

Assessment of contact exposure to biopesticide tank mixtures in different bee species under controlled laboratory conditions

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Abstract

*Bees are essential components of terrestrial ecosystems, providing crucial pollination services for both wild and cultivated plants. However, pollinator populations face increasing challenges due to a combination of anthropogenic pressures, including pesticide exposure and landscape modifications. While biopesticides are often promoted as environmentally friendly alternatives to synthetic chemical pesticides, the potential risks of tank mixtures containing multiple biocontrol agents to non-target insects remain insufficiently investigated. Here, we describe a laboratory-based assay developed to assess contact exposure in three bee species differing in their life profile. The lethal effects and behavioural abnormalities caused by biopesticide tank mixtures were assessed in the highly eusocial honey bee *Apis mellifera*, the primitively eusocial bumble bee *Bombus terrestris*, and the solitary mason bee *Osmia cornuta*. Standardized exposure tests were conducted under controlled laboratory conditions using adult individuals. The assessment included behavioural impairments, mortality, and species-specific sensitivity to the mixtures. The observed results highlight the need to consider the interspecific differences when evaluating the potential risks of external stressors such as pesticides on bees.*

Keywords: biopesticides, bees, contact exposure, pollinator health

Introduction

Social insects such as bees play vital roles in maintaining biodiversity and agricultural productivity. In recent decades, both managed and wild bee populations have been affected by multiple stressors including habitat loss, pathogens, and pesticide exposure. Among pesticides, biopesticides have gained increasing attention as components of sustainable pest management. Despite their natural origin, biopesticides are not inherently risk-free for nontarget organisms. Especially when applied as tank mixtures, combining different microbial or botanical active agents, potential additive or synergistic effects on non-target arthropods, such as pollinators, cannot be ruled out.

While the effects and risks of chemical synthetic pesticides on bees are well documented through standardized testing frameworks, comparable test systems for evaluating biopesticide mixtures are still missing. This work presents a controlled laboratory protocol designed to evaluate the lethal effects and behavioural abnormalities of contact exposure to biopesticide tank mixtures on adult individuals of three representative model bee species: the honey bee (*Apis mellifera* L.), the bumble bee (*Bombus terrestris* L.), and the solitary mason bee (*Osmia cornuta* Latreille).

Material and Methods

The experimental design was based on OECD 214 (Acute Contact Toxicity Test for Adult Bees) and OECD 247 (Bumble bee, Acute Contact Toxicity Test), with modifications to reflect tank mixture application under realistic spray conditions. Experiments were conducted under controlled laboratory conditions using adult bees from healthy laboratory-maintained colonies. Prior to exposure, all individuals were visually inspected to ensure

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vitality and normal activity. Here, the mixtures of Netzschwefel Stulln (796 g/kg sulfur) with NeemAzal-T/S (10.6 g/L azadirachtin), as well as the mixture of Netzschwefel Stulln with Kumar (850 g/kg potassium bicarbonate), were examined. These tank mixtures are used to prevent the development of resistances and to enhance overall efficacy, and in this case the tank mix of NeemAzal-T/S and Netzschwefel Stulln may also be employed as a strategy to reduce copper use.

On the day of application, bees were pre-cooled to facilitate handling. Two hours before treatment, the cages containing bees were placed at 2 °C in a refrigerator and subsequently transferred to electrical cooling boxes maintained at the same temperature. Immediately before application, the bees were briefly immobilized by placing them in plastic bags between crushed ice for approximately ten minutes. Immobilized individuals were then transferred to Petri dishes and positioned inside a spray chamber.

The application chamber was equipped with flat fan nozzles (TeeJet 9503 EVS) delivering a fine, uniform spray mist. Bees were sprayed at room temperature with the respective test solutions, ensuring full and even coverage of the body surface. Following exposure, bees were transferred back to stainless steel cages and returned to climate chambers set to 26 °C, 60% relative humidity.

Observations of behaviour and mortality were performed at 2, 4, 24, 48, 72, and 96 hours post-exposure. The described setup allows for standardized comparative assessment of contact effects between bee species while minimizing handling stress and ensuring reproducibility.

Results

Exposure to tested tank mixtures resulted in mortality up to 22.4% in honey bees, whereas it remained lower in mason bees (6.3%) and bumblebees (6.7%). The results indicate that honey bees exhibited the highest sensitivity, with up to 78% showing abnormal behaviours compared to less than 10% in the other two species.

Conclusion

Preliminary results indicate that honey bees exhibited the highest sensitivity. The observed results highlight the need to consider the interspecific differences when evaluating the potential risks of external stressors such as biopesticides on bees. Furthermore, while this study focused on adult contact exposure, future work has to include oral exposure routes and exposure of larvae to provide a more comprehensive understanding of risk across developmental stages and exposure pathways.

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