroa* management.

References

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