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Review

Bees as environmental and toxicological bioindicators in the light of pesticide non-targeted exposure

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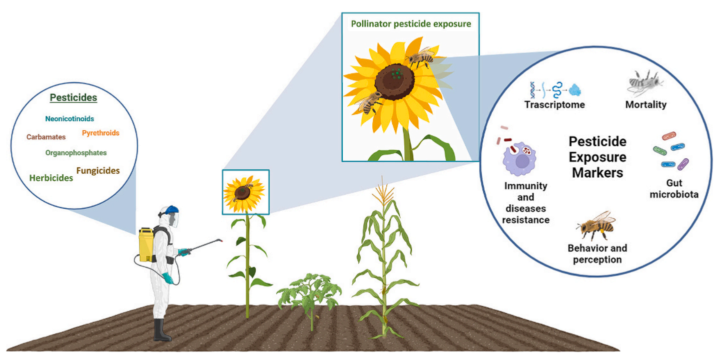
HIGHLIGHTS

- Bees are exposed to pesticides through various routes.
- Bees serve as effective bioindicators of environmental pesticide contamination.
- Standardized methods assess the impact of pesticides on bee health.
- Wild pollinators should be included in comprehensive toxicology studies.
- Pesticide bioaccumulation is detectable in food web organisms like the Asian hornet.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticides have a significant impact on the environment, harming valuable non-target organisms like bees. Honeybees, in particular, are ideal bioindicators of pesticide exposure due to extensive research on how pesticides affect their behavior, immunity, development, biomolecules, and detoxification. However, wild pollinators are less studied in terms of pesticide exposure, and their inclusion is essential for a comprehensive risk assessment. Additionally, food chain organisms, such as the Asian hornet, could serve as indicators of pesticide bioaccumulation. Addressing gaps in honeybee toxicology, understanding the limitations, and exploring the role of wild pollinators and insects as complementary indicators, along with advancements in risk assessment methodologies, could enhance predictive models. These models would help anticipate environmental pesticide impacts while reducing the need for costly, time-consuming research.

1. Introduction

The extent of human activities has significantly affected the environment, especially with consistent pesticide usage and has ushered in an age of a form of mass extinction of species. The term "insectageddon" was coined to define the loss of insect species in this age (Ptaszyńska, 2022; van der Sluijs, 2020) as the most rapid decline in the Animal kingdom compared to vertebrates (Pimm et al., 2014). The loss of biodiversity, especially within the insect communities, is one of prominent concern in environmental studies due to vast impact of insects on

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the biosphere (Blüthgen et al., 2023). In fact, the ratio between terrestrial and aquatic insects has shifted in favor of the latter (van Klink et al., 2020). It makes sense since human activities are mainly carried out on land where the consequences are more prominent and direct. In 2006, beekeepers in America and Europe reported high losses in their colonies to a phenomenon termed colony collapse disorder (CCD) in 2007 (van Engelsdorp et al., 2008; vanEngelsdorp et al., 2009). Studies have revealed that bee decline, whether domesticated or wild, was the cumulative effect of several factors including pesticide exposure and other anthropogenic activities. (Cox-Foster et al., 2007; Goulson et al., 2015; Meeus et al., 2018). With approximately 5.5 million species, insects have diversified enough to inhabit vast array of ecological niches meaning that they have evolved to be able to occupy most living environments (Stork, 2018). Moreover, multiple species of insects have evolved over several millennia to pollinate plants efficiently. The flagship species of such an important role thus far has been the honeybee, *Apis mellifera*. Their economic importance is not to be neglected as many countries rely on pollination for massive income reaching 153$ billion in annual income in 2005 (Gallai et al., 2009).

Honeybees are solidary insects and possess social immunity to fend against intruders and to maintain a hygienic environment. The possession of social immunity, however, has led to low genetic diversity in honeybees compared to other insects like the fruit fly, *Drosophila melanogaster*. (Evans et al., 2006). This may have rendered honeybees more prone to environmental stressors, as they do not possess sufficient variability in immune-related or detoxification genes relative to other insects such as *Drosophila* (Evans et al., 2006). Multiple studies have considered the sub-lethal effects of pesticide exposure in bees even in different combinations whether in vitro, in vivo, or in situ (Alaux et al., 2010; Chakrabarti et al., 2015; Harwood and Dolezal, 2020; Malladi et al., 2023; Stanley et al., 2015; Sukkar et al., 2024; Walderdorff et al., 2018; Wu et al., 2012).

The convenience of honeybees in environmental risk assessments lies in the ability to gather data from both individual beekeepers and large corporations alongside well-established regulatory test systems for evaluating acute and chronic toxicity in laboratory, semi-field, and field conditions (OECD, 1998a, 1998b, 2014, 2021). This data can also be derived from honey production rates, agricultural outputs, and global economic indicators. Thus, a base can be established for many parameters to be evaluated. The toll of pesticide usage and the effect of human activities on the environment can be evaluated more accurately than for other organisms especially when they are not as commonly present as honeybees. In addition, honeybees were found to be adequate proxies of other bee species for pesticide and heavy metal toxicity tests taking into consideration their inter-specific differences (Heard et al., 2017).

However, a more recent study in 2023 revealed that pollen collected by honeybees has fungicide residues while pollen collected by bumblebees in the same areas has mainly insecticide residues questioning the use of honeybees as substitutes for other insect species in toxicological studies (Zioga et al., 2023a). Nowadays, bumblebees are also included in pesticide toxicity testing representing wild pollinators (Gradish et al., 2019; Nicholson et al., 2024). Moreover, honeybees are a good indicator of herbicide and fungicide contamination in their proximal areas but that is not true for insecticides as found in the same study (Hung and Yiin, 2023). As in some aspects, honeybees may not give off the same outcome as other insects regarding the effects of pesticides on immune pathways (Malladi et al., 2023) or behavior but it is still an indicator of an effect that hinders normal function and an overall pointer to pesticide contamination and spread beyond the limit of the desired activity.

This review discusses the various aspects and parameters used in toxicological tests for pesticide exposure in honeybees and the potential of using domesticated hives as proxies for other insects and bioindicators of environmental contamination. Additionally, we highlight the effects of pesticide exposure on bees, the limitations of using honeybees as bioindicators, and alternative species that could be used for complementary studies or to provide a comprehensive view for building predictive models, thus reducing the need for extensive research.

2. Exposure to pesticides

2.1. Bees' non-targeted exposure

Pesticides are chemicals used to prevent the infestation of pests including fungi, undesired plants, rodents, and insects (WHO, 2018). When applied to plants, they are termed "Plant Protection Products". The application of pesticides, although regulated by organizations like the EPA (Environmental Protection Agency) and EFSA (European Food Safety Authority), often also affects non-target organisms like honeybees, butterflies, and beetles, with unexpected impacts. Nectar is the primary route of exposure for both adult *Apis* and non-*Apis* bees orally followed by pesticide exposure via air particle contact. For larvae, nectar, and pollen are the main sources of exposure (Boyle et al., 2019).

In the world of insects, assessing the effects of pesticides across a wide range of species is more complicated compared to other taxa due the insects' high diversity and the exposure to different risk factors simultaneously. The complexity of simultaneous risk factor exposure can be observed in the effect of amitraz on honeybee survival. While amitraz alone did not significantly impact survival, its presence alongside other pesticides, such as coumaphos or τ-fluvalinate, increased their toxicity, whereas the toxicity of amitraz itself remained unchanged (R. M. Johnson et al., 2013).

Pesticide residues from agricultural practices are always found in hives and hive products but especially in pollen (Y. Yang et al., 2023). At least 1 pesticide was present in pollen collected from Italy with multiple contaminations in 38 % of samples from hives in Italy from 2015 until 2018 (Tosi et al., 2018).

2.2. Neonicotinoids and pesticide interactions

Neonicotinoids are a class of systemic pesticides developed by Shell and Bayer Corp in the early nineties to treat sucking insects and protect plants such as the beetroot and sunflower. With a structure similar to nicotine, neonicotinoids act by binding to nicotinic acetylcholine receptors (nAChR) in insects fatally disrupting the nervous system (Bonmatin et al., 2015; Tomizawa and Casida, 2005). Although neonicotinoids were developed to treat sucking insects, they have been found to have a drastic effect on honeybees, with numerous studies focusing on imidacloprid, as it is the most widely used neonicotinoid. As systemic pesticides, they can migrate to all parts of the plants, including pollen and nectar. (Bonmatin et al., 2015). Neonicotinoids were also found to be one of the pesticide families most associated with collapsed colonies in a 2-year field study (Calatayud-Vernich et al., 2019).

Though claims have been made that the exposure of bees to neonicotinoids is low in the environment, a study published in 2015 by Kessler and colleagues state that honeybees and buff-tailed bumble bees actually prefer food that contains neonicotinoids like imidacloprid, thiamethoxam, and clothianidin in adult bees in the short-term (Kessler et al., 2015). Honeybees are also often exposed to multiple neonicotinoids at the same time. In fact, North America and Europe had the highest percentage of mixtures of neonicotinoid residues in honey in 2017 (Mitchell et al., 2017). Zioga et al. detected neonicotinoids like thiamethoxam and clothianidin in fields where they were not previously applied (Zioga et al., 2023b) putting the question of the true exposure and risk of neonicotinoids to honeybees and the environment. Arguably, wild pollinators face a greater risk of global decline compared to domesticated honeybees (Halvorson et al., 2021). However, a decrease in honeybee health is also evident, with neonicotinoids and their combinations identified as significant risk factors (Harwood and Dolezal, 2020; Lu et al., 2020; Y. Yang et al., 2023). The extensive focus on honeybees in toxicological studies may serve as a silver lining for predicting the effects of pesticides on other insects. Of course, complementary studies on different insect species are still necessary, but honeybees could provide a solid foundation for comparison and help offset the need for additional research on other insects that would require significant time and funding.

Pesticide interactions including synergism; antagonism and addition effect have been previously reported (Carnesecchi et al., 2019; Martin et al., 2021), therefore, using a wider range of pesticides might induce a synergistic effect that may not be directly visible in single pesticide evaluations especially when taking into consideration the different environmental factors. Carnesecchi et al. (2019) applied the Model Deviation Ratio (MDR) to quantify the magnitude of interactions between pesticides and their effect on honeybee health. Carnesecchi et al. (2019) found that 17 % of pesticide binary mixtures showed concentration addition effect, 11 % had antagonistic relationships while 72 % showed synergism while 55 % of synergism was between insecticides/acaricides and sterol-biosynthesis-inhibiting (SBI) fungicides where synergism is associated with CYP450 inhibition via toxicokinetic interactions as key mechanisms.

Evaluating pesticide interactions in toxicological studies can be challenging, particularly when considering synergism, antagonism, or concentration addition effects. Martin et al. (2021) reviewed 10 years of research on chemical interactions regarding the toxicological effects of chemicals and metals in a wide range of organisms and they found that 65 % of studies that state synergism or antagonism do not significantly deviate from the classification of additive effect. However, in the review of Martin et al. (2021), pesticide interactions reported slightly higher percentage of synergism and more observable in-vitro than in-vivo. In invertebrates, toxicological parameters such as immunotoxicity, neurotoxicity, endocrine disruption, and genotoxicity have been understudied. These factors should be carefully considered when evaluating potential risks to insects. It is also important to take into account previously reported or well-characterized interactions between pesticides in biological systems. In honeybees, context-specific pesticide-pesticide interactions have been reported, particularly concerning immunological endpoints such as reactive molecule production, phagocytosis, and alterations in immune pathway genes (Malladi et al., 2023; Sukkar et al., 2024; Sukkar et al., 2023a; Sukkar et al., 2023b). To properly assess the impact of pesticides on honeybees, it is crucial to consider their interactions with other risk factors, such as diseases or additional pesticides. Studies have shown that synergistic effects can occur when pesticides interact with these factors (Chakrabarti et al., 2015; Harwood and Dolezal, 2020; Hotchkiss et al., 2022; Paris et al., 2020; Tosi and Nieh, 2019).

3. Parameters used to assess the effect of pesticides

Regarding pesticide toxicity, several parameters are taken to consideration in honeybee research to understand the effects of pesticide on bee colonies and their environmental impacts. Parameters include survival, behavior, immunity, diseases resistance, gut microbiota, detoxification of pesticides, and larval toxicity assays (Fig. 1). In this section we discuss each of these parameters and their implications.

3.1. Survivability

The first checkpoint to analyze the effect of pesticides is the mortality rate and the direct impact on honeybee survival. The lethal doses or concentrations of pesticides are usually indicated by LD_{50} (median lethal dose; the dose that results in 50 % death of the population) or LC_{50} (median lethal concentration; the concentration that results in 50 % death of the population). Both LD_{50} and LC_{50} can vary for the same organism and pesticide depending on the mode and the duration of exposure. Standardized methods have already been established for

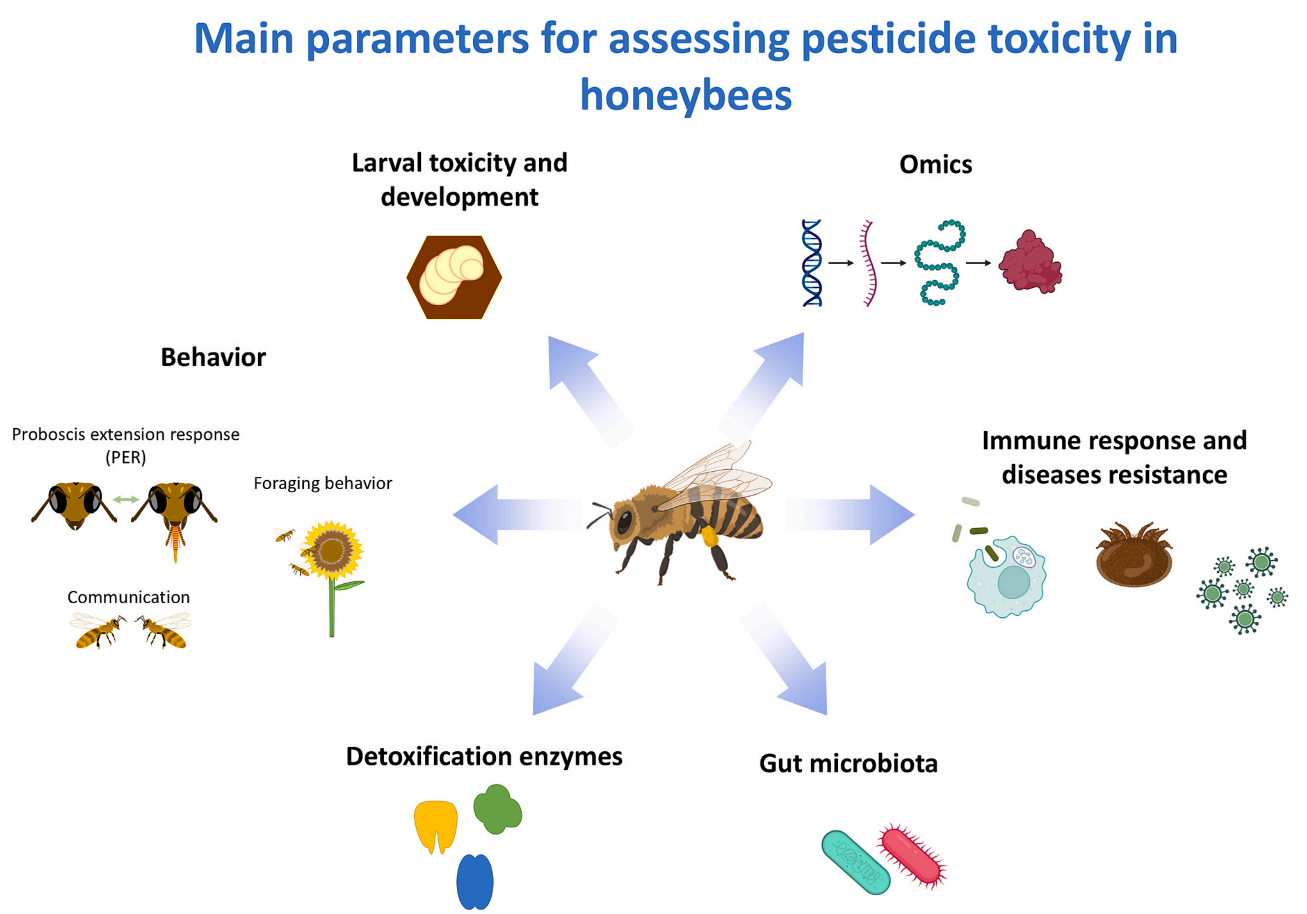

Fig. 1. Different parameters used to assess the effect of pesticides on honeybees.

toxicity testing on honeybee health (OECD, 1998a, 1998b, 2014, 2021). However, this parameter is not a reliable reference for assessing the applicability of pesticides. This is because pesticides may have sub-lethal effects that manifest in other parameters. The impact of sub-lethal doses of pesticides on different aspects of the organism must also be evaluated. Physiological and cellular impacts are taken into consideration when studying the effect of pesticides on honeybees as these parameters affect survivability. These are in addition to the complexity of honeybees as social insects, and their different ploidy even at tissue-specific levels (Aron et al., 2005) and variable development and interconnected organization.

3.2. Behavior

Honeybees are social insects that have a caste system with different roles ranging from pollen collection and attending to the brood and queen, egg laying, fertilization, and protecting the hive from pests and intruders (B. R. Johnson, 2010; Ono et al., 1995) in addition to sanitary practices including self-grooming and allogrooming or removing bees that are either dead or infected (De Roode and Lefèvre, 2012). This hygienic behavior aids in limiting bacterial and mite interactions in the hive (Larsen et al., 2019). In this case, the term “social immunity” is given to collective behaviors that limit pest activity or that are defensive.

These roles are complementary and interconnected which means that any effect on behavior could affect the whole hive. In this sense, honeybees can demonstrate the effect of pesticides on different behavioral aspects. When at times the effect is not directly visible on the behavior of a cast, the outcome of the hive collaboration and whole behavior could be an indicator referring to pesticide exposure.

Neonicotinoids were observed to affect foraging behavior. Imidacloprid fed at 26 ppb reduced foraging activity and non-significantly lowered recruitment behavior (Ohlinger et al., 2022). Even at 5 ppb, imidacloprid fed to larvae caused emerged bees to perform fewer foraging flights and induced early first-flight onset altering the functional organization of the hives (Colin et al., 2019). Sub-lethal doses of imidacloprid and thiamethoxam affected short-term olfactory memory in forager honeybees with sub-chronic exposure durations (Wright et al., 2015).

Glyphosate and imidacloprid decreased associative learning in honeybees yet the combination of these pesticides gave results similar to the control group (Mengoni Goñalons and Farina, 2018). In 5-day-old bees that were fed both imidacloprid and glyphosate, this observation was explained either by potential antagonism or by the lower consumption imidacloprid, which ultimately resulted in exposure to lower doses of the latter, masking its effect.

Given the complex nature of pesticide exposure, which varies in degree, interacts with other environmental factors, and is influenced by numerous risk factors, it is essential that risk assessments for pesticide use and its impact on honeybees be conducted from multiple perspectives. These assessments must account for not only the direct effects of pesticides on individual bees and colonies but also the broader ecological context, as well as potential interactions with other chemicals, diseases, or environmental stressors. Simply relying on a limited set of parameters, such as pesticide concentration in the hive, may fail to capture the full scope of the problem. Even when clear symptoms of pesticide exposure, such as colony decline or bee mortality, are observed, a deeper, multi-layered analysis is required to fully understand the underlying causes and interactions that contribute to these outcomes.

Flupyradifurone is a butenolide similar to neonicotinoids in binding to acetylcholine receptors and it targets mainly sucking insects (Nauen et al., 2015). However, at a sub-lethal dose of 1.4×10^{-5} mol/L, flupyradifurone favored early onset of foraging behavior in honeybees, even without any morphological changes to honeybee brains, reducing the time inside the hive leading to reduced in-hive activity and low life expectancy (Hesselbach et al., 2020). This also questions the applicability of flupyradifurone as an alternative to neonicotinoids. Tosi and Nieh (2019) discovered that flupyradifurone had a seasonal effect on worker bees where it is more toxic in the summer period and has more effect on forager bees than in-hive bees; a point they raised to concern since risk assessments schemes are usually limited to single pesticide exposure on in-hive bees for evaluation of toxicity. Tosi and Nieh (2019) also found synergism between flupyradifurone and propiconazole, a sterol biosynthesis inhibitor fungicide on abnormal behavior frequency.

Bees fed the pyriproxyfen insecticide (42 mg/L) have shorter foraging duration and an earlier first-forage age while bees fed the acaricide spirodiclofen (42 mg/L) had low pollen collection but with higher lipid content affecting the quantity and quality of pollen in the hive (Elizabeth Deeter et al., 2023). Thus, early-age flights, flight onsets, and flight durations are potential indicators for certain pesticide contamination but more studies are needed in order to understand the effect of pesticide cocktails in addition to other risk factors on honeybee behavior.

Proboscis extension response (PER) assay is also used to evaluate the sub-lethal effects of pesticides on honeybees and the assay has been applied to a multitude of pesticides including λ-cyalothrin, cypermethrin, τ-fluvalinate, triazamate, dimethoate, and other chemicals (Decourtye et al., 2005; De Stefano et al., 2014). Lambin et al. found that 5 to 20 ng/bee of imidacloprid stimulates gustatory responsiveness and increases PER (Lambin et al., 2001). In a more recent study, the PER assay was applied for field-realistic concentrations of glyphosate (2.5 μg/mL) and imidacloprid (1 ng/mL) with an observable effect of each pesticide but not with their combination (Mengoni Goñalons and Farina, 2018). Acetamiprid induced decreased PER, impaired long-term olfactory learning and increased locomotor activity at 0.1 ng/bee while thiamethoxam did not produce significant impairment (El Hassani et al., 2008). Indeed, it is observable that high concentrations of neonicotinoids are lethal but sub-lethal doses may stimulate the nervous system depending on the pesticide and mode of application (Tomizawa and Casida, 2005). In addition to foraging activity and PER, hive entrance and bee dance are also parameters for behavioral studies (Pham-Delégue et al., 2002).

Concerning non-*Apis* bees, pesticides have also been tested on stingless bees. The stingless bee species *Melipona quadrifasciata* showed reduced social communication regarding trophallaxis and attenuation after ingestion of acetamiprid (neonicotinoid) mixed with α-cypermethrin (Boff et al., 2018). As for bumblebees, pesticides such as imidacloprid and thiamethoxam affected flower choice, ability to collect pollen, foraging duration while clothianidin and sulfoxaflor affect colony development and reproduction (Raine, 2018).

3.3. Immunity and susceptibility to infection

The immune system of honeybees is one of the main aspects to study the effect of pesticides on pathogen resistance and vulnerability. Like other invertebrates, honeybees lack true lymphocytes and the ability to produce antibodies (Rowley and Powell, 2007). They mainly rely on their innate immune system. The arsenal of honeybees includes several processes for defense against diseases. Insects have a protective cuticle to prevent infection, however, if the cuticle is by-passed or infection occurs via other roots then the immune system goes into action(Menezes and Jared, 2002). After recognition of foreign bodies or pathogenic agents, defenses extend from the production of reactive molecules that destroy pathogens to cellular-mediated process such as phagocytosis, encapsulation, nodulation, and melanization (Larsen et al., 2019).

Pesticides were observed to affect the immune system of honeybees at different levels, whether cellular, genetic (Tesovnik et al., 2019) or humoral (Bartling et al., 2021). Imidacloprid, for example, decreased the phagocytosis in honeybees and bumblebees (Walderdorff et al., 2018). Synergism between pesticides was also observed by decreasing the production of defensive molecules like nitric oxide and hydrogen peroxide (Sukkar et al., 2023b). The neonicotinoid thiamethoxan synergism with *Nosema apis* infection reducing immune competence by decreasing encapsulation response and increasing honeybee mortality especially in drones before reaching sexual maturity (Grassl et al., 2018a).

The neonicotinoid thiacloprid, the fungicides fludioxynil and dimoxystrobin, and the herbicide pendimethalin were reported to alter the production of antimicrobial peptides in honeybees produced by the Toll and IMD (immune deficiency) immune pathways in addition to alteration of the cytochrome P450 detoxification genes and other immune components including NOS, Doux, and catalase of the oxidative response system (Bartling et al., 2021) at different time points.

The immune responses to viruses include RNA interference (RNAi) pathway which spans several phyla (Nayak et al., 2013; Rosa et al., 2018; Wang and He, 2019) and is confirmed in honeybees for viral defense including in response to the *Deformed Wing Virus* (DWV) and to *Israel acute paralysis virus* (IAPV) (Galbraith et al., 2015; Maori et al., 2009). However, pesticides were observed to increase viral titers in bee queens reared in-vitro suggesting an effect on the RNAi pathway (DeGrandi-Hoffman et al., 2013).

The immune response itself is not the only parameter to evaluate immunotoxicity or immune fitness. Hemocyte count and subclasses are also affected by neonicotinoid exposure in bee queens (Brandt et al., 2017) which could affect immune competence.

3.4. Gut microbiota

Gut microbiota in honeybees play key roles in their health. The microbiota usually consists of different bacterial taxa that affect different functionalities of the honeybee including diseases resistance and immunity. Bacterial composition varies between bee species and evolves with time within the same individual as well (Hotchkiss et al., 2022). Many pesticides alter bee gut microbiota mainly glyphosate (Motta et al., 2018).

Research on the effect of microbiota on bee performance is still lacking (Hotchkiss et al., 2022)., several studies have shown the effect of multiple stress factors with their different antagonism and synergism on survival, immunity, and disease resistance (Aufauvre et al., 2012; Bird et al., 2021; Bruckner et al., 2021; Grassl et al., 2018b; Harwood and Dolezal, 2020; Malladi et al., 2023; Shojaei et al., 2018; Tosi and Nieh, 2019). However, the association of gut microbiota shifts and change in health status, diseases resistance, foraging activity, and other parameters in not well understood at complex interaction levels that involves risk factors combinations.

3.5. Larvae and brood

Honeybee larvae offer a practical option for toxicological testing, depending on the specific parameters being studied. Larvae at the 5th instar stage (just before pupation) contain the highest amount of extractable hemolymph compared to other developmental stages, including adults (Borsuk et al., 2017; Negri et al., 2015; Sukkar et al., 2023a) reaching 35 μL of hemolymph per larva (Malladi et al., 2023; Sukkar et al., 2024). This makes them particularly suitable for studies focusing on immunotoxicity, as their hemocytes (immune cells) and any infections present in the hemolymph can be effectively analyzed. Standardized protocols for honeybee larvae acute and chronic toxicity have become robust and repeatable (De Souza et al., 2024). Numerous studies have utilized larvae to examine immune responses at both cellular and genetic levels, as well as microbial infections and their interaction including studies on immune pathways, enzymes, phagocytosis, and cell death (Crailsheim et al., 2013; Dai et al., 2018; Gregorc and Bowen, 2000; Malladi et al., 2023; Prezenská et al., 2019; Sukkar et al., 2024; Sukkar et al., 2023a; Sukkar et al., 2023b; Yu et al., 2021). Larvae of other species are also used for their convenience like larvae of wild bees including bumblebees (Krueger et al., 2021; Siviter et al., 2020; Walderdorff et al., 2018; Yordanova et al., 2022). The usage of larvae in toxicological tests also extends to other insect species such as *Drosophila* spp. (Ferdenache et al., 2019; H. Gao et al., 2024; Young et al., 2020), Black Soldier Fly (Bruno et al., 2021), greater wax moth (Said et al., 2019; Sohail et al., 2021), lacewing (Rugno et al., 2019; Shan et al., 2020), and other insects. Additionally, the effects of larval pesticide exposure can be used to assess the effects on developments and effects in the adult stage.

3.6. Omics approaches in honeybee toxicology: gene products and biomolecular insights

In 2006, the western honeybee genome has been reported paving the way for development in honeybee comparative genomics, toxicology, evolution studies, honeybee health and more (Toth and Zayed, 2021). Following the publishing of the honeybee genome consortium, an inventory of honeybee transcripts, termed the DETOXome, has been developed to determine the gene expression of five protein superfamilies, including cytochrome P450 monooxygenases (P450), carboxylesterases, glutathione S-transferases (GST), UDP-glycosyltransferases (UGT), and ATP-binding cassette (ABC) transporters, at different life stages and in various tissues. (Maiwald et al., 2023) in addition to glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and phenoloxidase (POx) (Almasri et al., 2022). The inventory could prove as a fit reference point for toxicology in honeybees regarding pesticide and toxin exposure. The genome, transcriptome and metabolome of honeybees are also included in toxicology assessment to identify differentially expressed genes (DEGs) and differentially expressed metabolites (DEMs) when exposed to pesticides or physiological markers (Dickey et al., 2023; J. Gao et al., 2022; Zhang et al., 2022), nutritional status (Schmehl et al., 2014), and detoxification pathways covering a wide range of pesticide interactions (Berenbaum and Johnson, 2015).

Molecular determinants of toxicity include identified P450s, which are considered crucial for understanding insecticide metabolism in honeybees (Bass et al., 2024). In a recent review, Bass et al. (2024) addressed broader concepts of indicators including the insecticide target sites and the microbiome and their role in pesticide detoxification in honeybees as well as the cuticle referring to the pharmacokinetics of pesticide contact toxicity in the latter. Additionally, the authors recommended protein structure prediction software like Alphafold and artificial intelligence software developed by DeepMind to build predictive models for honeybee toxicity studies and risk assessments.

4. Pesticide usage and honey production

Phiri et al. (2022) reported that the production of honey and the number of colonies have increased over 6 decades (until 2017) in Asia, South America, Africa, and Oceania. Still, honeybee colonies have decreased in Europe and North America by 11.6 % and 28.4 % respectively even when honey production increased. The lowest honey production increase was in Oceania. Correlation studies between pesticide usage, bee colony abundance, and honey production are needed to truly understand the effect of pesticides on the honey production parameter.

Furthermore, using pesticides without strict regulations may be more economically harmful than adhering to stringent guidelines and limiting their extensive use on crops. To elaborate, using pesticides may affect honey production in countries that rely on honey exportation or using pesticides that are inducing resistance in target organisms, but remain harmful to non-target organisms (Barman et al., 2022; Cuesta-Maté et al., 2021; Rodríguez-Dehaibes et al., 2005). However, the economic relevance is context-dependent as it is related to the degree of honey dependency for income compared to crops and other services that require the use of pesticides. In other words, a balance between the economic pros and cons of pesticide use must be established by considering its impact on honey production, pest infestation levels, and crop yields.

5. Alternative bioindicators

5.1. Wild bees

On several points, honeybees provide a convenient bioindicator since standard methods have already been developed for rearing and toxicological tests (De Souza et al., 2024; Mortensen et al., 2019; K.-C. Yang et al., 2021). However, the difference between honeybees and other bee species in terms of body size, foraging behavior, food consumption rate, and the pollen/nectar ratio result in differential exposure to pesticides between honeybees and wild bees. Henceforth, a comprehensive establishment of pesticide exposure and risk assessment of the environment must not be limited to honeybee species but extend to other species as well.

For example, examining the effect of the pyrethroid λ-cyhalothrin on different social or solitary bee species like *Andrena vaga, Bombus terrestris, Colletes cunicularius, Osmia bicornis, Osmia cornuta, Megachile rotundata, Apis mellifera,* results revealed that the insecticide had variable effect on the different bee species mortality and induction of abnormal behavior (Jütte et al., 2023) taking into account the different size of bees. The mentioned study stated that honeybees can be used as a substitute in some cases but some bee species are more sensitive, thus a broader range of models is needed for more realistic risk assessments. In addition, Raine and Rundlöf (2024) reviewed that non-*Apis* bees like bumblebees, stingless bees and solitary bees vary from honeybees and that the pesticide effect extrapolation would be limited in this case setting a necessity for more studies on *non-Apis* bees since they are under evaluated. In fact, foraging bumblebee queens are an additional exposure route to pesticides compared to honeybees which should be considered when comparing exposures to pesticides (Gradish et al., 2019).

5.2. Honeybee predators as bioaccumulation indicators: the Asian hornet

Many bee predators and pests are also good candidates to study pesticide application. The Asian hornet can be studied as a bioindicator of pesticide contamination; for instance, in a study in southwestern France with the highest pesticide concentrations detected in hornets in grasslands and forests in sub-urban areas (Tison et al., 2023). The study sheds light on bioconcentration of pesticides from honeybees (the prey of Asian hornets) and other sources which may continue through the food chain raising concern. The pesticides mainly found in hornets' nests were piperonyl butoxide (37.55 %), pyrimethanil (12.5 %), the amitraz metabolite DMFP (N-(2,4-dimethylphenyl)-formamide; 12.5 %), amitraz and cymiazole (4.5 %) (Tison et al., 2023). Amitraz is mainly used against *Varroa destructor* mites that parasitize honeybees (Filazi and Yurdakok-Dikmen, 2018). The fact that amitraz and its active metabolite DMFP are found in honeybee predators such as hornets indicates its persistence in the ecosystem and its bioaccumulation.

6. Limitations in using honeybees for toxicological assays

6.1. Cell lines

Currently, there are no commercially available cell lines of honeybees to conduct standardized studies. Cell lines from Hymenoptera only contribute to 1.4 % of established cell lines from insects, mainly sources from embryonic tissue followed by larval sources (Perera et al., 2023). The need for an established cell line is crucial to standardize toxicological tests, decrease individual variability between samples, and provide a continuous source of cells at any given time without the obligation of the time-consuming and laborious conditions of fresh extraction. Regarding honeybees, the AmE-711 cell line was established from embryonic tissues cultured in HB-1 medium (M. J. Goblirsch et al., 2013), however, it harbors the deformed wing virus (DWV) (Guo et al., 2020) but was stated later to be a fit cell model for insecticide toxicity test and complementary to whole organism studies (M. Goblirsch and Adamczyk, 2023). More cell lines from different tissues of honeybees are needed to evaluate the tissue-specific effects of pesticides and other risk factors.

6.2. Different sub-species and lineages

Different breeds of honeybees have different susceptibilities and differ in tolerance to diseases, wintering, and defense against pests. Many studies do not usually indicate the breed of the honeybees used. This could result in a gap in understanding the different effects of pesticides on domesticated bees as their variability translates into the variability in results for the same experimental treatments.

7. Discussion

A variation between lab and field experiments is evident when assessing colony loss and health in the presence of pesticides, suggesting that colonies can be more resilient in the field than indicated by laboratory experiments elucidate (Harwood and Dolezal, 2020). However, the complexity of field interactions must be depicted by including many parameters that influence the outcome simultaneously, including temperature variation whether long-term or with the day. In addition to the level of biodiversity richness within the area and the frequency of interaction with pathogens and pests. Ignoring the consideration of the parameters at the field level may build a base for an incomplete assessment of environmental risks to honeybees and biodiversity in general.

As social insects with a colony system, honeybees provide insights of the effects of pesticides on behavior which is crucial for maintaining hive function. The impact of pesticides on neurobiology and behavior may not be clear on non-social insects or insects that do not require complex individual and collective behavior giving honeybees an advantage as models in ecotoxicological studies on that end.

The debate arises on whether to equally prioritize wild pollinators to honeybees (Halvorson et al., 2021) and that the overall functional importance of honeybees can be compensated naturally. In fact, there is a concern of competition between wild bees and honeybees (Mouillard-Lample et al., 2023), and that honeybees affect wild bee abundance (Angelella et al., 2021). In addition, Weekers et al. (2022) concluded that apiculture of honeybees might competitively impact pollinator diversity where pollinators are relatively high. However, honeybees have co-evolved with human activity and their surrounding environment for millennia, and thus their implication in ecological functioning should not be underestimated. Additionally, unlike other domesticated animals including cattle, bee activity and functioning impact wide areas and are not limited to closed barns or fields.

There is limited evidence regarding the transfer of pesticides across terrestrial trophic levels, underscoring the need for further research on pesticide residues within the food chain (Tison et al., 2024). Bees, supported by extensive existing research, serve as practical bioindicators, highlighting their significance in this context. However, it is essential to consider other organisms when designing model systems to predict pesticide bioaccumulation. In addition to the Asian hornet as bioindicators for pesticide contamination and bioaccumulation (Tison et al., 2023), different insect orders including Hymenoptera, Diptera, Hemiptera, Neuroptera, Lepidoptera, Coleoptera were demonstrated as a good indicator for chlorpyrifos pesticide contamination in a study made in Chili over a duration of 14 months (Valdés et al., 2023). By incorporating a diverse range of organisms, it may be possible to use honeybees as a predictive model for environmental bioaccumulation, minimizing the need for extensive environmental studies. Furthermore, there is still a knowledge gap genetic variability spectrum of molecular markers in honeybees related to detoxification demanding further investigation (Bass et al., 2024). More research on pesticide toxicity in honeybee larvae is needed as well as stated by De Souza et al. (2024).

In this review we highlighted the benefits of bees as bioindicators comparing pesticide exposure studies made on honeybees and wild pollinators taking to consideration the effect of pesticides on their decline. Though previous research was prioritized towards domesticated honeybees with less consideration to wild pollinators, the collected data and research could be beneficial for future risk assessments when additional parameters are taken to consideration including further omics inclusion and pushing its advancement in toxicity tests. Other bioindicators should also be taken to consideration to understand the degree of bioaccumulation and bioconcentration of pesticides in the environment which could facilitate in understanding and predicting the effect of pesticide application in the environment. With the rapid advancement in artificial intelligence technology, including predictive software in risk assessments may prove robust and dependable.

CRediT authorship contribution statement

Dani Sukkar: Visualization, Investigation, Writing – original draft. **Jairo Falla-Angel:** Validation, Supervision, Funding acquisition, Writing – review & editing. **Philippe Laval-Gilly:** Validation, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that this study was conducted in the absence of any conflict of interest.

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Data availability

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

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