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Review

Biopesticides and insect pollinators: Detrimental effects, outdated guidelines, and future directions



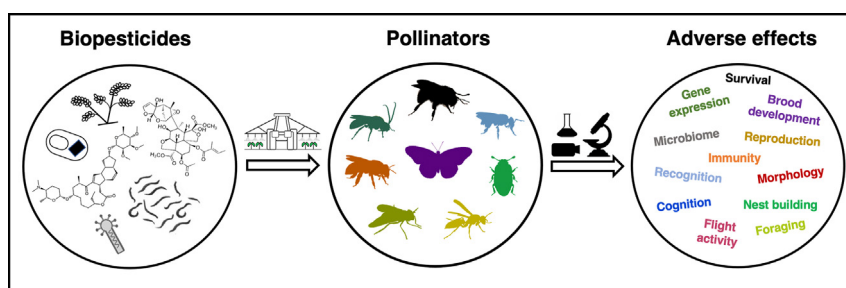
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HIGHLIGHTS

- Biopesticides have been gaining increased attention to develop more sustainable methods for pest management in agriculture.
- Current risk-assessment protocols fail to evaluate the detrimental effects of biopesticides non-target pollinators.
- Literature revision demonstrates that biopesticides cause a plethora of sublethal effects on insect pollinators.
- Research and policies gaps and future perspective are summarized.

GRAPHICAL ABSTRACT



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ABSTRACT

As synthetic pesticides play a major role in pollinator decline worldwide, biopesticides have been gaining increased attention to develop more sustainable methods for pest management in agriculture. These biocontrol agents are usually considered as safe for non-target species, such as pollinators. Unfortunately, when it comes to non-target insects, only the acute or chronic effects on survival following exposure to biopesticides are tested. Although international boards have highlighted the need to include also behavioral and morphophysiological traits when assessing risks of plant protection products on pollinators, no substantial concerns have been raised about the risks associated with sublethal exposure to these substances. Here, we provide a comprehensive review of the studies investigating the potential adverse effects of biopesticides on different taxa of pollinators (bees, butterflies, moths, beetles, flies, and wasps). We highlight the fragmentary knowledge on this topic and the lack of a systematic investigation of these negative effects of biopesticides on insect pollinators. We show that all the major classes of biopesticides, besides their direct toxicity, can also cause a plethora of more subtle detrimental effects in both solitary and social species of pollinators. Although research in this field is growing, the current risk assessment approach does not suffice to properly assess all the potential side-effects that these agents of control may have on pollinating insects. Given the urgent need for a sustainable agriculture and wildlife protection, it appears compelling that these so far neglected detrimental effects should be thoroughly assessed before allegedly safe biopesticides can be used in the field and, in this view, we provide a perspective for future directions.

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1. Insect apocalypse and the need for a sustainable agriculture

In recent years, a catastrophic decline of the entomofauna has been recorded worldwide. The rapidity and extent of the process have recently led scientists to rightfully define it the “Insect Apocalypse”. Many drivers contributing to this worrying phenomenon have been identified (Potts et al., 2010; Sánchez-Bayo and Wyckhuys, 2019), but the risk of pesticides for insects is undeniably overwhelming. Starting from the 1950s and '60s, a considerable number of synthetic pesticides have been designed and massively delivered in the crop field to control insect pests. As a result, residues of many harmful pesticides can be detected in natural environments and food products at concentrations well above recommended limits, with negative consequences on human health and devastating effects on non-target organisms (van der Werf, 1996; Damalas and Eleftherohorinos, 2011).

In the long-lasting battle between agricultural production and arthropod pests, the casualties of war have been represented by all those species of beneficial insects, which provide valuable key ecosystem services such as pollination, predation, seed dispersal, and nutrient recycling to an extent that they are often considered among ‘the little things that run the world’ (Steffan-Dewenter et al., 2005; Potts et al., 2010). Pollinators in general, and bees in particular, have faced a relentless decrease in recent years and such a decline concerned both wild and managed species (Brittain et al., 2010; Potts et al., 2010; Lever et al., 2014; Goulson et al., 2015; Potts et al., 2016). The pollinator decline is bound to have profound environmental and economic consequences in terms of maintenance of ecosystem stability and biodiversity and agricultural productivity due to their fundamental pollination services of wild and cultivated plants (Gallai et al., 2009). Given the well-known role of synthetic pesticides in wiping up pollinator populations worldwide (Brittain et al., 2010; Ndakidemi et al., 2016; Sponsler et al., 2019), the scientific community, followed by policy-makers and stakeholders, mobilized to push towards the adoption of more sustainable strategies such as biocontrol and integrated pest management (IPM) strategies. IPM actions, however, are not always explicitly “pollinator friendly”, and a new synthesis, defined as “integrated pest and pollinators management” (IPPM), has recently been proposed. IPPM includes all those strategies minimizing negative impact on pollinators while promoting synergies between crop pollination, pest control practices and ecosystem services (Egan et al., 2020). Ecofriendly alternatives developed in the last decades include, among others, all those agents of control defined as naturally occurring pesticides or biological pesticides (biopesticides), whose use should pave the way to an environmentally sustainable agriculture.

2. Biopesticides: definition and use

Biocontrol microbials, their insecticidal metabolic products, and other pesticides based on living organisms are sorted as biopesticides by the United States Environmental Protection Agency (EPA, 2021). Based on a

technical definition provided by the EPA, the hundreds of registered products enlisted as biopesticides can be classified into three main classes: (i) naturally-occurring biochemicals acting through non-toxic mechanisms; (ii) microbial entomopathogens; and (iii) plant-incorporated protectants from genetically engineered plants (Chandler et al., 2011). The term biopesticide, however, is usually applied to designate those commercial preparations containing living organisms (bacteria, viruses, single-celled eukaryotes, fungi and nematodes) and/or bioactive compounds (such as metabolites) produced directly from living organisms to control and suppress populations of pests (Ghosh, 2000).

Biopesticides have been used worldwide since the end of the 19th century. At present, they represent only a rather narrow niche in the annual global pesticide market (Arthurs and Surendra, 2019) with a value of about US\$ 3 billion worldwide, which corresponds to just 5% of the total crop protection market. Yet, it seems that their industry and market is finally coming of age (Glare et al., 2012). International organizations and institutions encourage their use, which is allowed in organic farming, with respect to more harmful active substances. As a result, after a few decades of relatively slow growth, the biopesticide market has accelerated its pace, growing at a rate of 10–15% per year versus 1–2% for synthetic pesticides (Thakore, 2006; Olson, 2015). Such a continuous rise is consolidating the biopesticides' position in the global market and some multinational companies are starting to include these products in their portfolios to keep up with the growing interest and demand for these products.

The increasing attention towards these environmentally sustainable agents of control is documented also by the growing body of scientific literature on the topic. In fact, while the overall number of papers reporting the word ‘biopesticide’ is rather low if compared to those reporting the term “pesticide” (4262 vs 133,462 on WoS for the period from 1985 to 2021), this number has steadily grown with respect to synthetic pesticides, especially in the last two decades, with hardly 309 papers containing the word biopesticide in WoS from 1985 to 2000 and the tenfold number of 3953 results corresponding to the period from 2001 to 2021 (Fig. 1).

3. Biopesticides: pros and cons

The mode of action of a biopesticide is a crucial component for its efficacy and resulting commercial success. While synthetic pesticides typically consist in a single chemical with a specific action, biopesticides rely on both chemical and biochemical approaches, thereby showing more complex mechanisms of action, which disrupt the biochemical, genetic or structural functions of their targeted pests (Hubbard et al., 2014). A large array of microbial biopesticides have been formulated from bacteria like *Bacillus thuringiensis* (Bt), entomopathogenic fungi (EF), such as *Beauveria bassiana* or *Metarhizium anisopliae*, viruses, single-celled eukaryotes, and entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae. Others have been derived from either microorganisms, as the neurotoxic insecticide Spinosad consisting of spinosyn A and D,

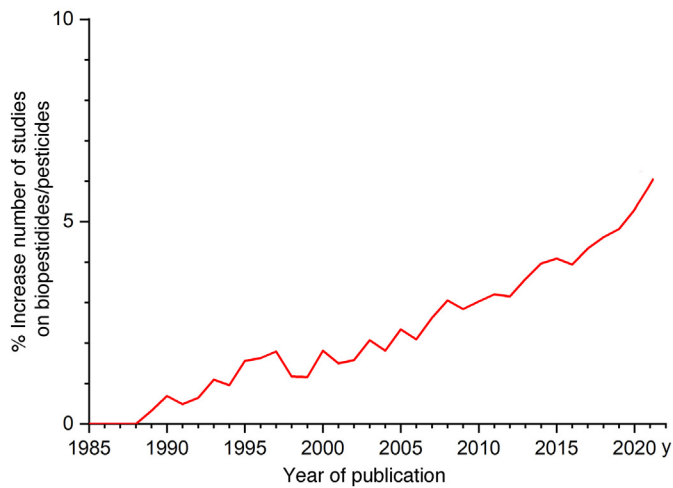


Fig. 1. Percentage increase in the number of studies reporting the word “biopesticide” with respect to those reporting the word “pesticide” from 1985 to 2021 (data obtained from <https://www.webofscience.com>). It is evident how the fraction of studies focusing on biopesticides is continuously growing over the years.

which is derived from the fermentation of the actinomycete *Saccharopolyspora spinosa*, or plants, as the allochemical azadirachtin, which is extracted from the Neem tree, *Azadirachta indica* (Copping and Menn, 2000). EF and EPNs attack their insect targets percutaneously, penetrating the host body where they proliferate, killing the insect host in the process (Shah and Pell, 2003; Lewis et al., 2006). Bacterial agents of control and microbial or plant products, such as Cry toxins, spinosyns or azadirachtin can be highly active after ingestion or through contact, causing rapid death in a wide range of insect pests (Mordue et al., 2010; Biondi et al., 2012; Vachon et al., 2012).

A number of advantages in the use of these biopesticides in agriculture have been demonstrated (Copping and Menn, 2000; Kumar and Singh, 2015) (Table 1). Biopesticides do not appear to have residue problem, which always draws significant concern for consumers, and efficacy can match that of conventional pesticides (Kumar, 2012). Comparative laboratory and field studies have demonstrated that the use of biopesticides may not only be safer for vertebrates and less damaging to non-target arthropods, but also more effective in the long-term control of specific pests (Peveling et al., 1999; Lomer et al., 2001; Arthurs et al., 2003). The mycoinsecticides based on *M. anisopliae*, for example, have been successfully used alongside synthetic organophosphates to control locusts and grasshoppers in Africa, Australia, and China. They showed a similar efficacy as the chemical pesticides, with a $\approx 75\%$ reduction in the pest population, but without threatening non-target epigeal arthropods other than orthopterans (Peveling et al., 1999; Lomer et al., 2001). Sometimes, however, field application of biopesticides can have a profound effect on non-target species, as for the bacterial pathogen Bt, used in the fight against lepidopteran pests in the US, but at the cost of a heavy decline of non-target

Table 1

Advantages and critical issues in the use of biopesticides compared to synthetic pesticides for pest control (in italics bold the advantages).

	Biopesticides	Synthetic pesticides
Toxicity	Low	High
Selectivity	High	Low
Dosage	Low	High
Exposure	Low	High
Speed of action	Slow	Rapid
Persistence	Short	Long
Shelf life	Short	Long
Manufacturing costs	High	Low
Registration costs	High	Low

Lepidoptera (Wagner et al., 1996; Peacock et al., 1998; Miller, 2000). Nonetheless, most of these biological agents of control appear to have only minor or negligible impacts on non-target species, such as pollinators (Goettel et al., 1990; Kumar, 2012). Thus, their safety represents one of the key features that has promoted their use in replacement of synthetic agrochemicals.

Because of the alleged safety towards non-target insect species, especially managed pollinators, honey bees, bumblebees, and mason bees have also been used as direct vectors to deliver biological control agents (BCA) against crop pests (Al Mazra'awi et al., 2006; Menzler-Hokkanen and Hokkanen, 2017; Maebe et al., 2021). The use of pollinating insects to spread BCA has its origin in the early '90s and in the last 15 years “pollinator-and-vector technology” has been systematically developed to improve sustainability, yield and quality in organic crop production (Smaghe et al., 2020). Honey bees, for example, have been used to vector BCA in open field and greenhouses (Kovach et al., 2000; Smaghe et al., 2020). Bumblebees represent the main vectors for dissemination of EF in greenhouses (Kapongo et al., 2008b). Mason bees have proven as efficient vectors for dissemination of BCA in apple and pear orchards (Menzler-Hokkanen and Hokkanen, 2017; Maccagnani et al., 2020).

4. Inadequate risk-assessment protocols

Specific regulations for the risk-assessment of biopesticides are not available so far, and the countries of the Organisation for Economic Co-operation and Development (OECD) entirely rely on the existing protocols for chemical pesticides. Such guidelines include evaluation of ecotoxicity to wildlife and beneficial insects, or rather those essentially classified as bees (i.e., *Apis mellifera* and *Bombus terrestris*) and terrestrial non-target arthropods other than bees (OECD, 2003). According to these protocols, the risk assessment for exposure of these organisms should include dose-response testing providing reliable ecotoxicological endpoints (lethal concentration 50, LC_{50} , lethal dose 50, LD_{50}) through chronic and acute oral/contact toxicity tests (OECD, 1998a, b 2013, 2017a). To measure sublethal effects or behavioral abnormalities, a specimen exposed to a given substance should fall into one of the three following categories: the individual is considered as (i) ‘unaffected’ if it shows ‘inconspicuous behavior (including natural occurring phases of inactivity)’; (ii) ‘affected’ when the exposed individual remains still upright or attempts to walk but displaying signs of reduced coordination, hyperactivity, apathy, cramps, rotations, shivering, increased self-cleaning behavior; and (iii) ‘moribund’ when the exposed individual is ‘unable to walk, shows very feeble movements of legs and antennae and only weak response to stimulation’ (OECD, 2017a, b, c).

The macroscopic motor abnormalities, classified as sublethal effects according to these guidelines, clearly represent only a small fraction of the possible behavioral/cognitive deficits or physiological alterations that may affect exposed specimens. A number of studies have highlighted how the spectrum of sublethal effects caused by exposure to bioactive substances on beneficial insects can in fact be vast (Desneux et al., 2007; Mommaerts and Smaghe, 2011; Siviter et al., 2018; Lu et al., 2020). Unfortunately, all these sublethal effects have been belatedly documented for synthetic agrochemicals, mainly because the abovementioned guidelines testing the safety of synthetic and biological pesticides do not adequately consider sublethals effects. Thus, it is crucial to learn from the experience on synthetic pesticides, and urgently update the existing risk-assessing protocols both for pesticides and biopesticides.

A second, often neglected issue when assessing biopesticides' safety resides in the fact that these substances, as for synthetic chemicals, have been mostly tested on very few commercially relevant non-target model species. Indeed, *A. mellifera* and *B. terrestris* alone serve as models for (bio)pesticide toxicity testing (Franklin and Raine, 2019; Klinger et al., 2019), and regulatory agencies do not require toxicity testing on other pollinators, despite the considerable variability across species (Borges et al., 2021).

Over 20.000 species have been described only for the group of bees, that is as vast as it is diverse in term of life history traits, individual body size, level of sociality and nesting habits (Michener, 2000). These differences

in ecology and life history among wild bee pollinators can influence routes and levels of exposure (Gradish et al., 2019; Chan and Raine, 2020). Therefore, the choice of very few representative species for testing the effects of (bio)pesticide risk assessment protocols appears excessively simplistic and highly inadequate to thoroughly assess the actual impact that these substances can have on wild entomofauna (Stoner, 2016; Franklin and Raine, 2019; Gradish et al., 2019).

Despite the growing body of research on the effects of synthetic pesticides on non-target insects, the attention paid to such relevant subjects with respect to biopesticides is still limited and fragmentary. A very recent review by Borges et al. (2021) has highlighted the challenges to test and assess the effects of microbial pesticides on honey bees and to expand the obtained results and risk assessment protocols to non-*Apis* bees, but without focusing on the array of potential adverse effects on other taxa of pollinators. Thus, in the present review, we provide an overview of the effects of the major classes of biopesticides described so far on pollinating insects (Fig. 2). We point out that a profound attention should be dedicated to testing for sublethal effects on non-target insect species to understand the effects of these agents of control on those behavioral features crucial for pollinators' health and survival.

5. Material and methods

Literature search was carried out using the Web of Science Core Collection (1st week of July 2021). We surveyed the peer-reviewed literature collecting records of biopesticide testing on different pollinating insects. The search terms used were based on three groups: (1) type of biopesticide (for example, enthomopathogenic fungi); (2) pollinator taxa (for example, bee); and (3) impact (for example, sublethal effect). The full search terms used are provided as supplementary materials. The results of our survey are summarized in Figs. 2, 3 as well as in Tables S1, S2, S3, S4, S5 and S6. Tables include summaries of type of biopesticide tested, target species, application mode, concentration, developmental stage, main effect, and reference.

6. Effects of biopesticides in insect pollinators.

6.1. Honey bees

Given that the Western honey bee *A. mellifera* is the most commonly managed pollinator in the world, it represents the most used insect model in the protocols assessing the effects of chemicals (OECD, 1998a, b, 2013, 2017a) and biopesticides (Fig. 2).

The potential impact of EF on honey bees has been tested both on immature and adult stages (Table S1). The enthomopathogenic spores, collected in the field by foragers, or spread directly on their bodies for entomovector dissemination (Menzler-Hokkanen and Hokkanen, 2017), or inside the hive to control parasitic mites (Meikle et al., 2008a), could pose a threat to both the brood and adults. Indeed, several isolates of both *B. bassiana* and *M. anisopliae* can grow at 34–35 °C (Davidson et al., 2003), which is the temperature the bees usually maintain within the nest. Formulations of *B. bassiana* have been tested to evaluate their efficacy against *Varroa* mites infestations in hives (Meikle et al., 2008a; Meikle et al., 2012b), and sporulation after death from bee pupae exposed to mites was observed under laboratory conditions. However, field studies on EF exposure of brood demonstrated that such treatments did not affect overall colony health (Meikle et al., 2008b; Meikle et al., 2012a). Several studies did not find adverse effects of *B. bassiana* and *M. anisopliae* on honey bees (Alves et al., 1996; Butt et al., 1998; Kanga et al., 2003; Al Mazra'awi et al., 2006). Nonetheless, in some cases exposure to *B. bassiana* and *M. anisopliae* has been proven to affect individual survival, development and physiology (Vandenberg, 1990; Butt et al., 1994) (Fig. 2; Table S1). Larval exposure to the fungus can alter AchE activity (Abdel Rasoul et al., 2013) and adult workers treated as pupae showed a significant weight reduction and an increased expression of immune-related genes (Hamiduzzaman et al., 2012). EF have been shown to affect also behavioral traits of honey bees both at the individual and colony level. An alteration in learning performance and sucrose responsiveness of workers exposed to *B. bassiana* conidia was documented: foragers displayed an enhanced sucrose responsiveness and


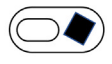
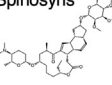
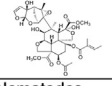

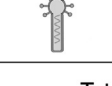
Biopesticide	Effect	Honey bees	Stingless bees	Bumblebees	Solitary bees	Butterflies/moths	Hoverflies	Beetles	Wasps
	negligible	18	2	13	2	1	-	-	-
	lethal	18	2	10	3	3	-	1	-
	sublethal	7	4	4	2	-	-	-	-
	negligible	27	3	4	-	5	-	1	-
	lethal	11	1	3	-	7	-	1	-
	sublethal	7	3	4	-	11	-	-	-
	negligible	-	-	-	-	-	-	-	-
	lethal	7	12	3	4	-	1	1	-
	sublethal	6	9	2	-	-	1	1	-
	negligible	1	1	-	-	-	-	-	-
	lethal	3	2	2	-	-	-	-	1
	sublethal	5	5	1	-	-	-	-	1
	negligible	2	-	-	-	-	-	-	-
	lethal	10	-	1	-	-	-	1	-
	sublethal	1	-	-	-	-	-	-	-
	negligible	6	-	2	3	-	-	-	-
	lethal	-	-	-	-	-	-	-	-
	sublethal	-	-	-	-	-	-	-	-
Total		118*	33*	44*	11*	20*	1*	4*	1*

Fig. 2. Overview of reviewed literature testing the potential effects of different biopesticides on different taxa of pollinating insects. The number of studies where a negligible, lethal or sublethal effect were demonstrated is listed in the table. The scale of grey is linked to the number of studies on the topic reporting a different effect (color code: white, no studies found; light grey, ≤ 5 studies; grey, between 6 and 10 studies; dark grey, ≥ 11 studies). Details on the protocols and results from the different studies are reported in the main text and in Tables S1–S6. *Papers testing multiple biopesticides or reporting multiple effects of the same biopesticide have been considered only once in the total.

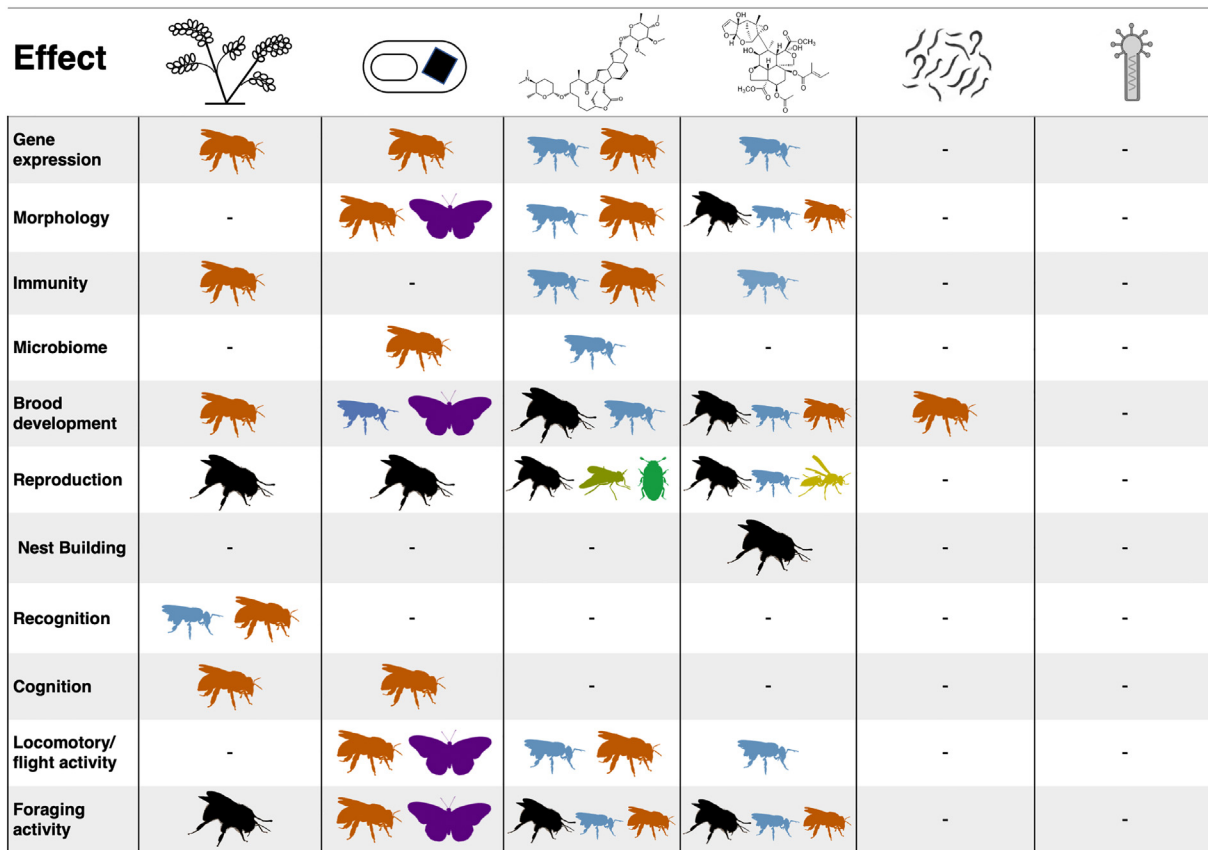


Fig. 3. Overview of the sublethal effects of biopesticides on different taxa of pollinating insects demonstrated in the reviewed studies (Tables S1–S6). The silhouette of pollinating insects representing the different taxa in each intersection between phenotypic trait and class of biopesticide demonstrate a sublethal effect on the peculiar trait in the taxa in exam.

inconsistent response to sucrose when tested with proboscis extension response (PER) (Carlesso et al., 2020). As sucrose responsiveness represents a critical determinant of the division of foraging labour, the recorded changes could unsettle the numerical ratio among sub-castes of foragers leading to suboptimal foraging at the colony level. Another study demonstrated that exposure to *B. bassiana* alters the cuticular hydrocarbons' (CHCs) profile of foragers disrupting the recognition system at the colony level. In fact, field bioassays showed that exposed bees are more easily accepted into foreign colonies by guard bees at the nest entrance, a fact that may favour the drift of foragers among colonies and the consequent spread of parasites and diseases (Cappa et al., 2019). The fungal biofungicide *Trichoderma* does not seem to have adverse effect on both the Western honey bee, *A. mellifera* (Kovach et al., 2000), and the Asian honey bee, *A. cerana* (Qiu et al., 2021), while *Cordyceps fumosorosea*, *Hirsutella* spp. and *Lecanicillium lecanii* proved toxic for bees at different developmental stages (Shaw et al., 2002; Espinosa-Ortiz et al., 2011).

As regards the effects of bacterial biopesticides, a meta-analysis carried out with 25 studies assessing the potential impact of Bt Cry proteins on honey bees showed no significant adverse effects on the development and survival of either larvae or adult workers under laboratory conditions (Duan et al., 2008; Hendriksma et al., 2011; Hendriksma et al., 2012). Although studies of acute toxicity performed under laboratory conditions may overlook sublethal effects that could potentially affect honey bees in the field, the results of this meta-analysis support the conclusion that the Cry proteins, often expressed in genetically modified crops (Mendelsohn et al., 2003), are unlikely to have negative direct effects on the survival of honey bees. Nevertheless, several studies on bacterial biopesticides showed a reduced survival of exposed bees (Vandenberg, 1990; Malone et al., 1999; Bailey et al., 2005; Hassona and Kordy, 2015; Renzi et al., 2016; Potrich et al., 2018) (Fig. 2; Table S2). Moreover, Bt-derived Cry-proteins induce

hypoactivity, enzymatic and morphostructural alterations in the midgut of workers and reduce their foraging activity, feeding behavior and learning performance (Ramirez-Romero et al., 2005; Ramirez-Romero et al., 2008; Renzi et al., 2016; D'urso et al., 2017). Recent studies have also highlighted that Bt can alter the gut microbiome of honey bees (Steinigeweg et al., 2022) while another bacterial biopesticide, *Bacillus amyloliquefaciens*, reduces the expression of genes linked to immunity in adult bees (Sabo et al., 2020).

The bioinsecticide spinosad proved to be highly toxic for honey bees (Peng et al., 2000; Bailey et al., 2005; Miles et al., 2012; Abdel Rasoul et al., 2013; Xavier et al., 2015; Cabrera-Marín et al., 2016; Challa et al., 2019) (Fig. 2; Table S3). In addition, spinosad affects foraging behavior (Cabrera-Marín et al., 2015) and induces transcriptional alterations of metabolic genes in the brain of adult bees at sublethal concentrations (Christen et al., 2019). In fact, exposed workers showed a significant down-regulation of genes encoding enzymes linked to oxidative phosphorylation and metabolism (Christen et al., 2019). Inhibition of acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) activities in different organs of exposed workers was also reported (Rabea et al., 2010). The same biopesticide induced structural changes in the midgut and Malpighian tubules and epithelia disorganization (Lopes et al., 2018). Sublethal concentrations can also perturb the behavioral activity of workers, causing a reduction in walking distance and speed, and an increase in resting time in comparison to control bees (Lopes et al., 2018).

Honey bees exposed to another biopesticide, the Neem tree-derived azadirachtin, manifested adverse effects (Mordue and Blackwell, 1993; Peng et al., 2000; Thompson et al., 2005; Nakasu et al., 2014; Weeks et al., 2018) (Fig. 2; Table S4). More than 90% of prepupae and pupae treated at the larval stage showed ontogenetic abnormalities with precocious and abnormal pigmentation on their mouthparts and other appendages (Peng

et al., 2000). Furthermore, azadirachtin affected colony overwintering, even when it had no apparent effects on the brood development (Thompson et al., 2005). This suggests that azadirachtin could have long-term adverse effects on bees exposed as brood. Azadirachtin interferes also with foraging behavior, by reducing the visitation rate of foragers in treated crop fields (Tschoeke et al., 2019).

As regards nematodes used as biopesticides, there are no records of EPNs naturally infecting bee colonies (Żóltowska et al., 2003). Honey bee workers and brood exposed to different EPN species (*Heterorhabditis baeteriophora*, *Steinernema carpocapsae*, *S. riobravis* and *S. glaseri*) under in-hive conditions showed a very low susceptibility to nematode infection (Kaya et al., 1982; Baur et al., 1995) probably because these EPNs cannot persist and infect bees at the temperatures maintained in the central part of the nest. However, given that under laboratory conditions other Heterorhabditidae and Steinernematidae species and strains were able to infest both larvae and adults (Żóltowska et al., 2003) (Fig. 2; Table S5), honey bees can be vulnerable to EPNs attack.

Honey bees are hosts for more than 20 viruses that typically persist in inapparent infections until activated by a stressor (McMenamin and Genersch, 2015). When potential stressors suppress the individual immunocompetence, virus replication is favoured and increased viral titres seem to be associated with the collapse of honey bee colonies (McMenamin and Genersch, 2015). Despite the fact that RNA viruses ascribed to the families *Dicistroviridae* and *Iridoviridae* are both found in collapsing colonies and potentially used as biopesticides (Bromenshenk et al., 2010; McMenamin and Genersch, 2015), specific studies testing their effects on honey bees are mostly lacking. Studies testing the impact of nuclear polyhedrosis viruses (NPVs), granulosis viruses and entomopoxviruses from different hosts on honey bees found no adverse effects, corroborating their host specificity and relative safety for use in microbial control programs (Cantwell et al., 1966; Knox, 1970; Morton et al., 1975; Heinz et al., 1995; Alves et al., 1996) (Fig. 2; Table S6).

Other novel biopesticides, such as the amino acid methionine or the spider venom peptide Hv1a/GNA, showed no adverse or detrimental effects on the survival of honey bees and their application appears safe (Nakasu et al., 2014; Weeks et al., 2018).

6.2. Stingless bees

Stingless bees are perennial highly eusocial bees, composed by more than 520 species with a tropical distribution (Michener, 2000). Despite their ecological importance and economic potential in the tropics (Grüter, 2020), only recently research has focused on the impact of pesticides and biopesticides on these pollinators (Tomé et al., 2012; Barbosa et al., 2015a).

Only few studies have tested the effects of EF on several species of stingless bees so far (Fig. 2; Table S1). In a first study, *B. bassiana* proved toxic to *Melipona scutellaris* workers (de Jesus Conceição et al., 2014). Another study (Toledo-Hernández et al., 2016) investigated the pathogenicity of commercial formulations of *B. bassiana*, *M. anisopliae*, and *C. fumosorosea*, to three economically important species of stingless bees, *Tetragonisca angustula*, *Scaptotrigona mexicana*, and *Melipona beecheii*. In all cases, individual workers were exposed to the recommended field concentrations and mortality was determined. Results showed a species-specific susceptibility to the three fungal biopesticides with *B. bassiana* and *C. fumosorosea* being less toxic (mortality rate lower than ≈30% in all bee species), while *M. anisopliae* being highly active especially against *T. angustula* (94.2% mortality). *B. bassiana* and *M. anisopliae* did not show significant detrimental effects on another stingless bee, *Meliponula ferruginea* (Omuse et al., 2022). Only one recent study has investigated the effects of EF on the recognition system of stingless bees demonstrating that guards of *T. angustula* at the colony entrance detect and repel nest-mates exposed to *B. bassiana*, treating them as foreigners and preventing their entry into the colony (Almeida et al., 2022).

As regards bacterial biopesticides, the potential adverse effects of Cry Bt-toxins on *Trigona spinipes* and *Melipona quadrifasciata* were assessed after ingestion of a contaminated diet during the larval stage. Exposure

did not cause lethal effects on the two species, even at higher doses than those commonly encountered in the field (Lima et al., 2013). Similarly, larval exposure did not affect locomotory behavior at adult stage in *M. quadrifasciata* (Seide et al., 2018). However, a delayed development was observed in exposed larvae. Exposure to spores of another bacterial biopesticide, *Lysinibacillus sphaericus* altered the behavior and foraging activity of workers of *Tetragonula carbonaria* (Shanks et al., 2017).

In recent years, a certain attention has been devoted to the impact of spinosyns on several species of stingless bees. Spinosad and spinoteram (another fermentation product of *Saccharopolyspora spinosa*) can prove highly toxic to these bees. In particular, a considerable reduction in adult survival after exposure to spinosyns was documented for *M. quadrifasciata* (Barbosa et al., 2015b; Piovesan et al., 2020), *Partamona helleri* (Tomé et al., 2015; dos Santos Araujo et al., 2019a), *Plebeia emerina* (Padilha et al., 2019), *Plebeia lucii* (Marques et al., 2020), *Scaptotrigona xanthotricha* (Tomé et al., 2015), and *Tetragonisca fiebrigi* (Padilha et al., 2020; Piovesan et al., 2020). In addition to the lethal effect, a plethora of sublethal effects on different developmental stages were demonstrated in several studies (Figs. 2, 3; Table S3). Exposure at the larval stage caused a delay in development and induced microbial and morphological changes in the midgut of *P. helleri* workers as well as alterations in their activity (dos Santos Araujo et al., 2019b). Spinosad exposure produced deformed pupae and impaired locomotor and flight ability in emerged adults of *M. quadrifasciata* (Barbosa et al., 2015b; Piovesan et al., 2020). Exposure of *M. quadrifasciata* workers also caused a decrease in the expression of the immune-related gene vitellogenin and an increase in the number of circulating hemocytes (Viana et al., 2020). In *P. lucii*, walking and flight were reduced without alterations of individual body mass and respiration rate (Marques et al., 2020). Finally, spinosad-based products affect foraging behavior at the individual and colony level, with a clear antifeedant effect on *Tetragonisca fiebrigi*, *Trigona fulviventris* and *S. mexicana* foragers and a reduction in colony strength of *S. mexicana* after exposure in the field (Gómez-Escobar et al., 2018). Conversely, the biopesticide did not discourage engaged foragers nor impaired collective foraging behavior over time in *Plebeia moureana* (Sánchez et al., 2012).

Several recent papers assessed the impact of azadirachtin in a few species of stingless bees. Exposure at the larval stage caused a dose-dependent increase in mortality of *M. quadrifasciata* larvae (Barbosa et al., 2015b). Sublethal effects were also observed in terms of reduced pupal body mass and deformities at the pupal stage that resulted in deformed adults after molting (Barbosa et al., 2015b). Azadirachtin decreased the expression of the immune-related gene vitellogenin, while increasing the number of circulating hemocytes in exposed workers of the same species (Viana et al., 2020). Azadirachtin was less toxic on *P. helleri* and *S. xanthotricha*, exhibiting negligible mortality on adult workers after exposure at the field-recommended concentrations (Tomé et al., 2015). Assessment of sublethal effects indicated that the bioinsecticide did not affect overall group activity of workers, but impaired individual flight take-off in the two species, potentially compromising foraging activity and colony integrity (Tomé et al., 2015). *P. helleri* exhibited a concentration-dependent azadirachtin feeding avoidance, with a subsequent reduced individual food consumption, which may impair the worker ability to forage and weaken the colonies (Bernardes et al., 2017). Finally, azadirachtin could also affect the colony fitness of *P. helleri* through its impact on the reproductive caste (Bernardes et al., 2018). The study demonstrated that queens' larvae exposure affected individual survival, delayed development time, caused deformations, and reduced the size of reproductive organs.

To date, the impact of EPNs and viral biopesticides has not been tested on stingless bees.

6.3. Bumblebees

Bumblebees are a well-known group of primitively eusocial insects, comprising approximately 280 species (Michener, 2000). Most *Bombus* species are distributed mainly in cold-temperate regions and are characterized by an annual colony cycle (Goulson, 2010). Despite the decline of many

wild bumblebee species in Europe and North America (Kosior et al., 2007; Cameron et al., 2011; Ghisbain et al., 2020; Rollin et al., 2020; Martinet et al., 2021), other bumblebee species are widely managed for crop pollination (Velthuis and van Doorn, 2006) and their susceptibility to synthetic pesticides has been intensively investigated (Osborne, 2012; Raine, 2018). A certain attention, although to a much lesser extent than synthetic substances, has been devoted also to the effects that biopesticides can have on these social bees (Mommaerts and Smaghe, 2011).

The toxicity of EF has been investigated on different bumblebee species (Fig. 2; Table S1). Most of these fungal pathogens (*Ampelomyces quisqualis*, *Clonostachys rosea*, *Gliocladium catenulatum*, *Hypocrea parasilulifera*, *C. fumosorosea*, *L. lecanii*, *Trichoderma atroviride*, *T. harzianum*) showed negligible effects (Kovach et al., 2000; Sterk et al., 2003; Van der Steen et al., 2003; Mommaerts et al., 2007, 2008, 2009; Reeh et al., 2014; Karise and Raimets, 2017; Akkoç et al., 2019; Demirozer et al., 2022) or slight toxicity (Mommaerts et al., 2012; Karise et al., 2016). The widely used *M. anisopliae* instead has been tested on workers and queens of the wild species *B. lucorum* and *B. lapidarius*, and the commercially available *B. terrestris* under laboratory conditions (Hokkanen et al., 2003). Exposure to the fungus caused an increase in mortality of both workers and queens. In exposed nests of *B. terrestris* no acute toxicity was observed in workers nor reduced sexuals' production was recorded. However, high conidia concentrations (comparable to those used in the field) provoked a severe worker mortality, a reduced sexuals' production, and a total colony loss within few weeks (Smaghe et al., 2013) (Table S1).

The impact of the other common EF, *B. bassiana*, has been largely tested on *B. impatiens* and *B. terrestris*. In greenhouses, inoculum dispensers releasing *B. bassiana* conidia were applied to the nest entrance of *B. impatiens* colonies used for crop pollination (Al Mazra'awi et al., 2006; Kapongo et al., 2008a). One study (Al Mazra'awi et al., 2006) reported no adverse impact of the biopesticide vectored by foragers on colony health, brood production, and bee activity. Conversely, in another study (Kapongo et al., 2008a), a significantly higher bee mortality was observed in exposed nests. Another experiment demonstrated that *B. terrestris* foragers sprayed with *B. bassiana* spread the infection to their nestmates upon returning to their nests (Hokkanen et al., 2003).

Toxicity and sublethal effects of the fungus at the individual and colony level were evaluated also in laboratory experiments on *B. terrestris* and *B. impatiens*. A noticeable reduction in individual worker lifespan (Karise et al., 2016), increase in mortality and colony infection rate (Hokkanen et al., 2003), reduction of foraging activity and production of sexuals (Mommaerts et al., 2009) were recorded after topical exposure to *B. bassiana* in dosages occurring when bumblebees are used in vectoring biopesticides. On the contrary, oral administration of *B. bassiana* did not affect worker survival nor other colony traits (Mommaerts et al., 2009). A similar experiment carried out on *B. impatiens* showed no adverse effect on worker survival, egg-laying, male production, or larval ejection rate. It is important to note, however, that laboratory studies have been carried out under conditions more similar to those of commercial bumblebee rearing, while underground feral colonies could offer more conducive temperature and humidity conditions for these fungal agents of control. Thus, the apparent safety of EF cannot be directly extended to all species of bumblebees and further experimentation investigating sublethal effects both in the laboratory and in the field is required. The fungal biopesticides based on *Trichoderma* spp. did not show instead adverse effects on *B. terrestris* (Mommaerts et al., 2008).

Bacterial biopesticides based on Bt, *Bacillus amyloqueliefaciens* (formerly *B. subtilis*) and their toxins are widely used in the biocontrol of insect and fungal pests, but, once more, relatively few studies have assessed their risk for both wild and managed bumblebees (Morandin and Winston, 2003; Mommaerts et al., 2009; Mommaerts and Smaghe, 2011; Ramanaidu and Cutler, 2013). These studies were carried out under laboratory conditions using micro-colonies of *B. terrestris*, *B. impatiens*, and *B. occidentalis* to evaluate worker mortality and sublethals effects on colony foraging ability, health, and reproduction. Results are contradictory, and the impact seems to vary mostly depending on the insect species and the

biopesticide administration modality (Table S2). Topical exposure to *B. amyloqueliefaciens* was not detrimental for worker survival nor for male production in *B. impatiens*, while oral administration adversely affected reproduction with slower oviposition and reduced sexuals' production (Ramanaidu and Cutler, 2013). Exposure to *B. amyloqueliefaciens* did not show acute toxicity in *B. terrestris* but resulted in high mortality and reduced colony reproduction after several weeks (Mommaerts et al., 2009). In colonies of *B. terrestris* exposed topically or via treated pollen to the two strains Bt *kurstaki* and Bt *aizawai*, no reduction in worker survival nor impairment of foraging behavior were recorded (Mommaerts et al., 2009) (Table S2). However, when administered through a feeding sugar solution, *B. t. aizawai* caused a 100% mortality and colony reproduction loss. Effects of Cry proteins (Cry1Ab and Cry1Ac) derived from Bt were also investigated in *B. impatiens*, *B. occidentalis*, and *B. terrestris*, but no lethal or sublethal effects were recorded on these species (Morandin and Winston, 2003; Babendreier et al., 2008). Bumblebee worker weights, pollen consumption, colony size, amount of brood, and the number of sexuals produced resulted all unaffected by the exposure to these biopesticides (Babendreier et al., 2008).

Further research focused on the impact of spinosyns (i.e., spinosad and spinetoram) on *Bombus* species. For contact toxicity, spinosad has been classified as highly toxic and, after oral exposure, both spinosad and spinetoram resulted toxic for bumblebees (Morandin et al., 2005; Besard et al., 2011). The impact of spinosad on colony health, including adult mortality, brood development, weight of emerging bees, and individual foraging efficiency, was monitored in colonies of *B. impatiens* (Morandin et al., 2005). While at unrealistically high dose the biopesticide heavily impacted colony traits leading to colony losses, acute and chronic exposure at more realistic field rates did not. Nevertheless, sublethal effects were observed in terms of impaired foraging in workers exposed during larval development. Other studies found that acute contact exposure to spinosad was moderately toxic for *B. impatiens* (Scott-Dupree et al., 2009), while acute or chronic oral administration of spinosad and spinetoram caused tremors, paralysis and worker mortality in *B. terrestris* (Besard et al., 2011).

The lethal and sublethal effects of azadirachtin were studied on *B. terrestris* in the laboratory (Fig. 2; Table S4). Microcolonies exposed to azadirachtin exhibited an increased mortality. Besides the lethal effect, azadirachtin drastically invalidated colony reproduction by inhibiting oviposition, cell building, and sexuals' production. Moreover, worker ovarian development and body mass of male offspring decreased with the increase of azadirachtin concentration, and exposure caused deformities and reduced lifespan in adult males. Finally, the bioinsecticide affected also individual foraging, evoking repellence at high concentrations upon foragers (Barbosa et al., 2015a) (Figs. 2, 3; Table S4).

EPNs might have adverse effects on bumblebees, but research testing the potential impact of EPNs on these pollinators is scant. *Heterorhabditis* sp. and *Steinernema* sp. caused a high mortality after *B. terrestris* workers were exposed to soil containing EPNs at the recommended field concentration (Dutka et al., 2015) (Fig. 2; Table S5). The results appear of particular concern since *Bombus* species are known to hibernate under rotten tree stumps, piles, leaf litter, and soil which might be a route of infection. Moreover, EPNs from the broad spectrum of commercial products could proliferate in the carcasses of infected dead bees, potentially infecting whole colonies.

As regards the effects of viruses used as biocontrol agents on bumblebees, research on the topic is still scarce. However, granuloviruses from the original host *Cydia pomonella* do not seem to have detrimental effects on *B. terrestris* worker survival, foraging behavior, and colony fitness (Mommaerts et al., 2009) (Fig. 2; Table S6).

6.4. Solitary bees

Wild solitary bees represent a widely heterogeneous group of insects belonging to the superfamily Apoidea (Michener, 2000). As already pointed out, they can largely vary in their morphology, body size, phenology, habitat, foraging flight distances, nutritional requirements, and nesting habits, but they invariably provide crucial ecosystemic services through

pollination in both natural landscapes and managed crops (Duchenne et al., 2020; Barraud et al., 2022; Hamroud et al., 2022). Although the conservation status of many of these species remains unknown, a growing number of them is facing a progressive decline over the years (Biesmeijer et al., 2006; Sánchez-Bayo and Wyckhuys, 2019). As for synthetic chemicals (Potts et al., 2010; Sgolastra et al., 2019), research evaluating the effects of biopesticides on solitary bees has been extremely scarce so far. The very little attention paid to the matter appears almost surprising, given that several solitary bee species, such as *Megachile rotundata*, *Osmia cornuta* and *Amegilla* spp., are commercially important managed pollinators (Pitts-Singer and Cane, 2011).

A comparative study demonstrated that the alfalfa leafcutting bee, *M. rotundata*, was more susceptible to *B. bassiana* with respect to honey bees under laboratory conditions, showing a more rapid lethal effect and twice the mortality than honey bees (James et al., 2012). The presence of *B. bassiana* was also documented in the gut of *M. rotundata* larvae collected in the field (McFrederick et al., 2014), although oral consumption of spore-contaminated food did not show obvious detrimental effects on the larval development under laboratory conditions (Goerzen et al., 1990). However, the same study, reported a high mortality in *M. rotundata* developing pupae exposed during the prepupal stage (Goerzen et al., 1990). Another entomopathogenic fungus, *Aspergillus parasiticus*, tested in the same study, showed no detrimental effects.

The toxicity of other two bioinsecticides, spinosad and spinoteram, was tested on larvae and adults of *M. rotundata* (Gradish et al., 2012). Spinetoram proved toxic for adult and immature stages. Treated larvae died before pupation or, occasionally, pupated without spinning a cocoon (Gradish et al., 2012). Other comparative studies investigated the direct contact toxicity of spinosad on adults of *M. rotundata*, *Osmia lignaria*, and *Nomia melanderi*, with respect to honey bees and bumblebees (Mayer et al., 2001; Mayes et al., 2003; Scott-Dupree et al., 2009). All studies invariably demonstrated the higher susceptibility of solitary bees to spinosad with bumblebees and honey bees being more tolerant than *O. lignaria* and *M. rotundata*.

Soil-dwelling bees can be infected by different species of nematodes in the wild (Hazir et al., 2010; McFrederick and Taylor, 2013); however, the possible lethal or sublethal effects of EPNs, such as *Steinernema* spp. or *Heterorhabditis* spp., on solitary bees has not been evaluated so far.

Similarly, also the potential impact of viral biopesticides in terms of direct toxicity or sublethal effects on solitary bees still represents a largely uninvestigated field. The only studies carried out on *M. rotundata* did not demonstrate significant detrimental effects (Goerzen et al., 1990; Barber et al., 1993; Kaupp et al., 2011).

As stressed above, despite the large number of solitary bees and their important ecological role, only a handful of commercially relevant species have been included in toxicological studies assessing the risk of biopesticide exposure. All these studies focused exclusively on the acute toxicity and did not investigate any sublethal effect on the tested species. Furthermore, in addition to the high sensitivity of the few tested species to bioinsecticides, many solitary bees often nest directly into crop fields, a fact that increases their chance of exposure to crop-protection products.

6.5. Butterflies and moths

Due to the elevated degree of host-plant specialization of many butterfly and moth species and their high vulnerability to habitat deterioration, the presence of Lepidoptera often represents a critical indicator of environmental quality (Erhardt and Thomas, 1991). Unfortunately, massive decline of some species and dramatic changes in butterfly and moth biodiversity from different geographic areas have been reported in the last decades (Sánchez-Bayo and Wyckhuys, 2019) and the steepest declines occur in those environments with a massive use of insecticides (Fox, 2013; Gilburn et al., 2015). Lepidoptera are particularly susceptible to both synthetic insecticides and biopesticides, which are often specifically designed to target them since many species represent destructive pests of commercially important crops (Sree and Varma, 2015). As a result, vast is the literature on

the effects of biopesticides on Lepidoptera, but most of it has focused on lepidopteran pests instead of ecologically relevant species. The studies that have considered the effects of biopesticides on non-target species of pollinating Lepidoptera have demonstrated that field application of these agents of control can dramatically impact them (Wagner et al., 1996; Peacock et al., 1998; Miller, 2000; Marvier et al., 2007).

EF have been widely investigated for control of virtually all groups of pestiferous arthropods, including Lepidoptera (Goettel and Hajek, 2001; Wraight et al., 2010). Their safety towards non-target lepidopterans has been stated without focusing attention of non-target pollinators apart from recording the non-target species abundance in treated versus untreated plots (Brinkman and Fuller, 1999). Fungal biopesticides can, in fact, represent a scourge for lepidopterans: in the case of commercially relevant species, fungal diseases have plagued sericulture, as many strains of these control agents can cause epizootics in the silkworm, *Bombyx mori* (Goettel et al., 1990).

The bacterial pathogen Bt, either directly delivered in the field or in the form of genetically modified Bt-crops expressing the toxic Cry-proteins, has been largely used in the US to fight lepidopteran pests, but at the cost of wiping up non-target lepidopterans in areas where the biopesticide has been applied (Wagner et al., 1996; Peacock et al., 1998; Miller, 2000; Marvier et al., 2007). A number of laboratory and field studies have investigated the potential effects of transgenic Bt crops on non-target Lepidoptera (Wraight et al., 2000; Hellmich et al., 2001; Sears et al., 2001; Stanley-Horn et al., 2001; Zangerl et al., 2001; Wolt et al., 2003; Anderson et al., 2004; Dively et al., 2004; Anderson et al., 2005; Mattila et al., 2005; Shirai and Takahashi, 2005; Wolt et al., 2005; Lang and Vojtech, 2006; Müller et al., 2012; Schuppener et al., 2012; Holst et al., 2013). These studies have evaluated effects of insecticidal Cry-proteins produced by these crops on survival, food consumption, larval, pupal and adult body mass, development time and behavior of different butterfly species. Overall, the adverse effects of Bt crops on non-target lepidopterans have been studied only in a dozen of species worldwide. Thus, due to restriction of data to such a limited number of species, it is difficult to draw general conclusions on the level of risk for butterflies and moths (Lang and Otto, 2010; Lang et al., 2019; Lang et al., 2020).

As Bt-derived Cry-proteins, also neurotoxic spinosins have a high level of activity and efficacy towards lepidopteran larvae (Sparks et al., 1998; Sparks et al., 2001). Yet, once more, the potentially adverse side effects on lepidopteran pollinators have been somehow neglected. Even an extensive review (Biondi et al., 2012) on the impact of spinosins on beneficial arthropods does not mention any lepidopteran species when reporting the cases of documented lethal and sublethal effects on pollinators.

Both EPNs and viral biopesticides can be very effective in the control of lepidopteran pests (Glazer et al., 1992; Moscardi, 1999), but they are regarded as safe for the environment, having little detrimental effects on non-target insect populations (Bathon, 1996; Ehlers and Hokkanen, 1996; Ehlers, 2001). However, despite their worldwide use as control agents of lepidopteran pests, specific studies testing their effects on non-target pollinating butterflies and moths are still lacking.

6.6. Hoverflies, beetles, and wasps

The attention towards the different global pollination communities has increased in recent years in light of the documented decline in bees and lepidopteran populations (Goulson et al., 2015; Sánchez-Bayo and Wyckhuys, 2019). Recent studies have revealed that also other insects, mainly some groups of flies and beetles, can provide important contributions to pollination networks and to the increase and maintenance of floral diversification (Bernhardt, 2000; Rader et al., 2016; Doyle et al., 2020; Rader et al., 2020; Raguso, 2020).

As regards the role of flies as pollinators, they are often recognized as generalized pollinators in alpine habitats and as specialized pollinators in brood-site deceptive plants mimicking fungi, feces, or carrion (Lefebvre et al., 2018; McCabe et al., 2019). Syrphid flies in particular visit many of the flowers pollinated by bees and butterflies, but their functional place in pollinator communities is still understudied (Rader et al., 2016). At the

adult stage, Syrphids feed almost exclusively on pollen, nectar, or honeydew, and this explains their contribution to pollination. Larvae of certain species are insectivorous, and they eat aphids, thrips, and other plant-sucking insects, being regarded as effective agents of biological control (van Rijn and Wäckers, 2016; Wotton et al., 2019; Rodríguez-Gasol et al., 2020). This peculiar lifestyle potentially brings both adult and immature stages in contact with commercial crops (van Rijn and Wäckers, 2016), thus exposing them to the risk of becoming collateral targets of synthetic agrochemicals and biopesticides (Bugg et al., 2008). However, despite their double beneficial role as pollinators and agents of biocontrol and their likely presence in managed crops (Rodríguez-Gasol et al., 2020), relatively little attention has been devoted on the adverse consequences that biopesticides application can produce on them. Most of the available literature focused on the impact that bioinsecticides can have on population dynamics and community characteristics of arthropod natural enemies (Williams et al., 2003; Arthurs et al., 2007; Jansen et al., 2014; Tian et al., 2015; Guo et al., 2016). The results of these studies are sometimes controversial. Some showed negligible adverse effects on non-target populations (Williams et al., 2003; Guo et al., 2016) or even a positive effect (Tian et al., 2015). Other studies have demonstrated that microbial and botanical insecticides, EF or EPNs, and transgenic Bt-crops can negatively affect non-target beneficial insects, such as hoverflies or parasitoid wasps, with significant reductions in their populations (Arthurs et al., 2007; Biondi et al., 2013; Jansen et al., 2014). However, since these studies were carried out mostly at the community level, it is not possible to extrapolate more detailed information about potential sublethal effects on pollinating Diptera. One exception is represented by the work of Moens and colleagues (Moens et al., 2011), which investigated the side effects of spinosad, alongside other synthetic insecticides, on the larvae of the hoverfly *Episyrphus balteatus*. This study evaluated both direct mortality of exposed larvae and sublethal effects of exposure by assessing the reproductive performance of adult hoverflies, originating from the surviving larvae. Spinosad proved highly toxic, killing 60% of treated larvae and the adults obtained from the surviving larvae did not succeed in laying eggs (Moens et al., 2011).

As regards the effects of biopesticides on coleopteran pollinators, the framework is somehow similar to what reported for lepidopteran and dipteran species. Several studies have begun to evaluate the effects of microbial biopesticides on beetle species that can act as both pest and pollinators (Glazer et al., 1999; Niu et al., 2018; Cottrell et al., 2019a, 2019b; Boston et al., 2020), but the attention is mainly focused on the lethal effects. Nonetheless, different families of Coleoptera are known to pollinate flowering plants. Adults can be commonly found on flowers, particularly in tropical ecosystems, feeding on both nectar and pollen (Sayers et al., 2019). Larvae can live on crop plants or among their root systems underground, preying on crop pests (Dixon and Dixon, 2000; Obyrcki et al., 2009). Thus, it would be important to also include these taxa when assessing the risks of biopesticides use on pollinators, especially considering that some of them have established highly specialized relationships with the plants that they visit (Armstrong and Irvine, 1989; Bernhardt, 2000; Aliscioni et al., 2017; Sayers et al., 2019).

In a recent review (Brock et al., 2021), it has been highlighted that also aculeate wasps both solitary and social can play an active role as pollinators, apart from their function as natural agents of control of other insect pests (Brock et al., 2021), and research on the potential impact of biopesticides has started to focus also on these species. In fact, it has very recently been demonstrated that chronic exposure to azadirachtin affects both individuals and colonies of the tropical paper wasp *Polistes versicolor*, increasing individual and colony mortality and impairing wasp reproduction (Teixeira et al., 2022). Given the important ecosystem services provided by these taxa, it would be essential to pay attention to the adverse effects that biopesticides could have on these organisms.

7. Conclusions and future directions

Biopesticide research has focused mainly on identifying more effective isolates and improving formulations and manufacturing technologies to

reduce costs and enhance products' shelf life and safety for human health. On the contrary, far less effort has been devoted to evaluating the actual safety and potential side effects of these substances on wild and managed pollinators.

Our review of the available literature has shown that different classes of biopesticides, besides their direct toxicity, can also cause a plethora of sublethal effects on pollinators (Fig. 3; Tables S1–S6). These effects can span from developmental, morphological, physiological, and behavioral alterations at the individual level, to disruption of social organization, division of labour and colony integrity in species with social lifestyle. Furthermore, the protocols adopted to assess the sublethal effects of biopesticides on pollinating insects do not follow precise guidelines and are often different, even when testing the same agent of control in the same species (Tables S1–S6). As a consequence, it becomes difficult to draw general conclusions and the resulting knowledge is still lacking and fragmentary.

Our in-depth revision work highlights several critical points on the current knowledge to achieve a more productive and sustainable agriculture using biopesticides that we sum up and discuss below:

- 1) Strikingly, most of these detrimental effects, have been investigated in honey bees and bumblebees, and, more recently, but to a lesser extent, in tropical stingless bees. Yet the scientific literature on the topic for other ecologically relevant groups, such as solitary bees, as well as lepidopterans, coleopterans, dipterans, and wasps is very scant. This lack of knowledge raises concerns because many of these wild species are already declining due to multiple causes. Unfortunately, it appears clear that most classes of biopesticides can have species-specific deleterious effects, making generalization across taxa a dangerous game. The recent review by Borges et al. (2021) has already pointed out that in the case of different microbial biopesticides, infectiveness and pathogenicity should be assessed in different life stages. We add that the same should be done in different taxa other than bees, since biopesticides can have multiple adverse effects on them (Figs. 2, 3). The same authors underline the issue of dosage and exposure in honey bees with respect to non-*Apis* bees, highlighting that both doses and time of exposure should be lowered to reveal more subtle effects that may impact bee health.
- 2) Another point that has recently draw considerable attention is the synergistic interaction among different agrochemicals that can amplify the deleterious effects towards non-target pollinators (Siviter et al., 2021a). However, the focus is, once more, represented by the synergistic effects of synthetic pesticides on honey bees and, to a lesser extent, non-*Apis* bees (Siviter et al., 2021a, 2021b). Thus, it appears compelling that in the future, the potential synergistic effects between biopesticides and between synthetic pesticides and biopesticides on pollinating insects should be more thoroughly evaluated both in the laboratory and in the field.
- 3) As regards the range of sublethal effects, apart from those already demonstrated, there are other aspects that would deserve attention and investigation. Concerning reproduction, which is a feature of uttermost importance for the conservation of declining pollinators, the literature has mainly evaluated visible morphological alterations in the reproductive apparatus or the production of individuals (Ramanaidu and Cutler, 2013; Barbosa et al., 2015a; Bernardes et al., 2018; Cottrell et al., 2019b; Fig. 3), while their reproductive behavior has largely been neglected. As for other behavioral traits, also the mating behavior (e.g., courtship displays, male attempts to copulate with receptive females, female willingness to mate, etc.) is likely to be affected by biopesticides. Future studies should therefore investigate their possible impact on these traits as well as on the reproductive physiology of an individual (e.g., number and viability of sperms, ovary development, egg fertility, oviposition rate, etc.).

Since the survival and success of many insect species is often mediated by symbiosis with microorganisms and most of the biopesticides are microbial agents or their products, the potential alteration of the microbiome induced by biopesticides that could affect the health of the individuals and colonies should be more thoroughly studied. The

colony integrity of social pollinators may, indeed, be jeopardized by those subtle detrimental effects undermining the cognitive abilities involved in collective behavior such as communication, recognition, and optimal foraging. Exposure to biopesticides has been demonstrated to affect these traits (Morandin et al., 2005; Mommaerts et al., 2009; Barbosa et al., 2015a; Cabrera-Marín et al., 2015; Bernardes et al., 2017; Cappa et al., 2019; Padilha et al., 2019; Carlesso et al., 2020; Almeida et al., 2022; Fig. 3; Tables S1–S6) but further research on how these products can interfere with the complex network of interactions among colony members is needed to understand to what extent subtle adverse effects on the exposed individuals can turn into a disruption of the social organization.

Overall, we strongly maintain that it would be highly desirable, if not utterly necessary, that different categories of sublethal effects, particularly those linked to fitness traits (i.e., reproductive capacity, foraging ability) and those impairing colony integrity in social species, should be investigated to fill the important knowledge gaps on potential sublethal effects of relevant biopesticides. If additional important sublethal effects caused by exposure to bioactive substances on different taxa of beneficial insects will be demonstrated, increased attention may be posed by policy-makers, industry, and stakeholders to update the existing risk-assessing protocols for testing the safety of these products. New risk-assessing protocols should therefore be considered in policy decisions and in good practice packages aimed at improving pollinators' health and conservation.

It is important to underline that the present review does not intend to condemn the use of biopesticides, as their application is undoubtedly highly preferable and more ecofriendly with respect to synthetic chemicals. Our aim, instead, is to increase awareness about the inadequacy of unique representative models in risk-assessment protocols and the urgent need for the development of comparative protocols testing multiple pollinator species, from solitary to social, to assess all the potential adverse side-effects that biopesticides could have on insect pollinators (Borges et al., 2021). Only through the careful evaluation of the lethal action and sublethal effects of biopesticides, and their potential synergistic interaction on a larger array of functional groups of pollinating insects, we will be able to successfully preserve the fragile ecological balance and the relationships among plants and pollinators (Klein et al., 2007).

The same commitment, attention and government support must be applied not only for the development of high-quality products, easier registration process, and mainstream acceptance of these substances in sustainable crop production, but also for research to assess the effects of these substances. More effort is necessary to promote productive collaborations among researchers, industry, policy-makers, stakeholders and farmers for the effective development and utilization of safer products, not simply for the benefit of mankind, but also for the environment, through the preservation of pollinating insects and the essential ecological and economic services that they provide.

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CRediT authorship contribution statement

D.B. and F.C. conceived the study. F.C. carried out literature search and wrote the first draft of the manuscript. D.B. and F.C. and R.C. contributed to the writing of final version of the manuscript.

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.

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