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## Oxidative stress in stingless bees exposed to pesticides and heavy metals: a systematic review

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**Abstract.** Stingless bees play a crucial role in maintaining biodiversity and supporting agricultural production; however, they are increasingly exposed to environmental contaminants associated with the intensification of anthropogenic activities. This qualitative systematic review aimed to synthesize and comparatively analyze the available evidence on the effects of pesticides and heavy metals on oxidative stress biomarkers in stingless bees, considering the evaluated species, classes of contaminants, exposure routes, and observed physiological responses. Literature searches were conducted in the Web of Science and Scopus databases via the CAPES Journals Portal, resulting in the inclusion of 18 studies. The findings indicate that insecticides, fungicides, herbicides, and heavy metals induce relevant sublethal effects, frequently converging on physiological pathways related to redox imbalance, such as the modulation of antioxidant enzymes and increased markers of oxidative damage. The review also highlights the predominance of laboratory-based studies and the concentration of data on a limited number of species, underscoring important knowledge gaps.

**Keywords:** Contaminants, Anthropogenic activities, Sublethal effects, Physiological responses, Systematic review.

### Introduction

Stingless bees belong to the order Hymenoptera, family Apidae, and tribe Meliponini, and are distributed across tropical and subtropical regions (Michener 2007). Task organization within the colony is performed by worker bees and includes nest construction, larval feeding, colony defense, and foraging activities (Grüter, 2020). Foraging, which consists of collecting pollen and nectar, represents the primary link between stingless bee workers and the external environment beyond the colony (Bueno *et al.*, 2023).

Brazil harbors a high diversity of stingless bees (Grüter, 2020), which, combined with the intensification of agricultural activities, makes these species particularly vulnerable to pesticide exposure (Botina *et al.*, 2020; Otesbelgue; dos Santos; Blochtein, 2018). These contaminants can be classified into different groups, notably insecticides, used to control agricultural pests and considered among the main risk agents for pollinators (Motta *et al.*, 2024); herbicides, applied for weed control and widely used in Brazil, especially in large monoculture areas (Prado *et al.*, 2023); and fungicides, employed

to manage fungal pathogens and generally regarded as having low toxicity to insects (Araújo *et al.*, 2025).

In addition to these classes, heavy metals may also be present in pesticide formulations or mobilized by intensive agricultural practices, which has markedly increased the environmental bioavailability of these elements and expanded the risk of exposure for bees (Feldhaar; Otti, 2020).

Among the consequences of bee exposure to these xenobiotics are physiological responses that disrupt cellular homeostasis, with oxidative stress standing out as a key mechanism (Kodríkef *et al.*, 2015). This process arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant systems to neutralize them, leading to damage to lipids, proteins, and nucleic acids (Simone-Finstrom *et al.*, 2016). In bees, such alterations may impair essential functions, including behavior, development, and foraging performance, with direct implications for colony maintenance.

Despite the increasing number of studies addressing toxicity in stingless bees, much of the available evidence remains fragmented,

concentrated on a limited number of species and pesticide classes, and frequently relies on *Apis mellifera* as a model species (Bogo *et al.*, 2024). Comparative integration of physiological responses, particularly those related to oxidative stress biomarkers, remains limited. In this context, this systematic review aimed to comparatively synthesize the available evidence on the effects of these contaminants on key oxidative biomarkers, considering chemical classes, exposure routes, and observed physiological responses, thereby contributing to a more realistic assessment of the environmental risks faced by these pollinators.

### Material and Methods

This study consists of a qualitative systematic review of the literature conducted following the framework proposed by Wancura; Milanesi; Cargnelutti (2025). Bibliographic searches were performed in the Web of Science and Scopus databases, accessed through the CAPES Journals Portal. The search strategy combined terms related to stingless bees, pesticides, heavy metals, and oxidative stress, including variations and combinations of these descriptors, using the Boolean operators AND and OR.

The retrieved records were exported in BibTeX format and subjected to duplicate removal using the RStudio software, with automated tools applied for file organization and management. The unified dataset was subsequently converted into a spreadsheet for screening and data extraction.

Article selection was carried out in three sequential stages: (i) title screening to identify potentially relevant studies; (ii) abstract evaluation, considering the presence of experimental or environmental exposure to pesticides and/or heavy metals and the measurement of biomarkers related to oxidative stress; and (iii) full-text reading of eligible articles to confirm compliance with the predefined methodological criteria.

Studies were considered eligible if they were empirical investigations with experimental data conducted on stingless bee species (tribe Meliponini) that assessed exposure to pesticides and/or heavy metals and measured biochemical, physiological, or molecular biomarkers associated with oxidative stress. Review articles, editorials, technical notes, studies lacking experimental data, investigations conducted exclusively with *Apis mellifera*, studies assessing only acute mortality without analysis of sublethal effects, and those that did not establish a direct relationship between contaminant exposure and oxidative responses were excluded.

The included studies underwent systematic data extraction, encompassing information on the evaluated species, contaminant class and type, exposure route, experimental design, analyzed biomarkers, and main observed effects. The study selection process is presented through a flowchart (Figure 1.), ensuring transparency and reproducibility of the review.

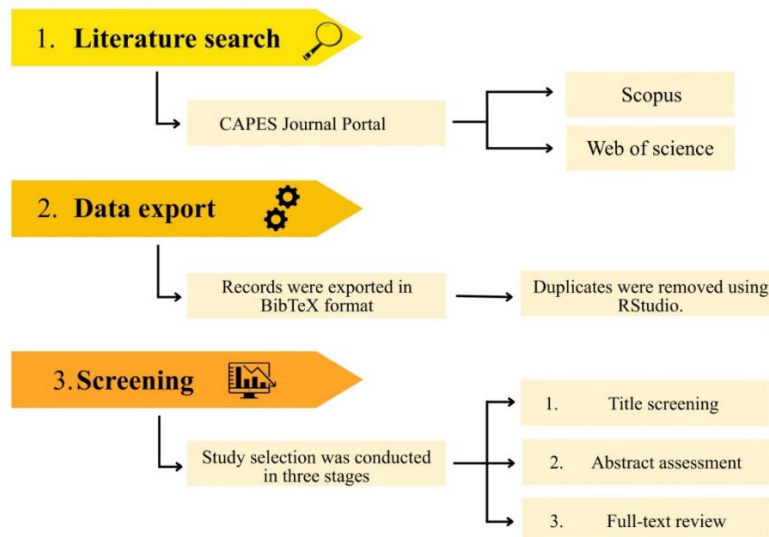


Figure 1. Flowchart summarizing the methodological steps followed in the systematic review

## Results and discussion

### Characterization of the Included Studies

Application of the eligibility criteria resulted in a final set of 18 studies, conducted predominantly

in Brazil and involving species from the genera *Melipona*, *Partamona*, *Plebeia*, *Scaptotrigona*, *Frieseomelitta*, *Tetragonisca*, and *Trigona* (Table 1). All selected studies were carried out under laboratory conditions, which allowed for a detailed

assessment of physiological and biochemical responses associated with oxidative stress. This pattern reflects the predominance of controlled experimental approaches in the investigation of sublethal effects, while also highlighting the limited availability of data obtained under environmentally realistic conditions.

#### *Physiological effects and patterns associated with insecticides*

Among pesticides, insecticides represent the most extensively investigated class and are associated with the most consistent sublethal effects in stingless bees (Bogo *et al.*, 2024). Neonicotinoids, in particular, exhibit a recurrent toxicity pattern by acting on nicotinic acetylcholine receptors, triggering neurophysiological alterations that are reflected in behavioral changes and biochemical responses associated with oxidative stress (de Souza *et al.*, 2024).

Consistently, the studies listed in Table 1 that addressed neonicotinoid exposure indicate that these compounds are capable of impairing the digestive tract, altering locomotor behavior, and inducing physiological responses associated with redox imbalance, frequently evidenced by changes in the activity of antioxidant enzymes. These patterns were observed across different species, including *Partamona helleri* (Motta *et al.*, 2024), *Frieseomelitta varia* (de Souza *et al.*, 2024), *Plebeia catamarcensis* (de Paula *et al.*, 2023), *Melipona scutellaris*, *Scaptotrigona postica*, *Tetragonisca angustula* (Lourencetti *et al.*, 2023), and *Melipona quadrifasciata* (Aguilar *et al.*, 2023).

In addition to neonicotinoids, other insecticides have also shown consistent patterns of sublethal toxicity in stingless bees. Compounds such as abamectin, acetamiprid, and chlorpyrifos impair essential physiological and behavioral functions, with relevant variation among substances belonging to the same chemical class. Overall, these insecticides affect feeding response, locomotion, survival, and development, in addition to inducing alterations in metabolic, immunological, and cellular stress-related processes (Ferreira *et al.*, 2022; Dorneles *et al.*, 2021), particularly when exposure occurs during immature stages (da Silva *et al.*, 2025). Such sublethal effects during larval development have direct implications for adult functional capacity and for the long-term maintenance of colony structure and performance.

#### *Herbicides: recurrent sublethal effects*

Herbicides, widely used in intensive agricultural systems, generally exhibit limited acute toxicity to stingless bees; however, the analyzed studies indicate consistent sublethal effects associated with prolonged exposure. These effects include behavioral alterations, locomotor impairment, reduced foraging efficiency, and modulation of enzymes related to oxidative stress, detoxification, and immune response, indicating disruption of physiological homeostasis (da Silva *et al.*, 2025; Fardeir-Gomes *et al.*, 2024).

Although glyphosate has demonstrated toxicity only at relatively high concentrations in some studies, suggesting a lower relative risk, assessments using commercial formulations have revealed mortality, disorientation, and motor impairment, with LD<sub>50</sub> values established at 24 and 48 hours (Ferreira *et al.*, 2023; Prado *et al.*, 2023). Collectively, these findings indicate that the impacts of herbicides are strongly dependent on formulation type, dose, and exposure duration, reinforcing the importance of evaluating sublethal effects in stingless bees.

#### *Fungicides: patterns of sublethal effects*

Although frequently regarded as having low toxicity to insects, the analyzed fungicides exhibited consistent sublethal effects in stingless bees, affecting multiple levels of biological organization. Overall, exposure to these compounds was associated with behavioral alterations, including changes in locomotion, social interaction, color preference, and reduced food intake, as well as physiological impairment, such as decreased survival and respiratory dysfunction (Almeida *et al.*, 2021; Gomes *et al.*, 2024; Araújo *et al.*, 2025).

At the cellular and tissue levels, alterations were observed in the digestive tract, Malpighian tubules, and metabolically active cells, including vacuolization, apoptosis, and an increased number of altered cells, indicating disruptions in absorption, excretion, and cellular homeostasis (da Silva *et al.*, 2024; Fardeir-Gomes *et al.*, 2024; Gomes *et al.*, 2024).

Concurrently, fungicides modulated enzymes associated with stress responses, detoxification pathways, and immune function, increasing energetic demand and impairing larval development, particularly under early-life exposure (da Silva *et al.*, 2025). Collectively, these findings indicate that fungicides can induce relevant physiological imbalances even in the absence of high acute toxicity, underscoring their importance in risk assessments for stingless bees.

**Table 1.** Studies selected from the bibliometric analysis addressing the exposure of stingless

Species	Pesticide name	Pesticide class	Exposure conditions	Results	Reference
Frieseomelitta varia, Melipona mondury and Trigonas pinipes	Difenoconazole	Fungicide	Exposure occurred through ingestion and contact	In F. varia, difenoconazole increased total hemocyte count (THC) and, via oral exposure, altered behavior and midgut morphology. In M. mondury, contact exposure decreased survival and THC, while oral exposure reduced locomotion and THC. In T. spinipes, contact exposure enhanced locomotion and social interactions.	Araújo et al. (2025)
Frieseomelitta varia	Clomazone, difenoconazole, and abamectin	Herbicide, fungicide and insecticide	First-instar newly emerged larvae were exposed for 48 hours to sublethal pesticide doses mixed into the larval diet.	They altered stress-related, detoxification, and immune enzymes, increasing energetic demand and impairing larval development	da Silva et al. (2025)
Partamona helleri	Thiamethoxam	Insecticide	Worker bees were orally exposed for 7 days to a sublethal concentration similar to that found in pollen grains.	It caused damage to the midgut, including vacuolization, apoptosis, oxidative stress, and enzymatic alterations, in addition to impairing bee locomotion.	Motta et al. (2024)
Partamona helleri	Fluazinam	Fungicide	Acute oral exposure of worker bees for 24 hours.	It reduced locomotion and food intake, caused intestinal alterations, oxidative stress, and cell death, and negatively affected behavior.	Gomes et al. (2024)
Frieseomelitta varia	Thiamethoxam	Insecticide	Acute exposure to thiamethoxam was performed to determine the median lethal concentration (LC50) and the median lethal time (LT50)	It reduced survival and altered enzymatic activities, affecting the abdomen more strongly than the head.	de Souza et al. (2024)
Frieseomelitta varia	Pyraclostrobin and thiamethoxam	Fungicide and insecticide	Oral exposure to the pesticides, either individually or in combination, for 48 h or 96 h	In the Malpighian tubules, vacuolization was the only damage observed, while broader morphological alterations in the analyzed organs impaired both excretion and absorption	da Silva et al. (2024)
Melipona scutellaris	Imidacloprid, pyraclostrobin and glyphosate	Insecticide, fungicide and herbicide	Oral exposure for 48 h to the pesticides, either individually or in combination.	They caused locomotor and cellular alterations, increased apoptosis, and resulted in a higher number of altered oenocytes and trophocytes.	Farder-Gomes et al. (2024)
Apis mellifera and Scaptotrigona postica	Fox Xpro	Fungicide	Exposure was conducted using a laboratory spray system	Bees positioned at the top of plants and those sprayed with fine droplets absorbed greater amounts of pesticide.	Freitas et al. (2024)

Species	Pesticide name	Pesticide class	Exposure conditions	Results	Reference
			designed to replicate field conditions, allowing the quantification of active ingredient absorption.	Stingless bees experienced higher contamination levels due to their lower body mass.	
<i>Tetragonisca angustula</i>	Clothianidin	Insecticide	Adult foragers were orally exposed for 6–8 h to a honey–water syrup contaminated with clothianidin.	Clothianidin residues were detected, and the species proved to be highly sensitive to the insecticide, even more so than other bee species reported in the literature.	Biscassi et al. (2024)
<i>Plebeia catamarcensis</i>	Thiamethoxam	Insecticide	Worker foragers from three colonies were orally exposed to sugar syrup contaminated with thiamethoxam after a 24-hour acclimation period.	Sublethal concentrations altered bee locomotion, causing hyperactivity or lethargy, which may impact small colonies and ecosystem services.	de Paula et al. (2023)
<i>Plebeia lucii</i>	Acephate and glyphosate	Insecticide and herbicide	Chronically exposed orally to food contaminated with different concentrations of commercial pesticide formulations.	Bee mortality increased with higher pesticide concentrations. Acephate reduced both lifespan and mobility, while glyphosate was toxic only at unrealistic concentrations, suggesting different risk levels for stingless bees.	Ferreira et al. (2023)
<i>Tetragonisca angustula</i>	Glyphosate	Herbicide	The study determined the 24- and 48-hour Median Lethal Dose (LD50) of the glyphosate-based herbicide formulation through ingestion	The doses caused mortality, motor impairments, excessive self-cleaning, and disorientation.	Prado et al. (2023)
<i>Melipona scutellaris</i> , <i>Scaptotrigona postica</i> , <i>Tetragonisca angustula</i> and <i>Apis mellifera</i>	Thiamethoxam	Insecticide	Forager workers of the three species were collected to evaluate the lethal concentration (LC50), estimate the lethal dose (LD50), and compare sensitivity to thiamethoxam.	Stingless bees showed greater sensitivity to thiamethoxam than <i>A. mellifera</i> . Among the species tested, <i>M. scutellaris</i> was the most sensitive, followed by <i>T. angustula</i> and <i>S. postica</i> , although the ranking varied depending on whether body weight was considered.	Lourencetti et al. (2023)
<i>Melipona quadrifasciata</i> and <i>Apis mellifera</i>	Imidacloprid	Insecticide	Bees were fed 0.1, 0.5, or 1 ng of imidacloprid diluted in a sugar solution.	Exposure reduced the innate food response and impaired learning and memory in a dose-dependent manner.	Aguiar et al. (2023)

Species	Pesticide name	Pesticide class	Exposure conditions	Results	Reference
<i>Partamona helleri</i>	Sulfato de cobre (CuSO <sub>4</sub> )	Pesticide/foiar fertilizer	Foragers were orally exposed to five CuSO <sub>4</sub> concentrations (5000, 1666.7, 554.2, 183.4, and 58.4 µg mL <sup>-1</sup> ), and the median lethal concentration (LC <sub>50</sub> ) was subsequently estimated.	There was an increase in mortality with rising CuSO <sub>4</sub> concentrations. The estimated LC <sub>50</sub> impaired locomotion, caused intestinal damage, and elevated oxidative stress and apoptosis.	Bernardes et al. (2022)
<i>Scaptotrigona aff. xanthotricha</i>	Abamectin and acetamiprid	Insecticides	Lethal doses (LD <sub>50</sub> and LD <sub>90</sub> ) and lethal time (LT) for both pesticides were assessed under contact exposure.	Exposure at LD <sub>90</sub> showed high toxicity for both pesticides: abamectin reduced survival more rapidly, while acetamiprid impaired locomotion and showed greater overall toxicity across the evaluated parameters.	Ferreira et al. (2022)
<i>Scaptotrigona bipunctata</i>	Chlorpyrifos	Insecticide/acaricide	Worker larvae reared in vitro were exposed to different concentrations of the pesticide.	Exposure reduced the probability of survival but did not alter development time, and the emerged bees were smaller, lighter, and 28% showed wing deformities.	Dorneles et al. (2021)
<i>Partamona helleri</i> and <i>Apis mellifera</i>	Imidacloprid and a fungicide mixture of thiophanate-methyl and chlorothalonil	Insecticide and fungicide	Forager workers of both species were orally exposed for 5 hours to sucrose solutions containing field-realistic doses of imidacloprid or commercial fungicide formulations.	Pesticide exposure altered color preference in a concentration-dependent manner, reduced locomotion with the insecticide, and decreased respiration with the fungicide mixture, while the individual fungicide components affected only color preference	Almeida et al. (2021)

### Heavy metals and convergence of physiological pathways

In general, metal exposure is associated with modulation of antioxidant enzymes and increased markers of oxidative damage, reflecting compensatory responses to redox imbalance. In *Scaptotrigona bipunctata*, exposure to copper sulfate resulted in a significant increase in catalase (CAT) and superoxide dismutase (SOD) activity, indicating activation of the antioxidant system in response to induced stress (Bernardes *et al.*, 2022). This biochemical response was accompanied by adverse physiological effects, including intestinal damage, locomotor impairment, and increased apoptosis; additional examples are presented in Figure 2.

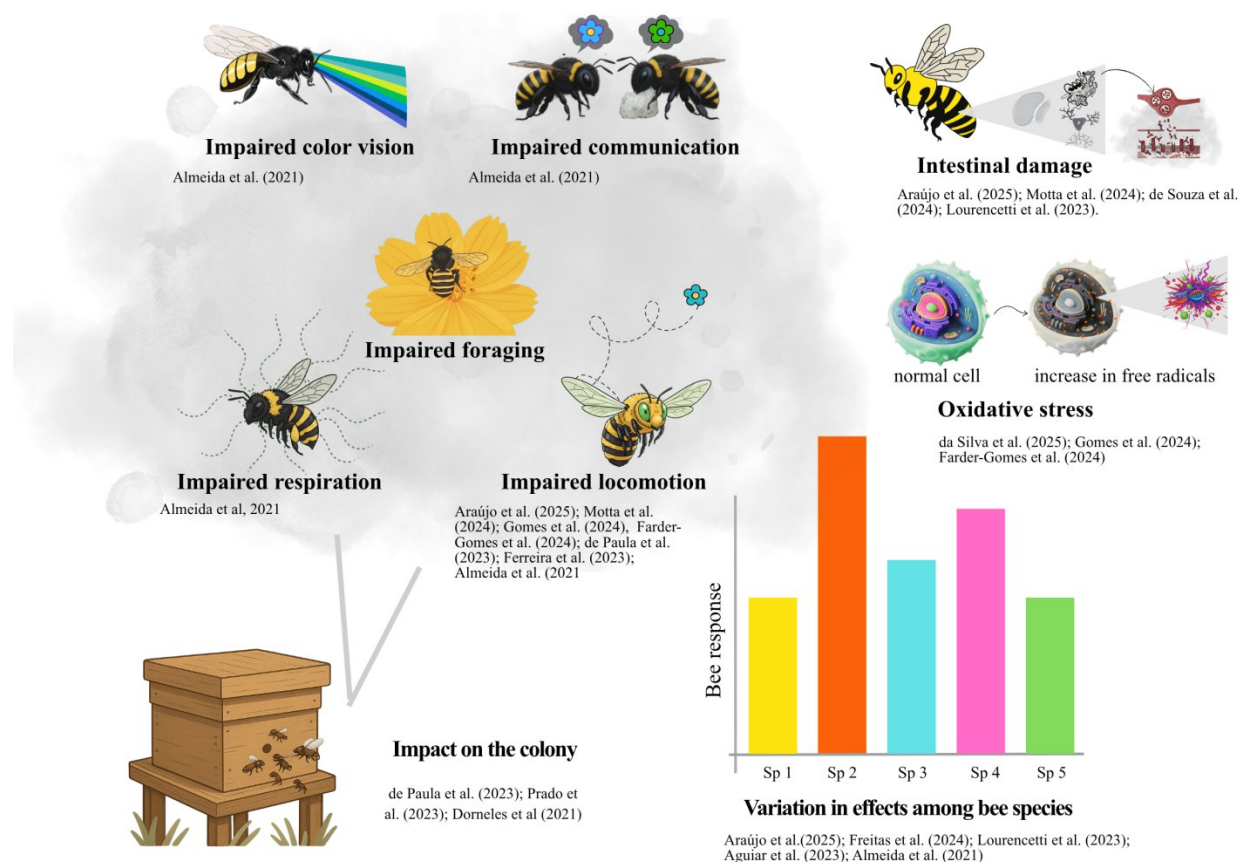
These findings indicate that, similarly to what has been observed for pesticides, heavy metals affect multiple levels of biological organization, integrating biochemical, cellular, and behavioral alterations. The convergence of these responses suggests that different classes of contaminants share similar physiological pathways of toxicity, particularly those associated with oxidative stress.

Furthermore, heavy metals may be present as impurities or secondary components in

commercial pesticide formulations, increasing the complexity of exposure scenarios in agricultural environments. In this context, the observed effects may result from the combined action of multiple contaminants, complicating the attribution of impacts to a single chemical agent and potentially amplifying oxidative stress responses

### Oxidative stress biomarkers and mixture effects

Oxidative stress biomarkers, such as catalase (CAT), glutathione S-transferase (GST), superoxide dismutase (SOD), acetylcholinesterase (AChE), and lipid peroxidation (LPO), constitute central tools for the integrated assessment of physiological health in stingless bees. The analyzed studies demonstrate that exposure to pesticides and heavy metals can trigger antioxidant and neurophysiological responses associated with redox imbalance, reflected by changes in enzymatic activity and indicators of oxidative damage. However, the magnitude of these responses varies according to species, contaminant type, dose, and exposure route (de Souza *et al.*, 2024; Motta *et al.*, 2024; Farder-Gomes *et al.*, 2024) and is further constrained by methodological heterogeneity among studies, particularly regarding the set of biomarkers evaluated.



**Figure 2.** Sublethal effects of bee exposure to xenobiotics. Key studies addressing different topics are highlighted. Illustration: images and design created using the Canva application.

Under environmentally realistic conditions, exposure occurs simultaneously and chronically to multiple contaminants, rendering assessments based on single substances insufficient for estimating ecotoxicological risks. Evidence indicates that mixtures of pesticides and metals can potentiate physiological imbalance, intensifying cellular stress, impairing immune responses, and exacerbating behavioral and functional alterations, with effects dependent on the chemical composition of the mixtures. Despite this, most studies still adopt simplified experimental designs, focusing on single-compound exposures or a limited number of species. This limitation underscores the need for investigations that integrate native species and regionally relevant contaminants to more realistically represent the exposure scenarios faced by stingless bee communities in agricultural environments.

### Conclusion

This systematic review demonstrated that, overall, the evaluated contaminants converge on similar physiological pathways associated with redox system imbalance in stingless bees. These responses are characterized by alterations in oxidative stress biomarkers, although the magnitude and, in some cases, the direction of the effects vary according to species, dose, exposure route, and duration.

Despite the consistency of the observed physiological patterns, the predominance of laboratory-based studies, the concentration of evidence on a limited number of species, and the scarcity of investigations addressing chemical mixtures reveal important gaps in understanding ecotoxicological impacts under environmentally realistic scenarios. These limitations hinder the extrapolation of results to field conditions and to the broader diversity of stingless bee species.

Collectively, the findings reinforce the relevance of oxidative stress biomarkers as integrative and sensitive tools for assessing stingless bee health, enabling the early detection of physiological alterations induced by environmental contaminants. Moreover, the results highlight the need for comparative and methodologically standardized approaches that incorporate multiple stressors, diverse exposure routes, and a broader range of species. Advances in this direction are essential for improving environmental risk assessment frameworks and for developing more effective conservation strategies for these essential pollinators.

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