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Bioinsecticides on honey bees: Exposure, sublethal effects, and risk assessment paradigms

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As synthetic pesticides contribute to the global decline of pollinators, biopesticides have gained attention as more sustainable pest management alternatives in agriculture. Despite their perceived safety, there is increasing evidence that bioinsecticides can harm honey bees, which are crucial pollinators of many commercial crops and key ecotoxicological models. This short review aims to summarize key studies on exposure pathways and sublethal effects of bioinsecticides on honey bees, highlighting outdated risk assessment paradigms and critical evaluation issues. We discuss the need for novel approaches, such as molecular techniques and AI technologies, to better understand and mitigate the effects of bioinsecticides on honey bees. We also highlight the importance of long-term field studies and ethical considerations in ecotoxicology to protect honey bees and promote sustainable agricultural practices.

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Introduction

Synthetic pesticides have become ubiquitous in our environment due to intensive agricultural practices to meet the growing human demand for food. Increased international trade has facilitated the global spread of crop pests leading to greater reliance on chemical pest control to guarantee adequate crop yields and stable production [1–3]. This widespread use of pesticides has not only contaminated agricultural landscapes, but also affected pristine areas and nature reserves [4,5]. The multitude of agrochemicals used in the ongoing battle against crop-destroying organisms has significant consequences for both human health and the environment [6,7]. Over the years, public awareness of the adverse effects of chemical insecticides on non-target organisms has progressively grown [8–11]. As a result, alternative crop protection strategies have been proposed and developed to mitigate the severe impact of synthetic insecticides [12]. These alternatives include naturally occurring insecticides or biological insecticides (Box 1) [13]. The market and use of these bioinsecticides has been steadily increasing due to their relatively high target specificity, low non-target toxicity, restrictions and bans on chemical insecticides, insecticide resistance and residue management [13,14]. Bioinsecticides are considered to be environmentally friendly and safe. Their efficacy in the long-term control of insect pests is comparable, if not superior, to that of synthetic substances, and their use is allowed in organic agriculture [12–14]. However, despite the common perception that 'organic' implies safety, recent research has shown that bioinsecticides can still have a plethora of detrimental effects on beneficial insects, especially pollinators and natural agents of control [15–17]. Among pollinators, the western honey bee, *Apis mellifera*, is a flagship species when considering the impact of agrochemicals. This is motivated by its: (i) worldwide distribution due to contemporary human-mediated introductions; (ii) ease of management; (iii) large-scale beekeeping operations that provide hundreds of colonies for economic interests; (iv) its vulnerability to these pollutants [18–21]. This species has served as an exemplary model organism in terrestrial ecotoxicology, acting as a surrogate for both *Apis* and non-*Apis* bees [20,22]. However, growing evidence shows that honey bees may not adequately represent the consequences of pesticide exposure for the vast and diverse group of solitary and social bee species. Thus, it is necessary to diversify model species in terrestrial ecotoxicology [22]. In addition, international risk assessment protocols and regulatory policies often fail to address the unique challenges posed by biological products compared to synthetic agrochemicals [15,23]. Here, we summarize key studies that have investigated the route of exposure and sublethal adverse effects of bioinsecticides on

Box 1. Biopesticides in focus: from definition to practical use.

Biopesticides, as defined by the United States Environmental Protection Agency (EPA), include microorganisms used in biological control, their insecticidal metabolites, and other pesticides derived from living organisms. The EPA classifies biopesticides into three main categories: (i) naturally occurring biochemicals with non-toxic mechanisms, (ii) microbial entomopathogens, and (iii) plant-incorporated protectants from genetically engineered plants. Biopesticides generally refer to commercial preparations containing living organisms (bacteria, viruses, fungi, etc.) or bioactive compounds from these organisms to control pests. Although biopesticides have been used since the late 19th century, they currently represent a small segment of the global pesticide market, valued at around US$3 billion, or 5% of the total. However, their market is growing rapidly at 10–15% per year compared to 1–2% for synthetic pesticides, driven by their acceptance in organic farming and Integrated Pest Management (IPM), and increasing demand for environmentally sustainable solutions [13,14]. This growing interest is reflected in the scientific literature, with a significant increase in research papers on biopesticides over the last two decades [15].

honey bees. We discuss the outdated ecotoxicological paradigms and the critical issues in risk assessment for honey bees. Finally, we highlight new technologies for investigating the true impact of these natural agents of control, taking into account recent findings on animal welfare and environmental sustainability.

Adverse effects of bioinsecticides on honey bees: from the individual to the colony

Honey bees can come into contact with bioinsecticides and other agrochemicals following multiple routes of exposure, and many contaminants are found in colonies and bee products [24,25]. Foragers may incorporate biological control agents (BCAs) orally or topically while gathering food resources for the colony or they may be sprayed during product application in the field [24,25]. Because of their perceived safety to bees, several bioinsecticides, such as the entomopathogenic fungi (EF) *Beauveria bassiana* and *Metharizium anisopliae*, have been applied directly inside hives to control *Varroa* mite infestations. This practice exposes queens, young house bees, brood and food stores to these bioinsecticides [26]. Furthermore, spores of entomopathogenic fungi and bacteria are applied topically through specifically designed dispensers to the body of foragers, using these pollinating insects as vectors to disseminate the BCA on crop flowers — a technology known as entomovectoring [27]. These techniques are adopted because ecotoxicological tests have shown no adverse effects of these BCAs on honey bees. However, the definition of “adverse effects” should not be limited to the mere lethality and ecotoxicological endpoints of the bioinsecticide, nor to the macroscopic motor abnormalities included in risk assessment guidelines [15,23]. Honey bees are social organisms and the effects on a single individual can reverberate throughout the complex social environment of the entire colony, jeopardizing its integrity and overall survival. Among the individual adverse effects of common bioinsecticides on honey bees, exposure to the entomopathogenic bacteria *Bacillus thuringensis* (Bt) and to Bt-derived Cry proteins can alter locomotor and enzymatic activity, as well as the midgut structure and gut microbiome of bee workers. This exposure reduces also their foraging activity and learning performance [15,28–31]. The neurotoxic metabolite spinosad, derived from the actinomycete *Saccharopolyspora spinosa*, can also induce structural alterations and epithelia disorganization in the midgut and Malpighian tubules [15,32,33]. It also causes transcriptional changes in metabolic genes in the brain, damage to mushroom bodies, reduced flight ability, respiratory rate, detoxification enzyme activity and hemocyte count in exposed bees [15,32,33]. These findings highlight how sublethal concentrations of the bioinsecticide can cause a wide range of deleterious effects, affecting structures and functions crucial for individual homeostasis and efficient colony task performance. Similarly to spinosad, azadirachtin, a bioinsecticide derived from the Neem tree *Azadirachta indica*, can be highly toxic to honey bees, affecting foraging activity in treated crop fields [15,34]. Sublethal effects on individual honey bees have also been documented following exposure to the EF *B. bassiana* [15,35,36]. This fungal bioinsecticide, among the various effects [15], affects learning and sucrose responsiveness of exposed foragers [35] and alters their cuticular hydrocarbon profiles, allowing the entry of foreign, fungus-exposed workers that are not recognized by nest entrance guards [36]. These individual effects reflect on the whole colony, as cognitive abilities and sucrose responsiveness are fundamental to the optimal division of foraging labor, and an efficient nestmate recognition system is essential to defend the colony against external threats and prevent pathogen outbreaks within colonies [35,36]. As regards entomopathogenic nematodes, viruses and essential oils used as bioinsecticides, honey bees have shown some sensitivity to several of these products under laboratory conditions [15–17]. However, studies testing their potential impact have demonstrated only minor sublethal effects or are still largely lacking [15,16].

Overall, research has shown that different classes of bioinsecticides can induce a wide range of sublethal effects on honey bees, ranging from subtle alterations in individual immunity, gene expression and microbiome, to impaired cognitive, kin recognition, foraging abilities and social immunity [15]. These effects often go undetected due to several critical issues: inadequate safety assessment procedures that typically focus only on lethal effects, intrinsic differences in the modes of action of bioinsecticides compared to synthetic chemicals, and unexpected interactions with other potential stressors.

Outdated risk assessment and critical issues when evaluating bioinsecticide effects on honey bees

The difficulty in assessing the impact of bioinsecticides on honey bees and other non-target organisms is mainly due to the inadequacy of current risk assessment protocols. These protocols are designed to test the impact of chemical insecticides and do not consider the crucial differences between biological and synthetic products. Despite attempts to harmonize pesticide legislation, the regulation of agrochemical use still varies greatly among countries in terms of safety guidelines and legal limits for the use of plant protection products [10,37]. Another critical issue when assessing the impact of biopesticides on honey bees and other non-target organisms lies in the difficulty of detecting and quantifying bioinsecticides using conventional analytical methods, partly due to their complex composition [38,39]. Furthermore, regulatory requirements should carefully consider the unique properties of several bioinsecticides during their process of approval [23]. For example, microbial bioinsecticides include living organisms or dormant spores that must first infect the potential hosts and then become pathogenic. For these products, toxicity testing alone may not be sufficient, and guidelines for testing non-target organisms designed for conventional synthetic substances might not be directly applicable to microbial insecticides [15,23]. Moreover, given the lag time between infection and pathogenicity, both acute and chronic toxicity tests adopted for chemical insecticides could fail to detect adverse effects of microbial bioinsecticides on honey bees and other non-target organisms. These effects may not become apparent for weeks after exposure, and without long-term monitoring of exposed individuals, the actual impact of these products could go unnoticed.

The inability to detect the full range of detrimental effects of insecticides over time is not limited to biological products or the use of honey bees as model organism. Current ecotoxicology paradigms for risk assessment do not include the possibility to monitor the impact of plant protection products used over large spatial scales and long periods of time [10,23]. Thus, a post-approval surveillance of the long-term effects of synthetic or biological insecticides on non-target organisms appears compelling to bridge the gap between regulatory frameworks and field-realistic situations of bioinsecticide use [10,40]. The honey bee, with its colonies that survive for several years and forage over large areas, all year round in tropical regions, represents an ideal model to evaluate the accumulation of bioinsecticides within nests over time and the subsequent adverse effects that may occur at both individual and colony level in the field. In addition, honey bees could be used to investigate the potential adaptation processes that these pollinators might employ to cope with natural plant protection products in the environment. Most ecotoxicological research focuses on laboratory experiments carried out over very short periods of time, highlighting the urgent need for long-term field studies to assess the effects of biological insecticides [15,40].

Furthermore, in a world where non-target organisms, such as honey bees and other pollinators, are exposed to a multitude of different stressors, it is crucial to consider the potential interactions that are likely to occur among them [11,21,41]. Bioinsecticides could be applied in the field alongside other plant protection products, such as herbicides or fungicides. These agents of control could interact in the bee body or accumulate over time in bee products, producing additive or synergistic effects that could be significantly different from those elicited by a single contaminant [41].

Finally, honey bees are exposed to commercial biopesticides that contain active ingredients and co-formulants, or ‘inerts’, that increase their effectiveness. These co-formulants can be toxic to non-target species [42,43]. However, studies on the effects of these formulations on bees are very limited. Other factors such as climatic conditions, floral resource availability and diversity may also interact with bioinsecticide exposure. Protocols should therefore be developed to assess the potential interactions of bioinsecticides with these variables, both in the laboratory and in the field, to understand the consequences in terms of adverse effects on honey bees.

Novel approaches to evaluate bioinsecticide impact on honey bees

In recent years, significant progress has been made in increasing attention towards the sublethal impact of plant-protection products on honey bees and other non-target organisms, as well as their potential interactions with various environmental stressors [5,9,11,15,41–43]. However, many challenges persist. International guidelines for safety testing still focus primarily on direct toxicity and lethality [15,23]. Ecotoxicological studies are often conducted under unrealistic laboratory conditions, failing to account for the co-occurring stressors found in natural environment and the impact of (bio) insecticides on behavior and complex interactions among individuals, especially in social species like honey bees. To address these issues, more holistic approaches that consider both the internal and external environments of an organism must be developed. This will improve the efficiency and sensitivity of pesticide research and regulation, ultimately enhancing the sustainability of agriculture. Molecular approaches, like RNA sequencing (RNA-seq), RNA interference and gene-editing, may be adopted to unravel the molecular determinants of honey bee sensitivity to bioinsecticides and to improve their resilience to these products

[44,45]. Changes in gene expression in specific tissues following exposure will help identifying which molecular pathways, structures and behaviors are affected by a particular bioinsecticide. This information can help predict the effects of different BCAs based on the alteration induced [44]. As already pointed out, estimating mixture toxicity would provide a more realistic approach to evaluate the risk of bioinsecticides to honey bees rather than testing individual substances or agents of control. However, practical limitations make it difficult to assess the effects of the many combinations of stressors that honey bees may encounter under field conditions. In such a complex scenario, machine learning, *in silico* predictions and AI technologies, which are capable of processing large datasets and developing models that integrate numerous potentially interacting variables, can serve as reliable, complementary tools to conventional approaches in pesticide mixture risk assessment [46]. In addition, the use of automated field RFID systems allowing the study of foraging performance by individual bees under different conditions will help to guide environmental policies that aim at enhancing pollinator health and pollination services [47,48]. These advanced techniques have the potential to improve our understanding of the cumulative effects of multiple stressors on honey bees under realistic conditions and facilitate more robust regulatory decisions regarding the use of bioinsecticides in agriculture. A final aspect that should be considered when dealing with safety testing of bioinsecticides using the honey bee as model organism is that recent research has begun to highlight that invertebrates, including bees, might also represent sentient beings capable of experiencing simple emotions and pain similar to vertebrates [49,50], and this brings forth ethical implications for the welfare and sustainability of the management of honey bees in wild, farmed and research contexts.

Conclusions

From effects on single genes, to the honey bees in the social milieu of their colony, bioinsecticides can cause developmental, morphophysiological, and behavioral changes at the individual level, as well as disruptions in social organization, division of labor and overall colony integrity. As the use of natural control agents increases worldwide, it is crucial to carefully assess all these subtle effects. To address these challenges, novel holistic and ethical protocols need to be developed and implemented in ecotoxicology. Long-term surveillance of the potential detrimental effects of bioinsecticides should be carried out under real field conditions. In addition, thorough studies of interactions between bioinsecticides and co-occurring environmental stressors are essential and require a multimodal approach. By taking these steps, we can better protect honey bees and recognize their essential ecological role and economic value. This approach will hopefully ensure that we effectively mitigate risks while promoting practices that support pollinator health and biodiversity conservation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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