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Impact of microorganisms and entomopathogenic nematodes used for plant protection on solitary and social bee pollinators: Host range, specificity, pathogenicity, toxicity, and effects of experimental parameters☆

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ABSTRACT

Pollinating bees are stressed by highly variable environmental conditions, malnutrition, parasites and pathogens, but may also by getting in contact with microorganisms or entomopathogenic nematodes that are used to control plant pests and diseases. While foraging for water, food, or nest material social as well as solitary bees have direct contact or even consume the plant protection product with its active substance (e.g., viruses, bacteria, fungi, etc.). Here, we summarize the results of cage, microcolony, observation hive assays, semi-field and field studies using full-size queen-right colonies. By now, some species and subspecies of the Western and Eastern honey bee (*Apis mellifera, A. cerana*), few species of bumble bees, very few stingless bee species and only a single species of leafcutter bees have been studied as non-target host organisms. Survival and reproduction are the major criteria that have been evaluated. Especially sublethal effects on the bees' physiology, immune response and metabolisms will be targets of future investigations. By studying infectivity and pathogenic mechanisms, individual strains of the microorganism and impact on different bee species are future challenges, especially under field conditions. Overall, it became evident that honey bees, bumble bees and few stingless bee species may not be suitable surrogate species to make general conclusions for biological mechanisms of bee-microorganism interactions of other social bee species. Solitary bees have been studied on leafcutter bees (*Megachile rotundata*) only, which shows that this huge group of bees (~20,000 species worldwide) is right at the beginning to get an insight into the interaction of wild pollinators and microbial plant protection organisms.

1. Introduction

The natural interaction between animals and plants as host organisms and their parasites or pathogens resulted in an evolutionary arms race of both, where both counterparts developed specific mechanisms to surpass each other. However, economically relevant plants used by humans in agriculture are more prone to pest organisms and have reduced possibilities to evolve naturally stable resistance mechanisms. Specific breeding and crossing systems help to improve the genetic constitution of crops, mostly being time-consuming and expensive. Current agricultural production (e.g., vegetable, fruit, and cereal) relies predominantly on large-scale application of chemical (synthetic) and non-chemical plant protection products to ensure farming efficiency and productivity. Apart from societal changes in terms of acceptance of chemical plant protection, in recent decades plant pathogens or pests have developed several resistance mechanisms against chemical pesticides, making them almost inefficient and impractical in agriculture. Newly developed pest and disease control products of both groups (chemical and non-chemical) must be evaluated for biosafety and environmental impact, traceability and fate in the environment and food chain, the use of mixtures, as well as the industrial production and development of delivery systems (Bonaterra et al., 2012).

Recently, several public reports showed a growing interest in developing biological control products to replace chemical products in more sustainable production systems (van Lenteren et al., 2018). Non-chemical-synthetic plant protection products (also known as biocontrol agents or biopesticides) are used to control plant diseases and pests (e.g., fire blight, soil-borne fungal diseases, and postharvest fruit

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fungal rot; Bonaterra et al., 2012; van Lenteren et al., 2018). They include microbial control agents based on microorganisms, natural substances derived from plants, and semiochemicals (pheromones) (Chandler et al., 2011; van Lenteren et al., 2018). Usually, bacteria, fungi (yeasts and other lower fungi), viruses and protozoans are considered as active substance of microbial plant protection products (Kabaluk et al., 2010). Though not considered as "microorganisms", here we also include entomopathogenic nematodes (exclusively in 3.4), as nematodes are often associated with toxin-producing bacteria (see 3.4), which are microorganisms of interest for the interaction with pollinators. However, different regulatory requirements for entomopathogenic nematodes among European countries are mentioned and regulation is not the same in these countries (Campos-Herrera, 2015; Richardson, 1996). The pesticidal activity of microbial control agents and nematodes (insecticide, acaricide, fungicide, and nematicide) is based on their pathogenic effect against the pest organism, toxic mode of action or by indirect activity such as competitive displacement of the target pest (Köhl et al., 2019a). In particular, antagonistic microorganisms might provide a long-term solution in the suppression of plant pathogens that is compatible with integrated pest management (IPM), by competition for nutrients, habitat, space and/or by the production of enzymes and antibiotics. A detailed description of all groups and debate on can be found in Francis et al. (2020), Köhl et al. (2019a) and Lacey et al. (2001, 2015). For decades, numerous microorganisms have been registered and used in different countries worldwide. Microorganisms and nematodes are either sprayed on large areas or locally applied, using sticks or tablets, seed coating or bacterial root colonization.

In the interaction context of plants, pests and microbial plant protection products, pollinators play a leading role. Pollinators transport plant pathogens causing severe diseases (by transmission, acquisition, and translocation). Especially honey bees and, for instance, *Osmia cornuta* disperse several bacteria (e.g., *Erwinia* sp., *Pseudomonas fluorescens, Bacillus subtilis*) causing plant diseases (Johnson et al., 1993; Maccagnani et al., 2009). However, pollinators can also transport and distribute microbial agents that are used for controlling plant diseases and pests (Maebe et al., 2020). While foraging, pollinators, in particular bees, can carry vegetative forms of bacteria and fungi as well as their dormant spores. A summary of tests on botanical aspects of pollination (e.g., stigma and pollen functions, fertilization, seed, and fruit set) and bees (honey, bumble, solitary bees) as vectors for microbial plant protection products has been published recently (Smagghe et al., 2020) and will not be considered here. Furthermore, we will not review application techniques (routes of exposure), formulations and additives, delivery of spray and problems, or effects of transgenic products on honey bees (*Apis mellifera*) and bumble bees (*Bombus* sp.). Details on these topics can be found elsewhere (Malone and Pham-Delegue, 2001; Preininger et al., 2018). We further refer to recent studies for details and critical discussion on physiological (enzymes, stress response, metabolism, immunity, etc.), morphological (histopathology, apoptosis), behavioural (learning, sensory, aggression, foraging/flight, hygienic behaviour, etc.) and reproductive (fertility, fitness) traits in terms of suitability for standardization and ecological relevance (Barascou et al., 2021; Di Noi et al., 2021). Future studies should focus on biological mechanisms and evolutionary consequences for bees in the context of the environment. In particular, fitness, and not survival alone, has to become a central aspect for risk assessment (Straub et al., 2020).

Hundreds to thousands of studies have been conducted to estimate potential lethal effects (lethal dose – e.g., LD_{50}) and sublethal effects (behaviour - foraging efficiency, reproduction - drone production, physiological effects, etc.), and to assess potential risk on individual bees as well as the full queen-right colony, mainly using chemical plant protection products. For microbial products, established standard methods used for non-microbial products must be reconsidered and might need adaptation (Borges et al., 2021). For example, test duration should be relatively longer than for standard bioassays, depending on the reproduction capacity of the microbial agent (Borges et al., 2021; Steinigeweg et al., 2021). Many other aspects should be considered when studying effects of microbial agents on host/non-host organisms. For instance, comparing fungal and bacterial agents, the method of exposure, as well as the possibility for production of toxins or other harming metabolites, are of crucial importance. Recently, Borges et al. (2021) summarized major knowledge gaps and limitations of current test guidelines to be used for microbial products, including suggestions for future improvement.

In recent years, new guidelines and guidance documents were published that specifically address the issue of pollinating bees. For example, the EFSA bee guidance document requires that the compatibility of agents and of inert components of chemical products with pollinators must be evaluated (EFSA, 2013), including the sublethal level. Another issue that may pose risks to bees are commercial formulations of microbial plant protection products that may contain additives as carrier for the active substances. In the EU, the approval of microbial active substances in plant protection products is realized at the strain/isolate level under the European Regulation (EC) 1107/2009. Data requirements are led out in Commission Regulation (EU) 283/2013 and 284/2013 for microbial active substances and the microbial plant protection product, respectively. Details on the regulatory processes related to risk assessment of microbial plant protection products for pollinators and the regulatory framework can be found in Köhl et al. (2019b) and Smagghe et al. (2020). This review has the intention neither to set up new guidelines or protocols for the risk assessment, nor to recommend limits or specifications of such documents.

Here, we summarize the status of bee studies and reported effects of microbial plant protection products and entomopathogenic nematodes, including their active substances, on solitary and social bees. Although all registered microorganisms are considered as harmless to bees and pollinators, the increasing use of microbial biocontrol agents and other biological control agents poses new environmental questions, which need to be addressed. For example, what is the impact of abiotic factors (for example in terms of climate change) that bees and microorganisms of microbial plant protection products are facing under natural conditions? How do other interaction partners (e.g., symbiotic and environmental bacteria or fungi, parasitoids, bee pests and pathogens, etc.) influence the interaction? Might there be any long-term transgenerational effects on bees? Unfortunately, most of such general questions cannot be answered now and methods need to be developed to address these questions. Open questions and topics we want to address in the current study, are i) Host range and specificity within bees - which organism infects which (non-)host bee species? ii) Criteria of an organisms' pathogenicity. Is infectivity and pathogenicity genus-, species- or strain-specific? Can results be generalized for other bee species or not? iii) Impact of experimental parameters - like observation time, temperature, group size, housing, and nutrition; iv) Exposure effects - natural environment *vs*. cage, effective dose *vs*. field application, exposure routes and consequences.

2. Material and methods

Literature search was conducted using the Web of Science Core Collection (2nd week of November 2020) with several search terms of the following style 'bee AND *the name of the microorganisms or genus of interest*'. Target organism and genus names were derived from books, literature reviews on microbial plant protection products and references therein. Relevant data were extracted from all articles that resulted from literature search and screening references therein. For many microbial organisms that are candidates for plant protection products, effects and observations were available and will be summarized here. For others, valid data are still pending. Future studies may fill those knowledge gaps. It is important to mention that we failed to find peer-reviewed scientific literature for several viruses, bacteria, fungi, fungi-like organisms, yeasts, and nematodes that are summarized in Table 1. All other tables (Tables 2–5) include summaries of effects on treated

Table 1
Microorganisms with useful activity as potential microbial plant protection agent. Not all mentioned microorganisms have been developed as microbial plant protection products.

Viruses	Bacteria	Fungi, Fungi-like organisms, and yeasts	Nematodes
Bacteriophage of *Pseudomonas tolaasii*; Granulovirus (*Adoxophyes orana*); Nucleopolyhedrovirus (*Helicoverpa armigera, Heliothis zea, Lymantria dispar, Mamestra brassicae, Neodiprion sertifer*, *Spodoptera exigua, Spodoptera littoralis*, species of the subfamily Lymantriinae); Pepino mosaic virus (PepMV)[a]; Reoviridae - Cypovirus; Zucchini Yellow Mosaik Virus	*Agrobacterium radiobacter;* *Azotobacter chroococcumis;* *Bacillus lentimorbus, B. licheniformis, B. popilliae, B. pumilis; Burkholderia cepacia;* *Erwinia herbicola* (= *Pantoea agglomerans*); *Pasteuria nishizawae;* *Pseudomonas aureofaciens, P. chlororaphis, P. putida, P. syringae, P. trivialis;* *Streptomyces griseoviridis, S. lydicus;* *Xanthomonas campestris* pv. *poae*	*Acremonium breve; Akanthomyces muscarius* alias *Lecanicillium muscarium; Aschersonia aleyrodis;* *Candida guilliermondii, C. oleophila, C. saitoana, C. sake; Conidiobolus thromboides;* *Coniothyrium minitans; Cryptococcus albidus, C. flavus, C. humicola, C. laurentii;* *Fusarium oxysporum* (non-pathogenic); *Gliocladium virens* (= *Trichoderma virens*); *Kloeckera apiculate;* *Lagenidium giganteum; Lecanicillium longisporum, L. muscarium;* *Metschnikowia fructicola, M. pulcherrima;* *Microdochium dimerum;* *Nomuraea rileyi;* *Paecilomyces lilacinus; Phlebiosis gigantea;* *Pichia anomala; Purpureocillium lilacinum;* *Pythium oligandrum; Rhodotorula glutinis;* *Saccharomyces cerevisiae* LAS02; *Sporothrix insectorum;* *Talaromyces flavus; Trichoderma asperellum T. atroviride, T. gamsii, T. lignorum, T. viride;* *Verticillium albo-atrum* alias *Verticillium dahliae, V. nonalfalfae*	*Deladenus siricidicola;* *Heterorhabditis heliothidis, H. marelatus, H. megidis;* *Neoaplectana dutkyi;* *Phasmarhabditis hermaphrodita;* *Romanomermis culicivorax;* *Steinernema kushidai*

[a] High percentage of positively tested bumble bees (potential vector) in greenhouses.

individuals/groups/colonies compared to their respective controls; sorted by microorganism and bee species (honey bees – different species, subspecies, and hybrids; bumble bees, stingless bees and leafcutter bees), including assay, application type, administration, agent concentration, commercial product (if given), experimental duration, bee's age and country.

3. Organisms, microorganism/nematode-bee interaction, observations, and effects on pollinating bees

3.1. Viruses

There are several insect-specific virus groups, which had been considered as potential control agents (Maramorosch, 1985). However, only baculoviruses are currently used as commercial insecticides to control a variety of pest insects. The viruses of the family *Baculoviridae* can be categorized on genus level: (1) *Alphabaculovirus* - nucleopolyhedroviruses (NPVs) of Lepidoptera, (2) *Betabaculovirus* - granuloviruses (GVs) of Lepidoptera, (3) *Gammabaculovirus* - NPVs of Hymenoptera, and (4) *Deltabaculovirus* - NPVs of Diptera (Harrison et al., 2018). Baculoviruses are highly host specific; therefore, it was not surprising they were not found to be cross-infective from the Lepidoptera to alternative hosts, like bees (Gröner, 1986).

Comparing all studies that have been conducted on honey bees (*Apis mellifera* sp., *Apis ceranae* sp.), bumble bees (*Bombus terrestris*) and leafcutter bees (*Megachile rotundata*) no effects on bees, in terms of mortality, larval development, foraging activity or colony health (e.g., egg production, brood rearing, drone production, bee mortality) were observed in the last six decades (Table 2). Only a single study described minimum or no effects on longevity, egg laying, brood care and development. However, even the authors mentioned that the effects might also resulted from the experimental setting (Morton et al., 1975). Even direct infections with virus particles (for example via feeding of infectious food) did not result in infected bees, confirming that baculoviruses and their commercial formulations do not replicate in bees and do not induce toxic, pathogenic effects. Also, iridoviruses (family *Iridoviridae*) and entomopoxviruses (family *Poxviridae*), which were evaluated for bee safety, did not induce pathological effects to bees.

3.2. Bacteria

From the early days of biological control research, bacterial control agents are in central focus of product testing and development. Strains of six species of bacteria (*Bacillus amyloliquefaciens, Bacillus cereus, Bacillus moritai, Bacillus thuringiensis, Lysinibacillus sphaericus* and *Pseudomonas fluorescens*), and specific strains of several subspecies (e.g. 18 subspecies of *Bacillus thuringiensis*) have been studied as microbial fungicides or insecticides, in particular for their effects on honey bees (*A. cerana* sp., *A. mellifera* sp.), bumble bees (*B. ignitus, B. impatiens, B. terrestris*) and an Australian stingless bee species (*Tetragonula carbonaria*) (Table 3). Feeding *B. moritai*, *L. sphaericus* or *P. fluorescens* (cultivated microorganism in sucrose solution) to honey bees did not affect longevity or colony health (Cantwell and Lehnert, 1979; Davidson et al., 1977; Meikle et al., 2012a; Vandenberg, 1990). However, a spray application of *L. sphaericus* on *T. carbonaria* colonies showed to reduce their foraging activity and affected the adult bee population and brood structure. Colonies even had classical brood disease symptoms (rotten smell; dark coloured larvae, fluid-like or dry) (Shanks et al., 2017). *L. sphaericus* may be a bacterial brood pathogen in stingless bees with specific potential virulence factors (Fünfhaus et al., 2018) but is also naturally associated with bees without showing symptoms (Cano and Borucki, 1995).

Nevertheless, for most interactions of bees and bacteria, more profound studies are needed. For example, feeding *B. cereus* (strain C-47) to forager honey bees resulted in medium mortality within days (Krieg, 1973). This single case is not enough for a recommendation or rejection of the bacterial species and the specific strain for potential usage as active ingredient. Another case is *Bacillus amyloliquefaciens* (strain QST 713). This strain has been assessed only as commercial product on honey bees (cage assay) and bumble bees (microcolonies), without effects on mortality, reduced or no effect on drone production and development (Mommaerts et al., 2009; Ramanaidu and Cutler, 2013; Sabo et al., 2020), and no effect on the gut microbiome of winter honey bees (Sabo et al., 2020) (Table 3). The very same studies also described negative effects (reduced drone production, delayed oviposition, and drone emergence) after feeding higher concentrations (~10^{11} CFU/l, higher than the recommended field concentration) (Ramanaidu and Cutler, 2013) or, in the most extreme case, high mortality and no drone production (Mommaerts et al., 2009). The latter study showed that the

Table 2
Testing of effects on honey, bumble, and leafcutter bees after application of baculoviruses and other insect pathogenic viruses as pure virus or commercial products. Viruses and virus-based commercial products are applied as insecticides, mainly with high specificity against Lepidoptera (see main text for details).

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
Alphabaculovirus – Nucleopolyhedroviruses (NPV)										
AcMNPV	*Apis mellifera*	cage, colony	food	sucrose solution	$7.5 \times 10^{4-5}$ OB/g	60 days	newly emerged	minimum or no effect on longevity, behaviour, brood production	USA	Morton et al. (1975)
wild-type and recombinant AcMNPV	*Apis mellifera*	cage	injection	culture medium	5×10^{4} budded virus particles/bee	9 days	newly emerged	no effect on bees	USA	Heinz et al. (1995)
Nucleopolyhedrovirus (of 13 different host organisms)	*Apis mellifera, A. m.* (Africanized), *A. m.* (Italian strain), *A. cerana indica*	cage, observation hive, colony	food, spray, contact, inhalation, in-forest spray	water, sucrose, honey solution	5×10^{7}-10^{9} OB/ml or g; 10^{3-8} OB/bee; 1.3–10 $\times 10^{9}$ OB/ colony; 5.5–247.5 $\times 10^{9}$ OB/ha	3, 10–22 days	newly emerged, young adults, forager, winter bees	no effect on mortality, bees, foraging activity, colony health (egg production, brood rearing, bee mortality), no infected bees	Brazil, Canada, Germany, India, UK, USA	Alves et al. (1996); Buckner et al. (1975); Cantwell and Lehnert (1979); Cantwell et al. (1966); Dhaduti and Mathad (1980); Doyle et al. (1990); Gröner et al. (1978); Kingsbury et al. (1978); Knox (1970)
Nucleopolyhedrovirus (of 2 different host organisms)	*Megachile rotundata*	cage	food	sucrose solution, pollen, nectar provision	1.2×10^{5} OB/bee; 10^{4-6} OB/bee	7 days	young larvae, newly emerged males	no effect on bees, larval development, no infected bees	Canada	Barber et al. (1993); Goerzen et al. (1990)
Betabaculovirus - Granuloviruses (GV)										
Granulovirus (of 5 different host organisms)	*Apis mellifera, A. m.* (Africanized)	cage, observation hive	food, spray, contact, inhalation	sucrose, honey solution	5×10^{6} OB/bee; 10^{8} OB/ml; 10^{10-12} granules/ml or g; 10, 50 $\times 10^{9}$ granules/colony	-, 3, 10 days	-, 3–13 day, forager, winter bees	no effect on mortality, bees, no infected bees	Brazil, Germany, USA	Alves et al. (1996); Cantwell et al., 1966; Gröner et al., 1978; Knox, 1970
Granulovirus (of 3 different host organisms) [a]	*Bombus terrestris*	microcolony, colony	topical, food	pure product, pollen, sprayed pollen, water, sugar solution	6.6×10^{12} OB/l; 5–6.6 $\times 10^{13}$ GV/l	-, 11 weeks	-, newly emerged	no effect on mortality, drone production, reproduction	Belgium	Mommaerts et al. (2009); Sterk et al. (2002)
other viruses										
grasshopper entomopoxviruses (2 different)	*Apis mellifera*	cage	food	sucrose solution	4×10^{8} spheroid inclusion bodies/ml	3–4 weeks	newly emerged	no effects on mortality, pathological effects	USA	Vandenberg et al. (1990)
Scapteriscus iridovirus	*Apis mellifera* (Africanized)	cage	food, spray	honey solution	0.13 mg tissue/bee; 8 mg tissue/ml	–	3–13 day	no effect on bees, no infected bees	Brazil	Alves et al. (1996)
Melanoplus sanguinipes entomopoxvirus	*Megachile rotundata*	cage	food	pollen, nectar provision	10^{3-5} OB/bee	–	young larvae	no effect on larval development	Canada	Goerzen et al. (1990)

[a] Commercial products: Capex 1% SC, Granupom 1% EC; OB: occlusion bodies.

Table 3
Testing of effects on honey, bumble, and stingless bees after application of bacteria, spores, or commercial products. Most bacteria and bacteria-based products listed here are used as insecticides, except for *Bacillus amyloliquefaciens* - QST 713 and *Pseudomonas fluorescens*, both are applied as fungicides. (Taxonomic details: *Bacillus amyloliquefaciens* - QST 713 aka *Bacillus subtilis* - QST 713, *Lysinibacillus sphaericus* aka *Bacillus sphaericus*).

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
Bacillus amyloliquefaciens - QST 713 *[A]	*Apis mellifera*	cage	food	sugar solution	3.9×10^6–1.7×10^7 CFU/ml	10 days	winter bees	no effect on mortality, behaviour, gut microbiome, decreased immune gene expression	Slovakia	Sabo et al. (2020)
Bacillus amyloliquefaciens - QST 713 *[A]	*Bombus impatiens*	microcolony	topical	control solution	9.52×10^9–1.90×10^{11} CFU/l	60 days	young bees	no effect on mortality, effect on drone development, increased drone production	Canada	Ramanaidu and Cutler (2013)
Bacillus amyloliquefaciens - QST 713 *[A]	*Bombus impatiens*	microcolony	food	honey solution	4.76×10^{10}–1.90×10^{11} CFU/l	60 days	young bees	no effect on mortality, reduced drone production at higher concentrations, delayed oviposition, drone emergence	Canada	Ramanaidu and Cutler (2013)
Bacillus amyloliquefaciens - QST 713 *[A]	*Bombus terrestris*	microcolony	food	sprayed pollen	7.5×10^9 CFU/l	11 weeks	newly emerged	no effect on mortality, drone production	Belgium	Mommaerts et al. (2009)
Bacillus amyloliquefaciens - QST 713 *[A]	*Bombus terrestris*	microcolony	topical, food	water, sugar solution	7.5×10^9 CFU/l	11 weeks	newly emerged	high mortality, no drone production	Belgium	Mommaerts et al. (2009)
Bacillus cereus - C-47	*Apis mellifera*	cage	food	sugar solution	unspecified	8 days	forager	medium mortality	Germany	Krieg (1973)
Bacillus moritai	*Apis mellifera*	cage, colony	food	sucrose solution	8.7×10^7/bee; 300×10^9/colony	–	-, newly emerged	no effect on longevity, colony health (egg production, brood rearing, bee mortality), no infected bees	USA	Cantwell and Lehnert (1979)
Bacillus thuringiensis, *B. t.* - BR 81, PS86Q3 *[B]	*Apis mellifera, A. m.* (Africanized)	cage, observation hive, colony	food, spray, contact, sprinkle, on crop, in-field	water, sucrose solution, sugar solution, microbial dust w/wo powdered sugar	0.04–2%; 5–30 g/colony; 3×10^8 spores/ml; 50×10^9 spores/colony; 1.78–28×10^4 spores/inch^2; 100 µg/10 mm^3; 65 cm^3/ha	-, 48 h, 11–21 days, weeks	-, forager	no effect on brood, adult bees, mortality, food consumption, colony development, no repellent effect on forager bees, no signs of poisoning, no disease effects	Argentina, Germany, Spain, USA	Cantwell et al. (1966); Fagúndez et al. (2016); Johansen (1962); Porcar et al. (2008); Stute (1963); Wilson (1962)
Bacillus thuringiensis *[C]	*Apis mellifera*?	colony	spray	0.2% Triton X-100	1%	months	–	no effect on adult bees and larvae, very low exotoxin concentrations in honey detected	UK	Burges (1976)
Bacillus thuringiensis	*Apis mellifera*	colony	food	water	1700 spores/bee	13 days	larvae	no effect on mortality, high larvae removal at very young stages (within 24 h), no effect on older brood removal	USA	Shimanuki et al. (1963)
Bacillus thuringiensis *[D]	*Apis mellifera*	cage	food	sucrose solution	0.06–1.67×10^9 spores/bee	14 days	–	no effect on bees till day 9, 100% mortality at day 11	USA	Cantwell et al. (1966)
Bacillus thuringiensis, B. thuringiensis - BR 81, BR 147, IPS 82, I/5 *[E]	*Apis mellifera, A. m.* (Africanized)	cage	spray, contact, food	water, sugar solution, candy paste (icing sugar + honey)	3–250×10^8 spores/ml or g	4–8 days	newly emerged, forager	increased mortality, variable mortality (dosage dependent), medium mortality, disintegrated midgut	Brazil, Egypt or Iraq, Germany	Ali et al. (1973); Krieg (1973); Libardoni et al. (2018)
Bacillus thuringiensis	*Bombus ignitus*	cage	injection	PBS	10^5 cells/bee	3–12 h	10-day old adults	increased PGRP-S gene expression in fat body and epidermis, increased expression (abaecin, apidaecin, defensin, hymenoptaecin) 12 h p.i.	Korea	You et al. (2010)
Bacillus thuringiensis var. *aizawai* *[F]	*Apis mellifera*?	cage, colony	spray, food	water, sugar solution	2.5, 5%	14 days, months	-, newly emerged	no effect on mortality, no adverse effects on colony life (egg	USA	Cantwell and Shieh (1981)

(*continued on next page*)

Table 3 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
								production, brood production, brood capping, honey production)		
Bacillus thuringiensis var. *aizawai* - ABTS-1857 *[G]	*Apis mellifera* (Africanized)	cage	food	candy paste	500 g/ha	24 h, 6 days	newly, emerged, forager	no effect on mortality, walking behaviour, no adulteration of midgut tissue, reduced vertical displacement	Brazil	Libardoni et al. (2021)
Bacillus thuringiensis var. *aizawai* - ABTS-1857 *[G], GC-91	*Apis mellifera*	cage, colony	food	honey solution, sugar solution (w/wo pollen), food jelly	40–24400 g/hl; 14–2730 ppm; 0.16–32 µg/larvae; 5 × 10^{10} CFU/l	4–96 h, 10–21 days, 4 weeks	-, larvae, newly emerged	increased mortality with increasing concentration, inhibited brood development, hypoactivity with reduced food consumption, midgut changes (morphostructure), gut microbiota dysbiosis (reduced bacterial abundance)	Germany, Italy	Steinigeweg et al. (2021), (2022); D'Urso et al. (2017)
Bacillus thuringiensis var. *aizawai* - ABTS-1857 *[G]	*Bombus terrestris*	microcolony, colony	topical, food	water, pure product, pollen, sugar solution	350 DMU/l; 0.01, 0.1%	-, 11 weeks	–	no effect on mortality, reproduction, drone production, foraging behaviour	Belgium	Mommaerts et al. (2010); Sterk et al. (2002)
Bacillus thuringiensis var. *aizawai* - ABTS-1857 *[G]	*Bombus terrestris*	microcolony, colony	food, spray, food	water, sugar solution, pollen	0.01–0.2%	10 days, 11 weeks	–	no effect on mortality (0.1%), reduced drone production; high mortality of eggs, larvae, and adults (0.2%)	Belgium, Korea	Kwon et al. (2003); Mommaerts et al. (2010)
Bacillus thuringiensis var. *alesti, B. t.* var. *anduze, B. t.* var. *berliner, B. t.* var. *dendrolimus, B. t.* var. *entomocidus, B. t.* var. *euxoae, B. t.* var. *galleriae, B. t.* var. *gelechiae, B. t.* var. *plebeja, B. t.* var. *sotto, B. t.* var. *subtoxicus* *[H]	*Apis mellifera, A. m.* (Hybrid)	cage, colony	contact, spray, food	powder added to comb foundation, solution sprayed to comb, solution diluted in liquid wax, sucrose solution	10^7 vegetative cells/ml; 10^8–70 × 10^9 spores/g	-, 9–16 days	-, newly emerged, adult bees, larvae	no effect on mortality, no harmful effects, without pathological effects, no infected bees	Czechia, Germany, UK, USA	Burges and Bailey (1968); Haragsim and Vankova (1968); Krieg and Herfs (1963); Vandenberg (1990)
Bacillus thuringiensis var. *alesti, B. t.* var. *anduze, B. t.* var. *bombycis, B. t.* var. *cazaubon, B. t.* var. *dendrolimus, B. t.* var. *entomocidus, B. t.* var. *euxoae, B. t.* var. *gelechiae, B. t.* var. *sotto, B. t.* var. *tenebrionis*	*Apis mellifera, A. m.* (Hybrid)	cage, colony	food, spray	sucrose solution	10^{6-8} spores/ml; 2 × 10^4-10^8 spores/g	-, 7–16 days	larvae, newly emerged, random age, adult bees	increased, medium to high mortality (only at highest dose)	Czechia, France, Germany	Haragsim and Vankova (1968); Krieg and Herfs (1962), 1963; LeComte and Martouret (1959)
Bacillus thuringiensis var. *israelensis* - 60-A	*Apis mellifera*	cage	food	spores + crystals, sucrose solution	10^8 spores/ml	7 days	forager	no effect on longevity	Germany	Krieg et al. (1980)
Bacillus thuringiensis var. *israelensis* - AM 65–52 *[I]	*Bombus terrestris*	colony	topical, food	pure product, pollen, sugar solution	7.2 × 10^4 ITU/l	–	–	no effect on mortality, reproduction	Belgium	Sterk et al. (2002)
Bacillus thuringiensis var. *kurstaki, B. t.* var. *kurstaki* - 61.33, ABTS-351, HD-1 *[J]	*Apis cerana, A. c. indica, Apis mellifera, A. m.* (Africanized), *A. m.*	cage, colony	spray, contact, food, on crop, in-field	water, candy paste, spray adjuvant, spores + crystals, sucrose solution, pollen,	11.8 × 10^6 IU/g; 500 g/ha; 0.25–8% w/w; 0.00008–10% w/v; 3	-, 24 h, 6–10 days, weeks	-, 1–5-day brood, newly emerged,	no effect on mortality, brood, colony development, walking and foraging behaviour, food	Brazil, Canada, Germany,	Bailey et al. (2005); Challa et al. (2019); Krieg et al. (1980); Libardoni et al. (2021);

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Table 3 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
	(Buckfast), *A. m. ligustica*			dipped, sprayed beeswax, comb, bee box	$\times 10^8$ spores/ml; 10^8 spores/colony		young bees, forager	collection; no alterations in midgut tissue	India, Spain, New Zealand	del Mar Leza et al. (2014); Malone et al. (1999); Potrich et al. (2018); Soni and Thakur (2011); Verma (1995)
Bacillus thuringiensis var. *kurstaki, B. t.* var. *kurstaki* - 61.33, ABTS-351, HD-1 *[J]	*Apis cerana indica, Apis mellifera, A. m.* (Africanized), *A. m.* (Hybrid), *A. m. ligustica*	cage, colony	spray, topical, food, strip	water and spray adjuvant, pollen, sucrose solution, honey solution, candy paste (icing sugar + honey), spores on starch background	0.25–8% w/w; 2.5–20 mg/ml; 3×10^8 spores/ml; 10^8 spores/colony	3–10 days	newly emerged, adult bees, forager	increased to high mortality (concentration dependent), reduced food consumption, reduced longevity (only for the highest concentration), slightly to moderately toxic, change in haemolymph amino acid composition, no alterations in midgut tissue	Brazil, Egypt, India, New Zealand	Brighenti et al. (2007); Challa et al. (2019); Hassona and Kordy (2015); Malone et al. (1999); Potrich et al. (2018); Soni and Thakur (2011)
Bacillus thuringiensis var. *kurstaki* - 4D1 (Cry+)	*A. m. mellifera*	cage	food	sucrose solution, 0.1% DMSO	1.4×10^3, 1.4×10^4 CFU/ml	25 days	newly emerged	no effect on mortality, feeding behaviour, reduced GST activity; ALP, GAPD and G6PD were modulated at day 10 p.a.	France	Renzi et al. (2016)
Bacillus thuringiensis var. *kurstaki* - 4Q7 (Cry-)	*A. m. mellifera*	cage	food	sucrose solution, 0.1% DMSO	1.4×10^3, 1.4×10^4 CFU/ml	25 days	newly emerged	slightly higher mortality, no effect on feeding behaviour, no effect on GST activity; ALP, GAPD and G6PD were modulated at day 10 p.a.	France	Renzi et al. (2016)
Bacillus thuringiensis var. *kurstaki, B. t.* var. *kurstaki* - ABTS-351 *[K]	*Bombus terrestris*	microcolony, colony	topical, food	pure product, water, pollen, sugar solution	1280 UAAK/l; 1%	-, 11 weeks	–	no effect on mortality, reproduction, drone production, foraging behaviour	Belgium	Mommaerts et al. (2010); Sterk et al. (2002)
Bacillus thuringiensis var. *thuringiensis, B. t.* var. *thuringiensis* - BI I/5 *[L]	*Apis mellifera, A. m.* (Hybrid)	cage, colony	food, spray	sucrose solution, spores + crystals	$1–3 \times 10^{7-8}$ spores/ml or g; 10^7 vegetative cells/ml	9 days	larvae, newly emerged, young bees, forager	no effect on mortality	Czechia, Germany	Haragsim and Vankova (1968); Krieg (1964); Krieg and Herfs (1963); Krieg et al. (1980)
Bacillus thuringiensis var. *thuringiensis* *[M]	*Apis mellifera, A. m.* (Hybrid)	cage	food	sucrose solution	10^6-3×10^8 spores/ml or g	16 days	newly emerged, adult bees	increased mortality, high mortality only at highest dose	Germany	Haragsim and Vankova (1968); Krieg and Herfs (1962), 1963
Lysinibacillus sphaericus, L. sphaericus - SSII-1	*Apis mellifera*	cage, colony	food	sucrose solution	range, 10^{4-8} spores/ml; $22–30 \times 10^9$/colony; diluted culture	14, 60 days	-, newly emerged	no effect on mortality, colony health (egg production, brood rearing, bee mortality), no infected bees	USA	Cantwell and Lehnert (1979); Davidson et al. (1977); Vandenberg (1990)
Lysinibacillus sphaericus (related to NRS-1693)	*Tetragonula carbonaria*	colony	spray	water	4×10^8 spores/colony	–	–	reduced forager activity, negative effects on worker behaviour, adult population and brood structure, classical brood disease symptoms	Australia	Shanks et al. (2017)
Pseudomonas fluorescens	*Apis mellifera*	cage	food	protein diet, sucrose solution	5.6×10^4–4×10^5 CFU/cage	–	–	no effect on longevity	France	Meikle et al. (2012a)

[a] *A Commercial product: Serenade; *B Commercial product: Hoe 2802, Hoe 2802 conc., Parasporin #276, Thuricide; *C Commercial product: Bakthane L69; *D Commercial product: Parasporin #276; *E Commercial product: Thuricide HP; *F Commercial product: Certan; *G Commercial product: FlorBac, Xentari, Xentari 10 WG; *H Commercial product: Bakthane L69, Thuricide 90TS5-8, Thuricide 90TS4-62; *I Commercial product: Vectobac 12 AS; *J Commercial product: Dipel, Dipel 2X, Foray 48B, Halt, Lipel; *K Commercial product: Dipel, Scutello 6.4 WP; *L Commercial product: Bathurin, Biospor 2802, Biotrol BTB, Thuricide 30B, Thuricide 90T; *M Commercial product: Bathurin; CFU: colony forming units; IU: international units; ITU: international toxic units; DMU, UAAK: abbreviations unclear (from Sterk et al., 2002).

Table 4
Testing of effects on honey, bumble, leafcutter, and stingless bees after application of fungi, fungi-like organisms, yeasts, yeast-like organisms and microsporidia, as pure living organism, spores, or commercial products. Most fungi or fungi-based commercial products listed here are used as insecticides, except for *Ampelomyces quisqualis, Clonostachys rosea, Gliocladium catenulatum, Hypocrea parapilulifera* + *Trichoderma atroviride* (1:1), *Trichoderma* sp. and the yeasts *Aureobasidium pullulans* and PBGY1, they are applied as fungicides. (Taxonomic details, Fungi: *Beauveria bassiana* aka *Cordyceps bassiana*, *Clonostachys rosea* aka *Gliocladium roseum*, *Gliocladium catenulatum* - J1446 aka *Clonostachys rosea f. catenulate*, *Cordyceps fumosorosea* aka *Isaria fumosorosea* or *Paecilomyces fumosoroseus*, *Metarhizium anisopliae* var. *acridum* aka *Metarhizium isolate* IMI 330189 or *M. flavoviride* or *M. acridum*, *Metarhizium anisopliae* - BIPESCO 5, F52 aka *Metarhizium brunneum*, *Verticillium lecanii* aka *Lecanicillium lecanii* or *Lecanicillium muscarium*; Microsporidia: *Antonospora locustae* aka *Nosema locustae* or *Paranosema locustae*).

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
Fungi, fungi-like										
Aspergillus parasiticus - SRS-Ap-86-1	*Megachile rotundata*	cage	topical, food	0.02% Tween 80, pollen/nectar provision	10^{2-6} spores/bee	-, 14 days	young larvae, prepupae	no effect on larval development	Canada	Goerzen et al. (1990)
Ampelomyces quisqualis *A	*Bombus terrestris*	microcolony	topical, food	aqueous solution, sugar solution, sprayed pollen	35×10^7 CFU/l	11 weeks	newly emerged	no effect on mortality, drone production	Belgium	Mommaerts et al. (2009)
Beauveria bassiana	*Apis mellifera* (Africanized)	colony	contact	0.01% Tween	10^8 spores/ml	–	reared queens	no negative effect on queen morphology - incl. midgut villi, increase in weight, shorter emergence time, no effect on breeding area of mated queens; no effect on worker hypopharyngeal glands	Brazil	Potrich et al. (2020)
Beauveria bassiana, B. bassiana - 53.67, 110.25, 01/110-Su, ARSEF 3687, ARSEF 3769, Bb05002, Bb-1, Bb-1333, BB008, CGMCC-13566, EABb 01/103-Su, EABb 04/01-Tip, EBCL 05002, GHA, IBCB 66, ICIPE 284, NY, UAMH 299, UAMH 4150 *B	*Apis mellifera, A. m.*? *A. m.* (Africanized), *A. m.* (Buckfast), *A. m. carnica, A. cerana cerana, A. c. indica*	cage, colony	food, contact, strip, spray, powder, on crop, in-field, immersed leaves	commercial product in water, spray adjuvant, spores on starch background, sucrose solution, food jelly, Entostat powder (carnauba wax) + hydrated silica, carnauba wax or wheat flour + hydrated silica, carnauba wax + hydrated silica or spores only, spores + corn flour, 0.01–0.05% Tween 80, 0.05% Triton-X-100	32×10^6–7.95×10^9 spores/g; 10^8 spores/ml; 10^8 spores/colony; 10^8–3.70×10^{10} CFU/g; 1.1×10^4 CFU/bee; 1.07×10^6 spores/bee; 3.8×10^6–9.2×10^7 CFU/cage; 10^7 spores/cage	-, 6–64 days, max.	-, 4–24 h, larvae, newly emerged, young bees, adult bees, forager	no effect on adult and brood mortality, adult bee mass, sealed brood, queen morphometry and emergence, movement of body parts, cleaning behaviour, foraging behaviour, adverse effects on bees, colony health (colony growth, adult bee weight, sealed brood), colony development, no obvious effect on bee population, no infected brood, few infected bees	Brazil, Canada, China, Egypt, France, India, Kenya, Spain, Turkey, USA	Ahmed and Abd-Elhady (2013); Akkoc et al. (2019); Al Mazra'awi et al. (2006a), Al Mazra'awi (2007); Challa et al. (2019); Colombo et al. (2021); García-Fernández et al. (2008); Jaronski et al. (2003); Meikle et al. (2007, 2008a, 2008b, 2009, 2012a); Omuse et al. (2022); Peng et al. (2020); Rosana et al. (2021); Sinia and Guzman-Novoa (2018); Soni and Thakur (2011)
Beauveria bassiana, B. bassiana - 447	*Apis mellifera* (Africanized), *A. m. carnica*	colony	dust	talc powder, pure spores	$1–7.5 \times 10^6$ spores/g; 8.0×10^{11} spores/colony	14 days	–	no to low mortality, no effect on colony health, infected bees	Brazil, Egypt	Alves et al. (1996); Sewify et al. (2015)
Beauveria bassiana, B. bassiana - 17–41, 431–33.99, 447,	*Apis mellifera, A. m.*? *A. m.* (Africanized),	cage, colony	food, topical, spray,	ambrosia syrup, spray adjuvant, 0.03–0.5% Tween 80, 0.01%	4–100 mg spores/ml; 10^{1-8} spores/ml; 10^{8-9} CFU/g;	3–21 days	larvae, pupae, newly	increased to high mortality (concentration dependent), highly	Australia, Brazil, Canada, China, France,	Al-mazra'awi (2007); Alves et al. (1996); Challa et al. (2019); Colombo

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Table 4 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
ARSEF 3769, Bb05002, BB001, CGMCC-13566, GHA, HBb1-12, IBCB 66, NI8, NY, PL63, TPB3 *[C]	*A. m. carnica, Apis cerana indica*		contact, powder	Silwet L77, 0.01–0.5% Polysorbate 80, aqueous solution, sucrose solution, candy paste (icing sugar + honey), spores + corn flour, candy paste (sugar + honey	10^3–5×10^6 spores/bee; 9×10^7–9×10^9 spores/cage		emerged, young bees, nurse bees, forager	variable pupal mortality (strain specific), transmission among bees, no alterations in midgut tissue, infected bees	India, Mexico, UK, USA	et al. (2021); Espinosa-Ortiz et al. (2011); Greco et al. (2019); James et al. (2012); Jaronski et al. (2003); Meikle et al. (2006); Peng et al. (2020); Portilla et al. (2017); Potrich et al. (2018); Shaw et al. (2002); Vandenberg (1990)
Beauveria bassiana - EBCL 05002	*Apis mellifera*	colony	powder	carnauba wax + hydrated silica	2.0×10^9 CFU/g	–	–	increased mortality, colony weight loss; Biopesticide contaminated with *P. fluorescens*	France	Meikle et al. (2012a)
Beauveria bassiana - GHA *[D]	*Apis mellifera*	cage	topical, powder	spore suspension, powder formulation	10^8 spores/ml; 4.4×10^{10}/g	12, 35 days	pupae, forager	increased mortality, reduced emergence, reduced body weight, infected bees; increased gene expression (hymenoptaecin, pUf68, BlCh), increased water loss, no effect on metabolic rate	Canada, Estonia	Hamiduzzaman et al. (2012); Karise et al. (2018)
Beauveria bassiana - ATCC 74040 *[E]	*A. m. ligustica*	cage, colony	topical	0.01% Triton X-100	10^9 spores/ml; 10^6 spores/bee	3, 14 days	forager	increased mortality, no effect on food consumption, higher sucrose responsiveness, higher resistance to extinction of appetitive responses to sucrose, higher proportion of specific (olfactory) learners, no effect on short-term or long-term memory, less responsive to odorants, lower aggression towards infected bees, altering CHCs bouquet (quantity), affects nestmate recognition	Italy	Cappa et al. (2019); Carlesso et al. (2020)
Beauveria bassiana - ATCC 74040 *[E]	*Bombus terrestris*	microcolony	topical, food	aqueous solution, sugar solution, treated pollen	3.45×10^7 CFU/l	11 weeks	adults	no or low mortality, reduced drone production (not for food – sugar solution)	Belgium	Mommaerts et al. (2007)
Beauveria bassiana - Bb-1, GHA *[F]	*Bombus impatiens, B. terrestris*	microcolony, colony	food, topical, spray, powder	spores + inert ingredients, spores + corn flour, control	10^9-1.37×10^{10} spores/g; 10^8 spores/ml; $2.33 \times$	-, 4 h, 7–60 days	-, young bees	no effect on mortality, movement of body parts, drone production,	Canada, Turkey	Akkoc et al. (2019); Al-mazra'awi et al. (2006b); Mommaerts et al.

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Table 4 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
				solution, honey solution, sucrose solution, treated pollen	10^9–4.66×10^{10} CFU/l			oviposition, drone development, colony health (brood production, activity), infected bees		(2007); Ramanaidu and Cutler (2013); Shipp et al. (2012)
Beauveria bassiana - GHA *[D]	*Bombus terrestris*	microcolony	food	sugar solution, sprayed pollen	2.5×10^{10} CFU/l	9–11 weeks	newly emerged	no effect on adult and larval mortality, reduced drone production, impaired behaviour	Belgium	Mommaerts et al. (2009)
Beauveria bassiana	*Bombus ignitus*	cage	injection	PBS	10^5 spores/bee	3–12 h	10-day old adults	no effect on PGRP-S gene expression in fat body and epidermis	Korea	You et al. (2010)
Beauveria bassiana - GHA *[D]	*Bombus terrestris*	cage	powder	spores + inert ingredients	4.4×10^{10}/g	max.	forager	no effect on metabolic rate (CO2 release) or water loss rate, reduced longevity at 2 different temperatures, infected bees	Estonia	Karise et al. (2016)
Beauveria bassiana - SF86-21	*Bombus terrestris*	colony/cage	spray	0.05% Tween 80	10^8 CFU/ml	5 weeks	–	transmission from infected to healthy workers, effect became weaker over time, at the end no difference in number of total workers	Finland	Hokkanen et al. (2003)
Beauveria bassiana - GHA + *Clonostachys rosea* - 88–710 *[G]	*Bombus impatiens*	colony	powder	spores + inert ingredients	6.24×10^{10} spores/g + 1.38×10^7 spores/g	–	–	no effect on mortality, infected bees, crop dependent effects	Canada	Kapongo et al. (2008b)
Beauveria bassiana - GHA, SF86-21 *[D]	*Bombus impatiens, B. terrestris*	cage, microcolony, colony	spray, sprayed flowers, topical, food, powder	0.05% Tween 80; aqueous solution; sugar solution, spores + corn flour, powder formulation	10^{4-8} CFU/ml; 9×10^9–2×10^{11} spores/g	-, 25–35 days, 11 weeks	-, newly emerged, adults, forager	increased to high mortality (dosage dependent), reduced drone production, no drone production, no effect on metabolic and water loss rate, infected bees	Belgium, Canada, Estonia, Finland	Hokkanen et al. (2003); Karise et al. (2018); Kapongo et al. (2008a); Mommaerts et al. (2007), 2009
Beauveria bassiana (Mycotech Bioproducts), *B. bassiana* - GHA, SRS-Bb-86-5 *[H]	*Megachile rotundata*	cage, in-field	food, spray	pollen/nectar provision, commercial product in water	10^{3-5} spores/bee; 3.5×10^{13} spores/ha	–	young larvae, adult bees	no effect on larval development, diapausing prepupae, emerging adults, infected bees	Canada, USA	Goerzen et al. (1990); Goettel and Jaronski (1997)
Beauveria bassiana (Mycotech Bioproducts), *B. bassiana* - 17–41, GHA, SRS-Bb-86-5, TPB3	*Megachile rotundata*	cage	topical, spray	0.01% Silwet L77, inert paraffin, 0.02% Tween 80	10^{2-6} spores/bee; 10^{13} spores/3.785 l/0.405 ha	10–14 days	prepupae, newly emerged	increased mortality with increasing concentration, high mortality (concentration dependent), infected bees	Canada, USA	Brinkman et al. (1997); Goerzen et al. (1990); James et al. (2012)
Beauveria bassiana - GHA, ICIPE 284, Bea-TNK *[D]	*Melipona beecheii, Meliponula ferruginea, Scaptotrigona mexicana, Tetragonisca angustula*	cage	spray, contact	0.01% Tween 80, 0.05% Triton-X-100	10^{8-9} spores/ml	10, 20 days	young bees, newly emerged	no or low effect on mortality (strain specific)	Kenya, Mexico	Omuse et al. (2022); Toledo-Hernández et al. (2016)
		cage		1% Tween 80	10^{5-8} spores/ml	10 days			Brazil	Conceição et al. (2014)

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Table 4 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
Beauveria bassiana - Biofungi 1	*Melipona scutellaris*		topical, contact				newly emerged	increased mortality with increasing concentration, lost mobility		
Beauveria bassiana - ESALQ-PL63	*Tetragonisca angustula*	colony	topical	0.05% Tween	10^6 spores/ml	2, 24 h	forager	very high rejection rate of nestmates by guard bees	Brazil	Almeida et al. (2022)
Beauveria brongniartii, B. brongniartii - GSES	*Apis mellifera*	cage, colony	spray, on forest, in-field	-, water	-, 10^{4-8} spores/ml	2–3 weeks	-, adult bees	no effect on mortality, brood development, no infected bees	Germany, Japan	Tsutsumi et al. (1998); Wallner (1988)
Clonostachys rosea *[I]	*Bombus impatiens*	colony	powder	powder formulation	10^6 CFU/g	–	–	no effect on foraging behaviour, aggression, or self-grooming	Canada	Reeh et al. (2014)
Cordyceps fumosorosea - 409.96, HP1	*Apis mellifera*	cage, colony	spray, topical	0.03% Tween 80, 0.01–0.5% Polysorbate 80	10^{1-8} spores/ml	10–21 days	larvae, pupae (day 7), 1 week old	dosage dependent mortality, low to high mortality within 2 weeks, infected bees	Mexico, UK	Espinosa-Ortiz et al. (2011); Shaw et al. (2002)
Cordyceps fumosorosea - APOPKA 97 *[L]	*Bombus terrestris*	colony	topical, food	pure product, pollen, sugar solution	4×10^8 CFU/l	–	–	no effect on mortality, reproduction	Belgium	Sterk et al. (2002)
Cordyceps fumosorosea - APOPKA 97 *[L]	*Bombus terrestris*	microcolony	topical, food	aqueous solution, sugar solution, treated pollen	10^6 CFU/l	11 weeks	adults	no to low mortality, no effect on drone production (except for food – sugar solution)	Belgium	Mommaerts et al. (2007)
Cordyceps fumosorosea - Ifu-lu 01	*Melipona beecheii, Scaptotrigona mexicana, Tetragonisca angustula*	cage	spray	spores + Celite 400, 0.01% Tween 80	10^9 spores/ml	20 days	young bees	no effect on mortality	Mexico	Toledo-Hernández et al. (2016)
Culicinomyces clavisporus	*Apis mellifera*	cage, colony	food, spray	sucrose solution	10^6 spores/ml	12–16 days	adult bees	no effect on mortality, colony, no infected bees	Australia	Cooper et al. (1984)
Entomophaga maimaiga - ARSEF 1400	*Apis mellifera*	cage	contact	direct contact with sporangia	$2.0–2.3 \times 10^5$ spores/cage	14 days	newly emerged	no increased mortality, no infected bees	USA	Vandenberg (1990)
Gliocladium catenulatum	*Apis mellifera*	cage	powder	flower	thin layer	35 days	forager	no effect of mortality, metabolic and water loss rate	Estonia	Karise et al. (2018)
Gliocladium catenulatum - J1446 *[J]	*Apis mellifera*	cage	powder	spores + kaolin	10^8 CFU/g	35 days	forager	increased mortality, water loss, no effect on metabolic rate	Estonia	Karise et al. (2018)
Gliocladium catenulatum	*Bombus terrestris*	cage	powder	flower	thin layer	35 days	forager	no effect of mortality, metabolic and water loss rate	Estonia	Karise et al. (2018)
Gliocladium catenulatum - J1446 *[J]	*Bombus terrestris*	cage, microcolony, colony	topical, food, powder	aqueous solution, sugar solution, sprayed pollen, spore product, spore product + corn starch (1:1), spores + kaolin	$4.5 \times 10^7–10^8$ CFU/g; 7.5×10^8 CFU/l	11 weeks, max.	newly emerged, forager	no effect on mortality, drone production, foraging, metabolic rate (CO2 release), increased cuticular and total water loss rate	Belgium, Estonia	Karise et al. (2016); Mommaerts et al. (2009, 2011)
Gliocladium catenulatum - J1446 *[J]	*Bombus terrestris*	cage, microcolony, minihive	powder	spores + kaolin, spore product	10^{7-9} CFU/g	35 days, 5 weeks	forager	increased mortality (depending on experimental setup),	Belgium, Estonia	Karise et al. (2018); Mommaerts et al. (2012)

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Table 4 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
								water loss, no effect on metabolic rate, drone production, colony health		
Hirsutella necatrix - 49.81, *H. thompsonii* - 71,75,77.82	*Apis mellifera*	cage	spray	0.03% Tween 80	10^8 spores/ml	21 days	1 week old	low to high mortality within 2 weeks, infected bees	UK	Shaw et al. (2002)
Hirsutella thompsonii, H. thompsonii - ARSEF 257, 1947, 3323, UF15858	*Apis mellifera*	cage, observation hive, colony	food, contact on a smooth surface, contact, spray	sucrose solution, spores on agar plates, 0.01% Triton X-100	2×10^8 spores/ml; 271 CFU/bee; 6×10^5/colony; 10-day old culture; 2.2×10^5/agar disk	-, 7–30 days	-, larvae (5th instar), pupae, newly emerged	no effect on bee and larval or pre-pupal mortality, colony health (egg production, brood rearing), no infected bees, normal behaviour of hatched bees	USA	Cantwell and Lehnert (1979); Kanga et al. (2002); Peng et al. (2002)
Hypocrea parapilulifera + *Trichoderma atroviride* (1:1) *[K]	*Bombus terrestris*	microcolony	topical, food	aqueous solution, sugar solution, sprayed pollen	1.25×10^5 CFU/l	11 weeks	newly emerged	no effect on mortality, drone production	Belgium	Mommaerts et al. (2009)
Lagenidium giganteum - ATCC 52675	*Apis mellifera*	cage	food	sucrose solution	10^5 oospores or 1.5×10^3 zoospores/ml	30 days	–	no effect on mortality, no infected bees	USA	Kerwin et al. (1988)
Metarhizium anisopliae, M. anisopliae - E9, 54.67, 5630, BIPESCO 5, F52, IBCB 425, ICIPE 62, ICIPE 78, Qu-M845, UAMH 9198, V245 *[M]	*Apis mellifera, A. m.*? *A. m.* (Africanized), *A. m.* (Buckfast), *A. m. carnica*	cage, colony	food, contact, spray, strip, dispenser, spray, powder	commercial product in water, pure spores, Biobeads, Crisco oil, granular sugar + spores, spores on starch background, spores + corn flour, candy paste (icing sugar + honey), sucrose solution, food jelly, 0.05% Triton-X-100	32×10^6-10^{10} spores/g; 10^{8-9} spores/ml; 10^8–93.6×10^{10} spores/colony; 2.5–9.36×10^{10} spores/strip; 0.25 g spores/g inoculum	-, 4–24 h, 10–62 days	-, larvae, young brood, newly emerged, young bees, forager	no effect on mortality (adults, brood), worker body weight, movement of body parts, queen morphometry and emergence, colony development (bee mortality, brood production), bee population, no or positive effect on colony health (adult bees, brood development), no alterations in midgut tissue	Brazil, Canada, Chile, Egypt, India, Italy, Kenya, Turkey, UK, USA	Ahmed and Abd-Elhady (2013); Akkoc et al. (2019); Butt et al. (1998); Carreck et al. (2007); Colombo et al. (2021); Ferrari et al. (2020); James et al. (2006); Kanga et al. (2003, 2006, 2010); Omuse et al. (2022); Potrich et al. (2018); Rodríguez et al. (2009); Sinia and Guzman-Novoa (2018); Soni and Thakur (2011)
Metarhizium anisopliae, M. anisopliae - 441–445.99, E9, HMa1-7, IBCB 425, ICIPE 7, ICIPE 20, ICIPE 69, Meta 92204, Qu-M845, V208, V245, *M. a. var. acridum, Metarhizium brunneum* - F52 *[N]	*Apis mellifera, A. m.* (Africanized), *A. m. capensis*	cage, colony	food, contact, topical, spray, powder, sprinkle, immersed leaves	aqueous solution, sucrose solution, pure spores, rice-grains, 0.01.-0.03% Tween 80, 0.01–0.5% Polysorbate 80, deodorized kerosene oils, oil + solvent, 0.01–0.05% Triton X-100, candy paste (sugar + honey)	0.04×10^5–5×10^6 spores/bee; 10^{1-10} spores/ml; 2×10^{12} CFU/l; 9×10^{11} CFU/kg; 1.1–5×10^{10} spores/colony	-, 3–56 days	larvae, pupae, newly emerged, 3–13 day old, forager	increased to high mortality (adults, brood), no alterations in midgut tissue, no effect on metabolic and water loss rate, infected bees	Brazil, Chile; Estonia; France, Kenya, Mexico, Netherlands, UK	Alves et al. (1996); Ball et al. (1994); Butt et al. (1994); Colombo et al. (2021); Danfa and Van der Valk, 1999; Espinosa-Ortiz et al. (2011); Gerritsen and Cornelissen (2006); Karise et al. (2018); Meikle et al. (2006); Omuse et al. (2022); Potrich et al. (2018); Rodríguez et al. (2009); Shaw et al. (2002)
Metarhizium anisopliae - 445.99	*Apis mellifera*	cage	powder	spore powder	0.5 g/cage	48 h, 6 days		high mortality (nurses > forager), infected bees	UK	Bull et al. (2012)

(*continued on next page*)

Table 4 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
							newly emerged, forager	(nurse > forager); differential gene expression between groups and infection - especially immune response, no effect on vitellogenin expression		
Metarhizium anisopliae - SF	*Apis mellifera* (Africanized)	cage	dipped	70% spores, 30% diatom powder in water	10^8 spores/ml	24 h, -	newly emerged workers, drones, and queens	increased mortality in heat-stressed worker; PO activity decreased in workers and queens, increased in drones; increased Hsp70 protein quantity in heat-stressed infected bees (all sexes and castes)	Mexico	Medina et al. (2020)
Metarhizium anisopliae - UAMH 9198	*Apis mellifera*	cage	topical	spore suspension	10^8 spore/ml	12 days	pupae	increased mortality, reduced emergence, reduced body weight, infected bees; increased gene expression (hymenoptaecin, pUf68, BlCh)	Canada	Hamiduzzaman et al. (2012)
Metarhizium anisopliae - Met 52	*Bombus terrestris audax*	cage	injection (2 μl)	Ringer solution	3×10^8 CFU/ml	–	adult bees	effect on insect fat body protein expression (278 proteins) with 2 major clusters (up-/down-regulated); up-regulated: immune response, fatty acid metabolism, detoxification; down-regulation: apoptosis, amino acid metabolism, carbohydrate metabolism	Ireland	Hester (2020)
Metarhizium anisopliae - V245, *Metarhizium brunneum* - F52 *O	*Bombus lapidarius, B. lucorum, B. terrestris*	cage, microcolony	contact, spray, powder	suspension, spore product, fungi on potato dextrose agar	10^8 CFU/ml; 10^{7-9} spores/g	20 days, 6 weeks	–	medium to high mortality, infected bees, reduced drone production	Belgium, Finland	Hokkanen et al. (2003); Smagghe et al. (2013)
Metarhizium brunneum - F52	*Bombus terrestris*	colony	spray, food	ready to use solution, sucrose solution	9×10^{11} CFU/kg	4 h, 7 days	–	no effect on mortality, movement of body parts	Turkey	Akkoc et al. (2019)
	Melipona beecheii, Scaptotrigona	cage	spray	0.01% Tween 80	10^9 spores/ml	20 days	young bees		Mexico	Toledo-Hernández et al. (2016)

(*continued on next page*)

Table 4 (*continued*)

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
Metarhizium anisopliae - Ma-lu 01, Meta-TNK	*mexicana, Tetragonisca angustula*							increased to high mortality (strain specific)		
Metarhizium anisopliae - ICIPE 7, ICIPE 20, ICIPE 62, ICIPE 69, ICIPE 78	*Meliponula ferruginea*	cage	contact	0.05% Triton-X-100	10^8 spores/ml	10 days	newly emerged	no effect on mortality, infected bees	Kenya	Omuse et al. (2022)
Nomuraea rileyi	*Apis mellifera* (Africanized), *Apis cerana indica*	cage, colony	spray, topical, food, on crop, in-field	sucrose solution, water, and spray adjuvant, 0.02% Tween 80	10^{6-8} spores/ml; 2×10^8 spores/l; 5×10^6 spores/bee	-, 4, 15 days	larvae, adults, 3–13 day old, forager	no effect on bees, mortality, foraging behaviour, no infected bees	Brazil, India	Alves et al. (1996); Challa et al. (2019); Mulimani and KulkaRni, 2004
Trichoderma harzianum - 1295–22, T39 *[P]	*Apis mellifera*	colony	powder, drip	sucrose solution	2×10^{10} CFU/g; 2.67 g Trichodex/l	–	–	no effect of bee health, brood development	Netherlands, USA	Kovach et al. (2000); van der Steen et al. (2004)
Trichoderma harzianum - 1295–22, T22, T39 *[Q]	*Bombus impatiens, B. terrestris*	microcolony, colony	topical, food, powder, sprinkle	water, sugar solution, sprayed pollen, pollen pure product	6×10^8–10^{10} CFU/l; 2×10^{10} CFU/g; 2.67 g Trichodex/l	11 weeks	newly emerged, adults	no effect on mortality, brood development, drone production, reproduction, bee health	Belgium, Netherlands, USA	Kovach et al. (2000); Mommaerts et al. (2007, 2009); van der Steen et al. (2004); Sterk et al. (2002)
Trichoderma harzianum - T22 *[Q]	*Bombus terrestris*	microcolony	topical, food	aqueous solution, sugar solution	0.6×10^9 CFU/l	11 weeks	adults	increased mortality, reduced drone production	Belgium	Mommaerts et al. (2007)
Trichoderma harzianum ATCC20476 + *T. polysporum* ATCC20475 (1:1) *[R]	*Bombus terrestris*	microcolony	topical, food	aqueous solution, sugar solution, saturated pollen	Binab-TF-WP: 2000 spores/ml; Binab-TF-WP-Konc: 330000 spores/ml; 10^5 CFU/g + 10^6 CFU/g; 1.25×10^6 CFU/l	-, 11 weeks	L3, L4 larvae, young worker, adults	no effect on mortality, drone production, larval development	Belgium	Mommaerts et al. (2007), 2008
Verticillium lecanii, V. lecanii - 74.67, - V1-1 *[S]	*Apis mellifera, A. m.*?	cage, colony	spray, food	water, sucrose solution, ready to use solution	1.7×10^4-10^8 spores/ml	10–56 days, max.	-, 4–24 h, 1–5-day brood, young bees, forager	no effect on bee and brood mortality, movement of body parts	India, Netherlands, Turkey	Akkoc et al. (2019); Gerritsen and Cornelissen (2006); Soni and Thakur (2011)
Verticillium lecanii - 1.72, 17.76, 19.79, 30.79, 74.67, 450.99, 453.99	*Apis mellifera, A. m.*?	cage, colony	spray, strip	0.03% Tween 80, spores on starch background	10^8 spores/ml or colony	10–21 days	1 week old	variable mortality, increased mortality, infected bees (strain specific)	India, UK	Shaw et al. (2002); Soni and Thakur (2011)
Verticillium lecanii - V1-1 *[T]	*Bombus terrestris*	colony	spray, food	sucrose solution, ready to use solution	10^8 spores/ml	4 h, 7 days	–	no effect on mortality, movement of body parts	Turkey	Akkoc et al. (2019)
Yeast, yeast-like										
Aureobasidium pullulans - AP-SLU6	*Bombus terrestris*	colony	powder	wheat-bran formulation	10^8 CFU/g	4 weeks	–	no effect on flight activity, colony development	Sweden	Iqbal et al. (2021)
PBGY1	*Apis mellifera*	colony	drip	sucrose solution	10^7 spores/ml	–	–	no effect on brood development	Netherlands	van der Steen et al. (2004)
PBGY1	*Bombus terrestris*	colony	sprinkle	water	10^7 spores/ml	–	–	no effect on brood development	Netherlands	van der Steen et al. (2004)
Microsporidia										
Antonospora locustae	*Apis mellifera*	cage	food	sucrose solution	$5 \times 10^{1-4}$ spores/bee	26 days	1 week old	no effect on bees, no infected bees	USA	Menapace et al. (1978)
Nosema meligethi	*A. m. capensis*	cage	food	sugar solution	1.3×10^6 spores/ml[a]	30 days	newly emerged	no effect on bees, no infected bees	Finland	Lipa and Hokkanen (1992)

[a] *A Commercial product: AQ10; *B Commercial product: Biovar, Bio-Power, BotaniGard, Nostalgist BL; *C Commercial product: Bio- Power; *D Commercial product: BotaniGard; *E Commercial product: Naturalis; *F Commercial product: BotaniGard, Nostalgist BL; *G Commercial product: BotaniGard + EndoFine; *H Commercial product: Mycotrol; *I Commercial product: Origro's Endophyte; *J Commercial product: Prestop-Mix; *K Commercial product: Binab-T-vector; *L Commercial product: Preferal, PreFeRal 20 WDG; *M Commercial product: Bio-Blast, Bioranza; *N Commercial product: Bio1020, Met52; *O Commercial product: Bio1020; *P Commercial product: Rootshield T 22, Trichodex; *Q Commercial product: Rootshield T 22, Trianum, Trianum- P, Trichodex, Trichodex 25 WP; *R Commercial product: Binab T-vector, Binab-TF-WP, Binab-TF-WP-Konc; *S Commercial product: Mycotal, Nibortem, Vertalec; *T Commercial product: Nibortem; CFU: colony forming units; MFRC: maximum field recommended concentration.

Table 5
Testing of effects on honey and bumble bees after application of nematodes as organism, eggs, or commercial products. (Taxonomic details: *Steinernema carpocapsae* aka *Neoaplectana carpocapsae*). All nematodes are usually applied as insecticides having a wide range of natural host organisms.

Microbial organism	Bee species	Assay	Application	Administration	Concentration	Duration	Age	Effect (on treated bees)	Country	References
Heterorhabditis bacteriophora, H. taysera	*Apis mellifera, A. m. carnica, A. m. ligustica*	cage, microcolony, colony	spray, food	water, 0.01% Triton X-100, sucrose solution	100-5000 nematodes/ml	3 days	-, adult workers	no effect on mortality, no infected bees	Egypt, USA	Baur et al. (1995); Shamseldean et al. (2004); Taha et al. (2016)
Heterorhabditis bacteriophora, H. bacteriophora - H222	*A. mellifera, A. m. ligustica*	cage, colony	contact, spray	0.01% Triton X-100, on tissue paper	1-20, 1000 nematodes/ml	48 h, 5 days	brood, larvae, pupae	increased brood mortality, high mortality (concentration dependent)	Czech Republic, USA	Baur et al. (1995); Hyrsl et al. (2017)
Heterorhabditis bacteriophora - HP88, *H. taysera, Heterorhabditis* sp. - S1	*A. m. carnica*	cage	contact	–	300-2400 nematodes/cup	–	–	tolerant to infections, infected bees	Egypt	Shamseldean et al. (2004)
Heterorhabditis bacteriophora - HP88, *H. taysera, Heterorhabditis* sp. - S1	*A. m. carnica*	cage	spray, food	-, sucrose solution	400, 5000 nematodes/ml	2 days	–	increased mortality, high mortality, no infected bees	Egypt	Shamseldean et al. (2004)
Heterorhabditis sp. + *Steinernema* spp. *A	*Bombus terrestris*	cage	contact	treated soil	10, 25, 50 nematodes/cm^2	4 days	–	high mortality (concentration dependent), infected bees	UK	Dutka et al. (2015)
Steinernema affinis, S. feltiae	*A. m. mellifera*	cage, colony	-, topical	-, nematode suspension	9-10 nematodes/ larvae	48 h	drone, worker larvae	infected bees (sex-specific variance), reduced protein content in worker larvae, change in enzyme activity (esterases, peptidases, proteases, glycosidases) in worker larvae	Poland	Żółtowska et al. (2003a), 2003b
Steinernema carpocapsae - Leningrad strain, *S. carpocapsae*	*Apis mellifera, A. m. ligustica*	cage, colony	spray, food	0.01% Triton X-100, sugar, honey, Hawaiian Punch solution	400-1600 nematodes/ml	7 days	worker bees, forager, brood	increased mortality, forager and brood mortality, infected bees	USA	Baur et al. (1995); Hackett and Poinar (1973); Kaya et al. (1982)
Steinernema carpocapsae - All, *Steinernema* sp. - EBNX, EGG4	*A. m. carnica*	cage	contact, spray, food	-, sucrose solution	300-5000 nematodes/cup	2 days	–	high susceptibility, increased mortality, no infected bees	Egypt	Shamseldean et al. (2004)
Steinernema carpocapsae, S. glaseri	*Apis mellifera, A. m. ligustica*	microcolony, colony	spray	0.01% Triton X-100	400-1600 nematodes/ml	7 days	brood, adult workers	no effect on mortality, behaviour, w/ wo infected bees	USA	Baur et al. (1995); Kaya et al. (1982)
Steinernema feltiae	*A. mellifera*	cage	contact	on tissue paper	1-20 nematodes/bee	48 h	larvae, pupae	high mortality (concentration dependent)	Czech Republic	Hyrsl et al. (2017)
Steinernema kraussei *B	*Bombus terrestris*	cage	contact	treated soil	10, 25, 50 nematodes/cm^2	4 days	–	high mortality (concentration dependent), low number of infected bees	UK	Dutka et al. (2015)
Steinernema riobravis	*A. m. ligustica*	microcolony, colony	spray	0.01% Triton X-100	1000 nematodes/ml	5 days	adult workers, brood	no effect on adult bee mortality, increased brood mortality, no infected bees	USA	Baur et al. (1995)
Steinernema scapterisci	*Apis mellifera*	cage	food, contact	water-saturated co[a]tton ball	8000 nematodes/ cage	3 days	adult bees	increased mortality	USA	Nguyen and Smart (1991)

[a] *A Commercial product: Grow Your Own; *B Commercial product: VineWeevil Killer.

application method significantly drives effects on bees as non-target organism. Feeding of sprayed pollen caused low mortality, whereas feeding bacteria-treated sugar solution or spraying contaminated water resulted in a strong reduction of fitness and survival. Nutrition and food quality, in particular pollen, seem to play a key role enhancing survival or reducing harmful effects of bacterial plant protection products (Steinigeweg et al., 2021). However, this is still not enough data to understand sufficiently the actual interplay of bacterial control agents and bees under natural conditions. Testing only the commercial product already showed contradictory results on mortality and reproduction, without giving deeper insight to potential effects and mechanisms of the specific bacterium. At least one study started to investigate physiological response upon treating caged winter honey bees. Feeding the *B. amyloliquefaciens* (strain QST 713) formulated product decreased innate immune gene expression in adult bees (Sabo et al., 2020), something that should be avoided during overwintering. Moreover, microbial plant protection products should be evaluated under realistic scenarios, as products might not be applied year-around and on all crops.

The most intensively studied entomopathogenic bacterium is *B. thuringiensis*. Several *B. thuringiensis* strains have been developed as biocontrol agents. Here, we will not review its application, the discovery and diversity of toxins (e.g., protease inhibitors, Cry or VIP proteins), transgenic plants and pollen, or insect-pathogenic effects on host and non-host insects that are not bees. For all these topics, we refer to Bravo et al. (2011), Krieg (1961, 1962) and Ricroch et al. (2018). The focus of this study lies on observations and effects for the interaction of bees, treated with vegetative cells, bacterial spores, the delta-endotoxin, or commercial products including spores. In the early times, when subspecies or strain classification was missing, the bacterium *B. thuringiensis* showed in most studies not to induce adverse effects on adult honey bees or larvae under colony conditions (Burges, 1976; Cantwell et al., 1966; Shimanuki et al., 1963) and no increased mortality until day 9 post-treatment in a cage assay using a fast-killing strain (Cantwell et al., 1966). Others observed variable or dosage-dependent increasing mortality (Table 3) and very recently anatomical changes, disintegrated midguts, or changes in the midgut morphostructure, have been described (Libardoni et al., 2018; D'Urso et al., 2017). Recently, reduced bacterial abundance of bacteria belonging to the honey bees' core gut microbiome was discovered in colonies feed with a product containing *B. thuringiensis* (strain ABTS-1857) (Steinigeweg et al., 2022). This case of gut microbiota dysbiosis might be caused by interaction of the bees' gut bacteria with the developing products' microorganism for nutritional resources and habitat space. How this may affect host bees' physiology and survival needs further investigation.

In the end, all studies using *B. thuringiensis* clearly showed that a combination of bacterial strain, study dose, exposure and observation time, host organism, application technique and environment, will result in non-relevant, or relevant sub-lethal or lethal effects, even for the same strain (see Table 3 for details). Classically, most authors studied mortality, reproduction, drone production, colony development, colony health, food consumption, and foraging behaviour. Recently, the research community is asking if this is enough to describe particularly sub-lethal effects. This led to rethink experimental assays and to try new criteria; like changes of the gut system (Libardoni et al., 2018; D'Urso et al., 2017), vertical displacement (Libardoni et al., 2021), walking behaviour (Libardoni et al., 2021) and studying host physiology. In bumble bees, *B. thuringiensis*-injected 10-day old adults showed higher *PGRP-S* gene expression in the fat body and epidermis, as well as increasing expression of antimicrobial peptide genes (e.g., *abaecin, apidaecin, defensin, hymenoptaecin*), few hours after injection (You et al., 2010). For forager honey bees, feeding a product including *B. thuringiensis* var. *kurstaki* (ABTS-351) not only caused high mortality, but also changes in haemolymph amino acid composition have been observed (Hassona and Kordy, 2015). These are the first steps for a better understanding what really happens when microorganisms meet alleged non-host individuals or colonies. However, even using highly related strains, common features or differences concerning (non-) harming activities have to be confirmed first. One example is *B. thuringiensis* var. *kurstaki* harbouring Cry-proteins (4D1-Cry^{+}) or not (4Q7-Cry^{-}). Missing the plasmid coding the Cry genes, not only affected mortality of honey bees consuming this strain, but it also showed to have comparable and opposing effects on enzyme activity (e.g., alkaline phosphatase, glucose-6-phosphatedehy-drogenase, glutathione-S-transferase activity and glyceraldeyde-3-phosphatedehydrogenase) (Renzi et al., 2016).

Residues of synthetic, non-organismic plant protection products can be measured using chromatographic and spectrophotometric methods; bacterial products, like the *B. thuringiensis* toxins, by chromatography or ELISA. Transmission experiments between hive matrices resulted in very low concentrations of the exotoxin leaching out from treated wax into honey (Burges, 1976). However, commercial strains are generally exotoxin negative. To our knowledge, for bacterial agents a single method has been applied so far. *B. thuringiensis* spores were detected in several matrices (e.g., flowers, nectar – stored and honey stomach, pollen, bee bread, larvae, and adult bees) of the treated honey bee colonies or after field spray application, with several orders of magnitude difference among matrices (Alkassab et al., 2022; Steinigeweg et al., 2021, 2022). The major findings were that spore loads decreased over time in nectar (honey stomach), pollen pellets and adult bees under field conditions, whereas loads increased under colony conditions in larvae or stayed unchanged in stored matrices (stored nectar, bee bread) (Alkassab et al., 2022; Steinigeweg et al., 2021). The maximum level detected for *B. t.* spores in honey bee larvae can assumed as approx. 10^3 CFU/-larvae, which might be tolerable as larvae were alive during sampling (Alkassab et al., 2022).

Finally, we recommend cautiousness for extrapolating observations and especially non-harmful effects from one strain to the entire species or even the genus. Future studies should not only focus more on the interaction of the bacterial organism and the bee; also, environmental factors (e.g., UV-radiation, humidity, temperature, wind, food quality, habitat quality, etc.) might be of central relevance for resilience, survival and finally residue enrichment of the bacterium or its spores. As already mentioned, current assessment schemes that are used for non-organismic agents must be re-evaluated, adapted, and modified for microorganisms (Steinigeweg et al., 2021). Test duration is one of the most essential modifications, as the full range of microorganisms need several minutes to days for one cycle of replication or reproduction within the bees' environment.

Alternatively, bees may try to avoid encountering bacteria and their products, like honey bees do with dried preparations even after adding sucrose (Johnson et al., 1993). They may recognize high concentrations of foreign bacteria (Fouks and Lattorff, 2011) and communicate this finding with their nestmates. The colony environment with division of labour and social interaction among nestmates cannot be neglected as additional parameter of this multilevel system.

3.3. Fungi and fungi-like organisms

The fungicidal and insecticidal activity makes fungi a versatile tool as mycoparasites, mycoinsecticides and mycoacaricides worldwide (de Faria and Wraight, 2007); including at least 19 different species tested in experimental assays (*Aspergillus parasiticus, Ampelomyces quisqualis, Beauveria bassiana, Beauveria brongniartii, Clonostachys rosea, Cordyceps fumosorosea, Culicinomyces clavisporus, Entomophaga maimaiga, Gliocladium catenulatum, Hirsutella necatrix, Hirsutella thompsonii, Hypocrea parapilulifera, Metarhizium anisopliae, Lagenidium giganteum, Nomuraea rileyi, Trichoderma atroviride, Trichoderma harzianum, Trichoderma polysporum, Verticillium lecanii*) (Table 4). Most fungi species have been assessed as mycoacaricide against *Varroa destructor*, a parasitic mite of honey bees (reviewed in Meikle et al., 2012b). Fungal diseases, like strawberry grey mould caused by *Botrytis cinerea*, can be controlled

successfully by applying specific fungi (e.g., *Gliocladium roseum*) to strawberry plants (Mommaerts et al., 2009). Free-flying pollinating bees can be used as entomovectors to disseminate naturally microorganisms antagonistic to fire blight (*Erwinia amylovora*), plant and fruit disease-causing fungi (cultures: blueberries, raspberries, strawberries) or pathogens of pollen beetles (*Meligethes aeneus*), mites, moths, and thrips (Butt et al., 1998; Smagghe et al., 2020). Especially bumble bees are used to apply *Trichoderma* against *Botrytis* in greenhouses.

Several fungal strains from different species showed no effects on honey bees (*A. cerana* sp., *A. mellifera* sp.), bumble bees (*B. impatiens, B. terrestris*), stingless bees (*Melipona beecheii, Scaptotrigona mexicana, Tetragonisca angustula*) and leafcutter bees (*M. rotundata*), based on the parameters examined so far. In brief: *A. parasiticus* - no effect on larval development of *M. rotundata*; *A. quisqualis, H. parapilulifera* + *T. atroviride* (1:1), *C. fumosorosea* (mostly), *T. harzianum* + *T. polysporum* (1:1) - no effect on mortality and reproduction of *B. terrestris*; *B. brongniartii, C. clavisporus, E. maimaiga, L. giganteum, N. rileyi* - no effect on mortality and brood development of *A. mellifera* or *A. c. indica*; *C. rosea* - no effect on foraging behaviour, aggression or self-grooming of *B. impatiens*; *C. fumosorosea* - no effect on mortality of *M. beecheii, S. mexicana* and *T. angustula* (for more details, see Table 4).

Strain specificity, as already mentioned for the bacteria, is likewise the major observation for the fungal microorganisms. Just as an example, some strains of *C. fumosorosea, H. thompsonii* and *V. lecanii* caused low to high mortality exclusively on honey bees, although other strains have no effect on mortality, behaviour, and colony health (mostly of registered strains and products) (Table 4). For bumble bees, treated colonies of *B. terrestris* (with *V. lecanii* strain V1-1) did not differ from untreated ones (Akkoc et al., 2019). On the other hand, the very same strain (*T. harzianum* T22) showed variable effects among replicate studies with bumble bees (Mommaerts et al., 2007, 2009). In some cases, only a single strain of a single species was used so far, in preliminary studies to assess its risk to bees. Spraying a spore solution of *H. necatrix* on honey bees caused very low mortality within 2 weeks and individuals showed signs of infection, in comparison to other entomopathogenic fungal strains (Shaw et al., 2002). Here again, we have to raise the question if screening microorganism strains for their pathogenicity on bees in laboratory assays is enough evidence to accept the microorganism as potential agent in agriculture. Probably this is not the case and further refinement is needed (e.g., acute vs. chronic exposure, short term vs. long term studies, laboratory studies vs. studies under field realistic conditions).

The two species attracting most attention as biocontrol agents are *B. bassiana* and *M. anisopliae* (incl. *M. anisopliae* var. *acridum*, *M. brunneum*). Reviews on their life cycle, evolution, biology, and safety can be found elsewhere (Stone and Bidochka, 2020; Zimmermann, 2007a, b). For *B. bassiana* (especially strain GHA) and *M. anisopliae* (strain F52), concentration and experimental setup-specific observations on behaviour and mortality of honey bees, bumble bees and leafcutter bees can be listed as already mentioned (Table 4). However, with the work of Hokkanen et al. (2003) the list of tested bee species can be extended. They are the only ones so far using wild caught bumble bees (*B. lapidarius, B. lucorum*). They showed comparable infectivity with *M. anisopliae* (strain V245) and mortality as for commercial *B. terrestris* colonies (Hokkanen et al., 2003). Only three studies used stingless bees as alternative hosts for strains of *B. bassiana* and found that strains GHA, ICIPE 284 and Bea-TNK do cause no or low mortality on *M. beecheii, Meliponula ferruginea, S. mexicana, T. angustula* (Omuse et al., 2022; Toledo-Hernández et al., 2016), while strain 'Biofungi 1' caused increased mortality with increasing concentration for *Melipona scutellaris* (Conceição et al., 2014). Spraying young stingless bees with *M. anisopliae* resulted in a strain-specific increase to high mortality (Toledo-Hernández et al., 2016); whereas contact exposure of *M. ferruginea* to strains of *M. anisopliae* had no effect on bee mortality (Omuse et al., 2022). In general, wild bees (e.g., Andrenidae, Colletidae, and Megachilidae) have been neglected so far, and stingless bees are understudied, as potential hosts or non-target organisms becoming in contact with microbial plant protection products while foraging for food or nest material. Future research activities may consider ground-nesting bees and use comparable studies (incl. cavity nesting bees) to estimate risk on bees with different life cycles and habitats.

Despite rapid development in methodology and sensitivity, most of the studies still focus on mortality only, reproduction and development. Less than 10 studies set their focus on studying bee physiology after having contact with *B. bassiana* or *M. anisopliae*. Treating *B. terrestris* forager (*B. bassiana* strain GHA) did not affect metabolic rate (CO_2 release) or water loss rate (Karise et al., 2016). Injecting *B. bassiana* in 10-day-old adult *B. ignitus* caused no effect on *PGRP-S* gene expression in the fat body and epidermis (You et al., 2010). However, treating honey bees with the strain GHA or *M. anisopliae* (UAMH 9198, F52) resulted in increased gene expression (e.g., *hymenoptaecin, pUf68, BlCh*) and increased water loss (Hamiduzzaman et al., 2012; Karise et al., 2018). Infections with *M. anisopliae* may generally induce changes in gene expression (immune response, but not *vitellogenin*, of nurses and forager honey bees; Bull et al., 2012) and protein expression (immune response, metabolism, detoxification, and apoptosis in the fat body of *B. terrestris audax*; Hester, 2020). These few results and other studies on non-bee insects already showed that the knowledge on the interaction of entomopathogenic fungi with the hosts' immune system is limited (reviewed by Qu and Wang, 2018) and should be extended to the innate immune system but also social immunity of managed and wild bees. In particular, social immunity, the behavioural response towards mycosis (the active infection by a specific fungus), may have a central function in social bees. Under natural conditions, infected bees killed by the fungus might be removed from the colony right before fungal sporulation can be detected. This undertaker behaviour will reduce the risk of reinfection of healthy individuals (Alves et al., 1996).

Very recently, few scientists started to investigate behavioural changes after topical application of *B. bassiana* (ATCC 74040). Treated foragers (*A. m. ligustica*) had higher sucrose responsiveness, higher proportion of specific learners, but were less responsive to odorants, and finally the application did not affect short-term or long-term memory (Carlesso et al., 2020). The authors propose that the fungal exposure may interfere with the octopaminergic signalling cascade within the nervous system (Carlesso et al., 2020). The very same strain and treatment may also affect nestmate recognition via altering the cuticular hydrocarbon bouquet (Cappa et al., 2019). In this case, non-treated honey bees showed lower aggression towards infected once. This is the first honey bee study with a hint that infections with an atypical non-host-disease associated microorganism may manipulate the bees' chemical communication within the hive. In contrast to honey bees, stingless bees (*Tetragonisca angustula*) showed season-independent strong rejection behaviour towards *B. bassiana* (strain ESALQ-PL63) exposed pollen forager (Almeida et al., 2022). Topical exposure resulted in variance of amounts of cuticular hydrocarbon alkanes, maybe explaining the observed changes in nestmate recognition. To gain a deeper insight into the interaction of bees and fungi, alternative criteria, and methods (e.g., queen morphometry, movement of body parts, cleaning behaviour, anatomy of midgut tissue and villi, or development of worker hypopharyngeal glands; Table 4) haven been suggested and should be evaluated for their suitability as study parameter.

Comparing all studies, for assessing the potential risk to bees a clear differentiation between observations based on the pure organism or the commercial product including the microorganism is needed. For example, *G. catenulatum* did not harm honey bees and bumble bees in cage and colony settings. However, its plant protection product (including kaolin) increased mortality and water loss rate on adult *A. mellifera* and *B. terrestris* (Karise et al., 2016, 2018; Mommaerts et al., 2012), without any effect on the metabolic rate (Karise et al., 2018). The substrate kaolin has been suggested as substance inducing the effects observed in these studies (Karise et al., 2016, 2018; Mommaerts et al., 2012). In earlier studies using the similar commercial product (including

kaolin), the same authors did not find effects on mortality, drone production and foraging (Karise et al., 2016; Mommaerts et al., 2009, 2011). This shows again that authors must describe very precisely, if the pure organisms or the commercial product was used in a study, and more data are needed for making general conclusions on the applicability of the product and the related risk for bees.

Other fungi-like organisms, the yeast PBGY1, the yeast-like fungus *Aureobasidium pullulans* (AP-SLU6) and two species of microsporidia (*Antonospora locustae, Nosema meligethi*) did not cause infections or had any detrimental effects on *B. terrestris* and honey bees (*A. mellifera, A. m. capensis*) (Iqbal et al., 2021; Lipa and Hokkanen, 1992; Menapace et al., 1978; van der Steen et al., 2004). Screening for yeasts as active organism against insects or other microorganisms may has an enormous potential, if host-specificity can be guaranteed. Yeasts are actually everywhere, and bees encounter them regularly while foraging for nectar (e.g., *Metschnikowia* spp., a common nectar symbiont).

The environment, in its totality of all abiotic factors (wind, humidity, temperature, and light) is determining the specific microclimate that is optimal for successful host infection, germination and spore production on fruiting bodies. From comparative studies, testing entomopathogenic fungi for their activity against the ectoparasitic mite *V. destructor*, we already know that fungal development is highly dependent on temperature (Davidson et al., 2003), and infections with *B. bassiana, C. fumosorosea, H. necatrix, H. thompsonii, M. anisopliae* and *V. lecanii* resulted in successful reproduction of the fungus (Table 4). Using two bee relevant temperatures (18 and 28 °C), both microclimates resulted in reduced longevity of *B. terrestris* foragers after treatment (covering the workers with powder) with commercial products including different fungal organisms, but reduction was highest at 18 °C (Karise et al., 2016). Similar observations were described for exposed (*M. anisopliae* strain SF) heat-stressed Africanized honey bees compared to individuals not heat-stressed at their larval stage. Infected workers showed increased mortality (mean hazard ratio shift from approx. 2.65 to more than 5). Enzyme activity (phenoloxidase) decreased in infected workers and queens but increased in infected drones. All infected heat-stressed sexes and castes had increased Hsp70 protein quantities (Medina et al., 2020). These studies are first steps, but definitely not enough, to unravel the interaction of ambient temperature and host environment for optimal survival and development of the fungus. Nevertheless, even with the limited knowledge it is obvious that temperature, in combination with host specificity, should move into focus when assessing the risk to bees. For solitary bees, temperature might be a less important factor than for social bees, with constant temperature and humidity within their colonies. Even nest temperatures of eusocial bees differ (honey bees – 34 ± 2 °C *vs*. bumble bees – 30 ± 2 °C) (Goulson, 2010), hence driving fungal development. However, social bee foragers leaving the hive, bees at the nest entrance and even sexuals during their mating flights, all are exposed to low and high ambient temperatures.

3.4. Nematodes

Species and strains of the genus *Heterorhabditis* and *Steinernema* were assessed, mainly using cage assays, for their insecticidal effects on honey bees and bumble bees (Table 5). For both nematodes, a concentration dependent increase to high mortality was detectable for honey bee (*A. mellifera* sp.) brood and adults, with apparent variability for the different species and strains. In some cases, *H. bacteriophora* and *S. riobravis*, effects on mortality differed strongly between adult bees (no effect) and brood (increased to high mortality) (Table 5). The same can be seen for the majority of studies using microcolonies and normal size colonies, with no effects on adult bees but increased brood mortality (Table 5). Only a few studies, mainly using spray applications, described no effect on mortality or behaviour (*S. carpocapsae* – aka *Neoaplectana carpocapsae*, *S. glaseri*; Baur et al., 1995; Kaya et al., 1982). For bees successfully infected with nematodes, the parasites showed active reproduction within its new host, however without any general pattern (Table 5). Nematode reproduction might also be species-specific or an interaction of the host, its parasite, and the environment. Some honey bees (*A. m. carnica*) even showed to be tolerant towards infections with *H. bacteriophora* - HP88, *H. taysera*, or *Heterorhabditis* sp. - S1 (Shamseldean et al., 2004). Infections were confirmed by reproducing nematodes.

Bombus terrestris has been tested only by applying two commercial products (contact exposure) in cage assays (Table 5). In both cases, concentration dependent high mortality was observed, with bees successfully infected by the nematodes (lower number for *S. kraussei* than for the combination of *Heterorhabditis* sp. + *Steinernema* spp.) (Dutka et al., 2015).

In general, infection success and infectivity of nematodes is influenced by humidity and temperature (Kaya et al., 1982). As only few studies used full-size colonies with constant environmental conditions, infectivity has to be tested for the different nematode species on colony level, to see if the observed effects from cage assays are reproducible. In particular, the strains-specific infection intensity might be dependent on temperature (Hyrsl et al., 2017; Żółtowska et al., 2003b). Temperature resistant nematode strains are known, they may survive in colonies and actually do survive in cage assays in the incubator, usually at lower temperatures (below 25–30 °C).

Nematodes of the genus *Steinernema* harbour symbiotic bacteria (*Xenorhabdus* spp.), which may be a co-factor driving host susceptibility by causing septicaemia in infected bees (Shamseldean et al., 2004). This parasite-host system (bee, nematode, and its bacterial symbiont) might be responsible for differences observed in enzyme activity between controls and infected honey bees (*A. m. mellifera*). Infected worker larvae showed reduced protein content and changes in enzyme activity (e.g., esterases, peptidases, proteases, glycosidases) (Żółtowska et al., 2003b). The latter was the first approach to unravel potential physiological response of the host, the honey bee, to understand potential mechanisms explaining the reduced longevity upon successful infection by the nematode. It has to be mentioned that *Heterorhabditis* nematodes harbour as well symbiotic bacteria (of the genus *Photorhabdus*; Abd-Elgawad, 2021), which may play a significant role for the host-parasite system and need further attention in future studies.

4. General discussion and conclusion

Comparing all studies evaluated here, it can be summarized that some microorganisms have no lethal (and if evaluated no sub-lethal) effects on non-target organisms like bees, but for other studies high variability can be detected. This observation massively constrains making general assessments for specific microorganism species. The test systems used are mostly not standardised or not possible to be standardised for bee species not included in general guidance documents and test protocols. To get comparable results, biotic (e.g., host species, age and sex, reproductive status, nutritional status, parasites, and pathogen) and abiotic (e.g., temperature, humidity, ventilation, day-night light rhythm) factors should be adjusted for all bee species to reduce non-microbial induced variance down to a minimum. On the other hand, natural variance causing large fluctuation in spatiotemporal, environmental and phenological conditions cannot be ignored to simulate the most natural environment in test settings. Semi-field and field data revealed that the sensitivity of plant pathogens and non-target organisms is highly diverse towards their biocontrol agents. In contrast, repeated application of microbial plant protection products can induce resistance of target organism against its specific agent, as known for *B. thuringiensis* and C. pomonella granulovirus (Bardin et al., 2015). Generally, repeated application is a critical topic, as some organisms used in plant protection products are common members of the natural environment where they are applied. However, for most organisms the natural background concentration varies strongly relative to the matrix analysed. Genotypes or sub-species will differ strongly between applied products and natural microorganism diversity. Resistance, paired with

less sensitive isolates of plant pathogens, strongly reduces the efficacy in the field. Microorganisms added into the environment may induce undesired non-target effects; such as 1) displacement of beneficial microorganisms (e.g., gut symbionts; Steinigeweg et al., 2022); 2) being allergenic or toxicogenic to plants, animals, or humans by the production of secondary metabolites; 3) being pathogenic to plants or animals by the agent itself or due to contaminants; or 4) leading to horizontal gene transfer of adverse characteristics to non-target microorganisms (Köhl et al., 2019a; Montesinos, 2003). On the other hand, some bacteria or fungi produce secondary metabolites with insecticidal activity (Inamdar et al., 2014; Islam et al., 2021; Yun et al., 2013). Thus, metabolites produced by microorganisms might be even more critical than the organism itself. Future approaches have to characterize the organism's metabolic profile, as well as the relevance of their production under field conditions, to assess its risk to non-target organisms.

To increase efficacy, distribution and to reduce material (little waste) and residues (diminish hazard), managed bees are used as entomovectors for various microbial agents in crops (Smagghe et al., 2020). Even if still being in the experimental phase, entomovectoring provides direct delivery to plant targets (e.g., blossoms or leaves) and reduces time and money associated with conventional sprays for farmers. Most studies using powdery products that were applied via entomovectors or have been assessed in optimising dispenser systems, did not detect physiological changes in bees, except for an increase in water loss rate. This observation was explained by an increase in cuticular water loss but not due to respiratory water loss (Karise et al., 2016). As already mentioned, the substrate kaolin may be the causative substance inducing cuticular water loss and not the microorganism itself. Therefore, substrates and additives should also be tested and assessed singly and in combination with the microorganism. Additives can be exogenous protectants that allow the preservation of cell viability during dehydration. Dry formulation products (wettable powders, dusts, and granules; Schisler et al., 2004) have the advantage of being easily transportable and storable.

Not only product compounds but also the application device itself seem to affect microorganism dissemination and probably the interaction with the bee vector, at least under greenhouse conditions. For example, two-way dispensers had no negative effects on bumble bees and were more efficient, whereas one-way dispensers were less suitable (Mommaerts and Smagghe, 2011). With one-way devices, bees try to minimize powder contact, as they need to move in and out the hive through the powder, which also increases unintended product transport in the hive. The two-way solution where bees use separate ways for in- and outward movement reduce this risk. Even the length of the dispenser affects cleaning behaviour and finally the loading per individual (Mommaerts et al., 2010).

Very few studies investigated microorganism and microbial plant protection product transmission among bees (*A. mellifera, Bombus terrestris*) in the field or among colonies in green houses (Greco et al., 2019; Hokkanen et al., 2003). Transmission from infected to healthy workers became weaker over time (Hokkanen et al., 2003), which may be dependent on worker density and number of interactions. For honey bees, drifting of workers and drones among hives may increase the likelihood of being infected in non-treated colonies and enhance the distribution range of the microorganism (Kanga et al., 2003; Meikle et al., 2006, 2007). On the other side, self- and allo-grooming, the cleaning behaviour of bees, resulted in rapid decline in microorganism units per bee, which may enable a shorter period between several applications (Meikle et al., 2007, 2008a; 2008b, 2009). Behavioural and intrinsic physiological mechanisms interact to reduce potential microorganism burden of the individual organisms. Future studies should investigate more intensively the mechanisms by which synthetic, biological, and microbial plant protection products affect the non-target organisms' physiology, including insect humoral and cellular immunity (James and Xu, 2012). When studying host-pathogen interactions to characterize infectivity and pathogenicity of the (micro-)organisms, measurements and observations must be undertaken using the living organism, depending on the mode of action of the commercial product. Microorganism growth, as classical trait to study infectivity, has to be differentiated strongly from microorganism growth in or on the dead host organism using resources from a rotting food source. If this fact is not clear for the microorganism of interest, suitable studies have to be conducted to confirm the microorganisms' pathogenicity (e.g., survival assays using larvae or adults, etc.).

In conclusion, we will try to sum up current knowledge for the questions we have been asking at the beginning of this study:

i) Host range and specificity - Which organism infects which (non-)host species?

For most microorganism and nematode species, the non-target organism host range within the application range is not clear. However, highly specific baculoviruses did not harm honey bees, bumble bees and leafcutter bees tested so far. For all other biocontrol organism groups considered in this study (bacteria, fungi, nematodes) future studies need to investigate non-target solitary bees, a much larger group of bees worldwide compared to social bees. Such knowledge gaps and limits of experimental approaches are well known for solitary bees (Lehmann and Camp, 2021).

ii) Criteria of an organisms' pathogenicity. Is infectivity genus-, species- or strain-specific? Can results be generalized for other bee species or not?

Infectivity and pathogenicity are highly species- and strain-specific for the bee species tested and under the experimental setting of the respective study. For the (micro-)organism species, not showing any negative effect on bees, it is not recommended to generalize such observations. We advise to define clearly the organism species at strain level, using state-of-the-art methods, like whole genome sequencing (or whole-genome multilocus sequence typing) combined with phenotyping or other biochemical methods.

iii) Impact of experimental parameters - like observation time, temperature, group size, housing, etc.:

As already mentioned, the experimental setting, in particular application method, bees' age, time, nutrition, and housing are additional factors that should be considered when interpreting results of infection studies. Temperature is a critical factor, with social bees having quite constant temperature and humidity levels in their colonies, and solitary bees as well as solitary stages/tasks of social bees known to be highly affected by the environmental temperature.

iv) Exposure effects - natural environment *vs.* cage, effective dose *vs.* field application, exposure routes and consequences:

A major driver of bee survival, effects on reproduction and physiology, is the assay itself. The other parameters also affect observations, but compared to the assay itself, are of lesser relevance. Group size and having a natural environment *vs*. cages strongly influence host response, in particular for social bee species. Stress is induced by testing caged bees isolated from their queen, which undoubtedly make them more than usual susceptible to potential infections. Therefore, infectivity and toxicity assays with caged social bees have to be interpreted with caution, and the EPA recommended that 30-day whole-hive tests should be used instead (Goettel and Jaronski, 1997). Nevertheless, tests with caged honey bees and bumble bees under laboratory conditions result in higher stress levels of the test organisms but provide an absolute worst case scenario and if the study duration is of sufficient length, can still contribute to a robust data set on lower tier level.

Finally, a nearly neglected view from the ecological perspective is

recommended as fundamental future basic research. Genotype-by-genotype interaction, the individual interaction of specific genotypes of the microorganism and specific bee genotypes, should be considered in future experimental designs. This topic gets even more complicate for genotype-by-genotype-by-environment interactions. Previous studies on honey bees and bumble bees already confirmed the importance of the genotype and environment on disease susceptibility, survival, and bee behaviour (Barribeau et al., 2014; Büchler et al., 2014; Meixner et al., 2014; Uzunov et al., 2014). Having in mind the variance among different microorganisms and entomopathogenic nematodes tested by now (Tables 3–5); we can only speculate that effects might be even more complex for the interaction with specific genotypes of solitary and social bee species and sub-species.

Credit author statement

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Declaration of competing interest

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

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References

Abd-Elgawad, M.M.M., 2021. *Photorhabdus* spp.: an overview of the beneficial aspects of mutualistic bacteria of insecticidal nematodes. Plants 10, 1660. https://doi.org/10.3390/plants10081660.

Ahmed, A.A., Abd-Elhady, H.K., 2013. Efficacy of two fungus-based biopesticides against the honeybee ectoparasitic mite, *Varroa destructor*. Pakistan J. Biol. Sci. 16, 819–825. https://doi.org/10.3923/pjbs.2013.819.825.

Akkoc, S., Karaca, I., Karaca, G., 2019. Effects of some entomopathogen fungi on *Apis mellifera* L. and *Bombus terrestris* L. J. Nat. Appl. Sci. 23, 157–163. https://doi.org/10.19113/sdufenbed.477889.

Al Mazra'awi, M.S., 2007. Impact of the entomopathogenic fungus *Beauveria bassiana* on the honey bees, *Apis mellifera* (Hymenoptera: apidae). World J. Agric. Sci. 3, 7–11.

Al Mazra'awi, M.S., Shipp, J.L., Broadbent, A.B., Kevan, P.G., 2006a. Dissemination of *Beauveria bassiana* by honey bees (Hymenoptera: apidae) for control of tarnished plant bug (Hemiptera: Miridae) on canola. Environ. Entomol. 35, 1569–1577. https://doi.org/10.1093/ee/35.6.1569.

Ali, A.-U.-D.D., Abdellatif, M.A., Bakry, N.M., El-Sawaf, S.K., 1973. Studies on biological control of the greater wax moth, *Galleria mellonella* I. Susceptibility of wax moth larvae and adult honeybee workers to *Bacillus thuringiensis*. J. Apic. Res. 12, 117–123. https://doi.org/10.1080/00218839.1973.11099737.

Alkassab, A.T., Beims, H., Janke, M., Pistorius, J., 2022. Determination, distribution and environmental fate of *Bacillus thuringiensis* spores in various honeybee matrices after field application as plant protection product. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-022-19414-5.

Al-mazra'awi, M.S., Shipp, L., Broadbent, B., Kevan, P., 2006b. Biological control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: thripidae) by *Bombus impatiens* (Hymenoptera: apidae) vectored *Beauveria bassiana* in greenhouse sweet pepper. Biol. Control 37, 89–97. https://doi.org/10.1016/j.biocontrol.2005.11.014.

Almeida, F.C.R., Magalhaes, D.M., Favaris, A.P., Rodríguez, J., Azevedo, K.E.X., Bento, J. M.S., Alves, D.A., 2022. Side effects of a fungus-based biopesticide on stingless bee guarding behaviour. Chemosphere 287, 132147. https://doi.org/10.1016/j.chemosphere.2021.132147.

Alves, S.B., Marchini, L.C., Pereira, R.M., Baumgratz, L.L., 1996. Effects of some insect pathogens on the Africanized honey bee, *Apis mellifera* L. (Hym., Apidae). J. Appl. Entomol. 120, 559–564. https://doi.org/10.1111/j.1439-0418.1996.tb01652.x.

Bailey, J., Scott-Dupree, C., Harris, R., Tolman, J., Harris, B., 2005. Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. Apidologie 36, 623–633. https://doi.org/10.1051/apido:2005048.

Ball, B.V., Pye, B.J., Carreck, N.L., Moore, D., Bateman, R.P., 1994. Laboratory testing of a mycopesticide on non-target organisms: the effects of an oil formulation of *Metarhizium flavoviride* applied to *Apis mellifera*. Biocontrol Sci. Technol. 4, 289–296. https://doi.org/10.1080/09583159409355337.

Barascou, L., Brunet, J.-L., Belzunces, L., Decourtye, A., Henry, M., Fourrier, J., Le Conte, Y., Alaux, C., 2021. Pesticide risk assessment in honeybees: toward the use of behavioral and reproductive performances as assessment endpoints. Chemosphere 276, 130134. https://doi.org/10.1016/j.chemosphere.2021.130134.

Barber, K.N., Kaupp, W.J., Holmes, S.B., 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: lymantriidae). Can. Entomol. 125, 1055–1066. https://doi.org/10.4039/Ent1251055-6.

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., Nicot, P.C., 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Front. Plant Sci. 6, 566. https://doi.org/10.3389/fpls.2015.00566.

Barribeau, S.M., Sadd, B.M., du Plessis, L., Schmid-Hempel, P., 2014. Gene expression differences underlying genotype-by-genotype specificity in a host–parasite system. Proc. Natl. Acad. Sci. U.S.A. 111, 3496–3501. https://doi.org/10.1073/pnas.1318628111.

Baur, M.E., Kaya, H.K., Peng, Y.S., Jiang, J., 1995. Nonsusceptibility of the honey bee, *Apis mellifera* (Hymenoptera: apidae), to steinernematid and heterorhabditid nematodes. J. Nematol. 27, 378–381.

Bonaterra, A., Badosa, E., Cabrefiga, J., Francés, J., Montesinos, E., 2012. Prospects and limitations of microbial pesticides for control of bacterial and fungal pomefruit tree diseases. Trees (Berl.) 26, 215–226. https://doi.org/10.1007/s00468-011-0626-y.

Borges, S., Alkassab, A.T., Collison, E., Hinarejos, S., Jones, B., McVey, E., Roessink, I., Steeger, T., Sultan, M., Wassenberg, J., 2021. Overview of the testing and assessment of effects of microbial pesticides on bees: strengths, challenges and perspectives. Apidologie 52, 1256–1277. https://doi.org/10.1007/s13592-021-00900-7.

Bravo, A., Likitvivatanavong, S., Gill, S.S., Soberón, M., 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochem. Mol. Biol. 41, 423e431. https://doi.org/10.1016/j.ibmb.2011.02.006.

Brighenti, D.M., Carvalho, C.F., Carvalho, G.A., Brighenti, C.R.G., Carvalho, S.M., 2007. Bioactivity of *Bacillus thuringiensis* var. *kurstaki* (berliner, 1915) to adults of *Apis mellifera* linnaeus, 1758 (Hymenoptera: apidae). Cienc. E Agrotecnol 31, 279–289. https://doi.org/10.1590/S1413-70542007000200003.

Brinkman, M.A., Fuller, B.W., Leubke, K.G., Hildreth, M.B., 1997. Susceptibility of adult alfalfa leafcutting bees (Hymenoptera: Megachilidae) to *Beauveria bassiana* infection. J. Agric. Entomol. 14, 393–397.

Büchler, R., Costa, C., Hatjina, F., Andonov, S., Meixner, M.D., Le Conte, Y., Uzunov, A., Berg, S., Bienkowska, M., Bouga, M., Drazic, M., Dyrba, W., Kryger, P., Panasiuk, B., Pechhacker, H., Petrov, P., Kezić, N., Korpela, S., Wilde, J., 2014. The influence of genetic origin and its interaction with environmental effects on the survival of *Apis mellifera* L. colonies in Europe. J. Apicult. Res. 53, 205–214. https://doi.org/10.3896/IBRA.1.53.2.03.

Buckner, C.H., McLeod, B.B., Kingsbury, P.D., 1975. The effect of an experimental application of nuclear polyhedrosis virus upon selected forest fauna. Report CC-X-1010, Chemical Control Research Institute Ottawa, Ontario pp.25.

Bull, J.C., Ryabov, E.V., Prince, G., Mead, A., Zhang, C., Baxter, L.A., Pell, J.K., Osborne, J.L., Chandler, D., 2012. A strong immune response in young adult honeybees masks their increased susceptibility to infection compared to older bees. PLoS Pathog. 8, e1003083 https://doi.org/10.1371/journal.ppat.1003083.

Burges, H.D., 1976. Persistence of *Bacillus thuringiensis* in foundation beeswax and beecomb in beehives for the control of *Galleria mellonella*. J. Invertebr. Pathol. 28, 217–222. https://doi.org/10.1016/0022-2011(76)90125-7.

Burges, H.D., Bailey, L., 1968. Control of the greater and lesser wax moths (*Galleria mellonella* and *Achroia grisella*) with *Bacillus thuringiensis*. J. Invertebr. Pathol. 11, 184–195. https://doi.org/10.1016/0022-2011(68)90148-1.

Butt, T.M., Ibrahim, L., Ball, B.V., Clark, S.J., 1994. Pathogenicity of the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* against crucifer pests and the honey bee. Biocontrol Sci. Technol. 4, 207–214. https://doi.org/10.1080/09583159409355328.

Butt, M., Carreck, N.L., Ibrahim, L., Williams, I.H., 1998. Honeybee-mediated infection of pollen beetle (*Meligethes aeneus* Fab.) by the insect-pathogenic fungus, *Metarhizium anisopliae*. Biocontrol Sci. Technol. 8, 533–538. https://doi.org/10.1080/09583159830045.

Campos-Herrera, R., 2015. Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection. Springer International Publishing, Cham, Switzerland. https://doi.org/10.1007/978-3-319-18266-7.

Cano, R.J., Borucki, M.K., 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science 268, 1060–1064. https://doi.org/10.1126/science.7538699.

Cantwell, G.E., Lehnert, T., 1979. Lack of effect of certain microbial insecticides on the honeybee. J. Invertebr. Pathol. 33, 381–382. https://doi.org/10.1016/0022-2011(79)90042-9.

Cantwell, G.E., Shieh, T.R., 1981. Certan – a new bacterial insecticide against the greater wax moth, *Galleria mellonella* L. Am. Bee J. 121, 424–431.
Cantwell, G.E., Knox, D.A., Lehnert, T., Michael, A.S., 1966. Mortality of the honey bee, *Apis mellifera*, in colonies treated with certain biological insecticides. J. Invertebr. Pathol. 8, 228–233. https://doi.org/10.1016/0022-2011(66)90133-9.
Cappa, F., Petrocelli, I., Dani, F.R., Dapporto, L., Giovannini, M., Silva-Castellari, J., Turillazzi, S., Cervo, R., 2019. Natural biocide disrupts nestmate recognition in honeybees. Sci. Rep. 9, 3171. https://doi.org/10.1038/s41598-019-38963-3.
Carlesso, D., Smargiassi, S., Sassoli, L., Cappa, F., Cervo, R., Baracchi, D., 2020. Exposure to a biopesticides interferes with sucrose responsiveness and learning in honey bees. Sci. Rep. 10, 19929. https://doi.org/10.1038/s41598-020-76852-2.
Carreck, N.L., Butt, T.M., Clark, S.J., Ibrahim, L., Isger, E.A., Pell, J.K., Williams, I.H., 2007. Honey bees can disseminate a microbial control agent to more than one inflorescence pest of oilseed rape. Biocontrol Sci. Technol. 17, 179–191. https://doi.org/10.1080/09583150600937485.
Challa, G.K., Firake, D.M., Behere, G.T., 2019. Bio-pesticide applications may impair the pollination services and survival of foragers of honey bee, *Apis cerana* Fabricius in oilseed brassica. Environ. Pollut. 249, 598–609. https://doi.org/10.1016/j.envpol.2019.03.048.
Chandler, D., Bailey, A.S., Tatchell, G.M., Davidson, G., Greaves, J., Grant, W.P., 2011. The development, regulation and use of biopesticides for integrated pest management. Phil. Trans. R. Soc. B. 366, 1987–1998. https://doi.org/10.1098/rstb.2010.0390.
Colombo, F.C., Maciel, R.M.A., Abati, R., Raulino-Domanski, F., Longhi, S.J., Costa-Maia, F.M., de Souza Vismara, E., Lozano, E.R., Potrich, M., 2021. Do *Beauveria bassiana* and *Metarhizium anisopliae* affect worker survival and the production of Africanized *Apis mellifera* queens? J. Apicult. Res. 60, 260–269. https://doi.org/10.1080/00218839.2020.1828237.
Conceição, P. de J., Neves, C.M. de L., Sodré, G. da S., Carvalho, C.A.L. de, Souza, A.V., Ribeiro, G.S., Pereira, R. de C., 2014. Susceptibility of *Melipona scutellaris* latreille, 1811 (Hymenoptera: apidae) worker bees to *Beauveria bassiana* (bals.). Vuill. Sociobiology 61, 184–188. https://doi.org/10.13102/sociobiology.v61i2.184-188.
Cooper, R., Hornitzky, M., Medcraft, B.E., 1984. Non-susceptibility of *Apis mellifera* to *Culicinomyces vlavisporus*. Aust. J. Entomol. 23, 173–174. https://doi.org/10.1111/j.1440-6055.1984.tb01939.x.
Danfa, A., Van der Valk, H.C.H.G., 1999. Laboratory testing of *Metarhizium* spp. and *Beauveria bassiana* on Sahelian non-target arthropods. Biocontrol Sci. Technol. 9, 187–198. https://doi.org/10.1080/09583159929776.
Davidson, E.W., Morton, H.L., Moffett, J.O., Singer, S., 1977. Effect of *Bacillus sphaericus* strain SSII-1 on honey bees, *Apis mellifera*. J. Invertebr. Pathol. 29, 344–346. https://doi.org/10.1016/S0022-2011(77)80041-4.
Davidson, G., Phelps, K., Sunderland, K.D., Pell, J.K., Ball, B.V., Shaw, K.E., Chandler, D., 2003. Study of temperature-growth interactions of entomopathogenic fungi with potential for control of *Varroa destructor* (Acari: mesostigmata) using a nonlinear model of poikilotherm development. J. Appl. Microbiol. 94, 816–825. https://doi.org/10.1046/j.1365-2672.2003.01871.x.
de Faria, M.R., Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control 43, 237–256. https://doi.org/10.1016/j.biocontrol.2007.08.001.
del Mar Leza, M., Llado, G., Petro, A.B., Alemany, A., 2014. First field assessment of *Bacillus thuringiensis* subsp. *kurstaki* aerial application on the colony performance of *Apis mellifera* L. (Hymenoptera: apidae). Span. J. Agric. Res. 12, 405–408. https://doi.org/10.5424/sjar/2014122-5056.
Dhaduti, S.G., Mathad, S.B., 1980. Effect of nuclear polyhedrosis virus of the armyworm *Mythimna* (*Pseudaletia) separata* on colonies of *Apis ceranae indica*. J. Apicult. Res. 19, 77–78. https://doi.org/10.1080/00218839.1980.11100000.
Di Noi, A., Casini, S., Campani, T., Cai, G., Caliani, I., 2021. Review on sublethal effects of environmental contaminants in honey bees (*Apis mellifera*), knowledge gaps and future perspectives. Int. J. Environ. Res. 18, 1863. https://doi.org/10.3390/ijerph18041863.
Doyle, C.J., Hirst, M.L., Cory, J.S., Entwistle, P.F., 1990. Risk assessment studies: detailed host range testing of wild-type cabbage moth, *Mamestra brassicae* (Lepidoptera: noctuidae), nuclear polyhedrosis virus. Appl. Environ. Microbiol. 56, 2704–2710. https://doi.org/10.1128/aem.56.9.2704-2710.1990.
Dutka, A., McNulty, A., Williamson, S.M., 2015. A new threat to bees? Entomopathogenic nematodes used in biological pest control cause rapid mortality in *Bombus terrestris*. PeerJ 3, e1413. https://doi.org/10.7717/peerj.1413.
D'Urso, V., Mazzeo, G., Vaccalluzzo, V., Sabella, G., Bucchieri, F., Viscuso, R., Vitale, D. G.M., 2017. Observations on midgut of *Apis mellifera* workers (Hymenoptera: apoidea) under controlled acute exposures to a *Bacillus thuringiensis*-based biopesticides. Apidologie 48, 51–62. https://doi.org/10.1007/s13592-016-0448-4.
EFSA, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). EFSA J. 11, 3295. https://doi.org/10.2903/j.efsa.2013.3295.
Espinosa-Ortiz, G.E., Lara-Reyna, J., Otero-Colina, G., Alatorre-Rosas, R., Valdez-Carrasco, J., 2011. Susceptibility of larval, pupal and adult honey bees to isolates of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Sorokin) and *Paecilomyces fumosoroseus* (Wize). Interciencia 36, 148–152.
Fagúndez, G.A., Blettler, D.C., Krumrick, C.G., Bertos, M.A., Trujillo, C.G., 2016. Do agrochemicals used during soybean flowering affect the visits of *Apis mellifera* L.? Span. J. Agric. Res. 14, e0301 https://doi.org/10.5424/sjar/2016141-7492.
Ferrari, M.C.F., Favaro, R., Mair, S., Zanotelli, L., Malagnini, V., Fontana, P., Angeli, S., 2020. Application of *Metarhizium anisopliae* as a potential biological control of *Varroa destructor* in Italy. J. Apicult. Res. 59, 528–538. https://doi.org/10.1080/00218839.2020.1736814.
Fouks, B., Lattorff, H.M.G., 2011. Recognition and avoidance of contaminated flowers by foraging bumblebees (*Bombus terrestris*). PLoS One 6, e26328. https://doi.org/10.1371/journal.pone.0026328.
Francis, F., Jacquemyn, H., Delvigne, F., Lievens, B., 2020. From diverse origins to specific targets: role of microorganisms in indirect pest biological control. Insects 11, 533. https://doi.org/10.3390/insects11080533.
Fünfhaus, A., Ebeling, J., Genersch, E., 2018. Bacterial pathogens of bees. Curr. Opin. Insect Sci. 26, 89–96. https://doi.org/10.1016/j.cois.2018.02.008.
García-Fernández, P., Santiago-Álvarez, C., Quesada-Moraga, E., 2008. Pathogenicity and thermal biology of mitosporic fungi as potential microbial control agents of *Varroa destructor* (Acari: mesostigmata), an ectoparasitic mite of honey bee, *Apis mellifera* (Hymenoptera: apidae). Apidologie 39, 662–673. https://doi.org/10.1051/apido:2008049.
Gerritsen, L., Cornelissen, B., 2006. Biological control of *Varroa destructor* by fungi. Proc. Neth. Entomol. Soc. Meet. 17, 125–132.
Goerzen, D.W., Erlandson, M.A., Moore, K.C., 1990. Effect of two insect viruses and two entomopathogenic fungi on larval and pupal development in the alfalfa leafcutting bee, *Megachile rotundata* (Fab.) (Hymenoptera: Megachilidae). Can. Entomol. 122, 1039–1040. https://doi.org/10.4039/Ent1221039-9.
Goettel, M.S., Jaronski, S.T., 1997. Safety and registration of microbial agents for control of grasshoppers and locusts. Mem. Entomol. Soc. Can. 129 – Supplement S171, 83–99. https://doi.org/10.4039/entm129171083-1.
Goulson, D., 2010. Bumblebees. Behaviour, Ecology and Conservation. Oxford University Press, New York.
Greco, M.K., Coates, B., Feil, E.J., 2019. High tech research reveals preferential feeding in honey bees. J. Apicult. Res. 58, 471–477. https://doi.org/10.1080/00218839.2019.1582838.
Gröner, A., 1986. Specificity and safety of baculoviruses. In: Granados, R.G., Federici, B. A. (Eds.), The Biology of Baculoviruses. Vol. I - Biological Properties and Molecular Biology. CRC Press, Boca Raton, Fl, USA, pp. 177–202.
Gröner, A., Huber, J., Krieg, A., Pinsdorf, W., 1978. Tests of two Baculovirus preparations on honey-bees. Nachrichtenbl. Deut. Pflanzenschutzd. 30, 39–41.
Hackett, K.J., Poinar, G.O., 1973. The ability of *Neoaplectana carpocapsae* weiser (steinernematidae: rhabditoidea) to infect adult honeybees (*Apis mellifera*, apidae: Hymenoptera). Am. Bee J. 113, 100.
Hamiduzzaman, M.M., Sinia, A., Guzman-Novoa, E., Goodwin, P.H., 2012. Entomopathogenic fungi as potential biocontrol agents of the ecto-parasitic mite, *Varroa destructor*, and their effect on the immune response of honey bees (*Apis mellifera* L.). J. Invertebr. Pathol. 111, 237–243. https://doi.org/10.1016/j.jip.2012.09.001.
Haragsim, O., Vankova, J., 1968. Effet pathogène compare de 12 souches de Bacillus thuringiensis Berl. sur l'abeille domestique et son couvain. Ann. Abeille 11, 31–40. https://doi.org/10.1051/apido:19680103.
Harrison, R.L., Herniou, E.S., Jehle, J.A., Theilmann, D.A., Burand, J.P., Becnel, J.J., Krell, P.J., van Oers, M.M., Mowery, J.D., Bauchan, G.R., 2018. ICTV Report Consortium. ICTV virus taxonomy profile: Baculoviridae. J. Gen. Virol. 99, 1185–1186. https://doi.org/10.1099/jgv.0.001107.
Hassona, N.M., Kordy, A.M., 2015. Relationship between toxicity of certain pesticides to the honey bee, *Apis mellifera* L. (Hymenoptera: apidea) foragers and their haemolymph amino acids. Middle East J. Appl. Sci. 5, 19–25.
Heinz, K.M., McCutchen, B.F., Herrmann, R., Parrella, M.P., Hammock, B.D., 1995. Direct effects of recombinant nuclear polyhedrosis viruses on selected nontarget organisms. J. Econ. Entomol. 88, 259–264. https://doi.org/10.1093/jee/88.2.259.
Hester, R., 2020. A Proteomic Analysis of the Buff-Tailed Bumblebee (*Bombus Terrestris*) Fat Body - an Immune Perspective. PhD Thesis. Maynooth University, Maynooth, Ireland.
Hokkanen, H.M.T., Zeng, Q.Q., Menzler-Hokkanen, I., 2003. Assessing the impacts of *Metarhizium* and *Beauveria* on bumblebees. In: Hokkanen, H.M.T., Hajek, A.E. (Eds.), Environmental Impacts of Microbial Insecticides - Need and Methods for Risk Assessment. Kluwer Academic Publishers, pp. 63–71.
Hyrsl, P., Dobes, P., Vojtek, L., Hroncova, Z., Tyl, J., Killer, J., 2017. Plant alkaloid sanguinarine and novel potential probiotic strains *Lactobacillus apis, Lactobacillus melliventris* and *Gilliamella apicola* promote resistance of honey bees to nematobacterial infection. Bull. Insectol. 70, 31–38.
Inamdar, A.A., Zaman, T., Morath, S.U., Pu, D.C., Bennett, J.W., 2014. *Drosophila melanogaster* as a model to characterize fungal volatile organic compounds. Environ. Toxicol. 29, 829–836. https://doi.org/10.1002/tox.21825.
Iqbal, M., Jützeler, M., França, S.C., Wäckers, F., Andreasson, E., Stenberg, J.A., 2021. Bee-vectored *Aureobasidium Pullulans* for Biocontrol of Grey Mould in Strawberry. Phytopathology (*Early Online*). https://doi.org/10.1094/PHYTO-05-21-0205-R.
Islam, W., Adnan, M., Shabbir, A., Naveed, H., Abubakar, Y.S., Qasim, M., Tayyab, M., Noman, A., Nisar, M.S., Khan, K.A., Ali, H., 2021. Insect-fungal-interactions: a detailed review on entomopathogenic fungi pathogenicity to combat insect pests. Microb. Pathog. 159, 105122. https://doi.org/10.1016/j.micpath.2021.105122.
James, R.R., Xu, J., 2012. Mechanisms by which pesticides affect insect immunity. J. Invertebr. Pathol. 109, 175–182. https://doi.org/10.1016/j.jip.2011.12.005.
James, R.R., Hayes, R., Leland, J.E., 2006. Field trials on the microbial control of Varroa with the fungus *Metarhizium anisopliae*. Am. Bee J. 146, 968–972.
James, R.R., McGuire, M.R., Leland, J.E., 2012. Susceptibility of adult alfalfa leafcutting bees and honey bees to a microbial control agent, *Beauveria bassiana*. Southwest. Entomol. 37, 13–21. https://doi.org/10.3958/059.037.0102.
Jaronski, S.T., Goettel, M.S., Lomer, C.J., 2003. Regulatory requirements for ecotoxicological assessments of microbial insecticides - how relevant are they? In: Hokkanen, H.M.T., Hajek, A.E. (Eds.), Environmental Impacts of Microbial Insecticides - Need and Methods for Risk Assessment. Kluwer Academic Publishers, pp. 237–260.

Johansen, C., 1962. Impregnated foundation for wax moth control. Glean. Bee Cult. 90, 682–684.
Johnson, K.B., Stockwell, V.O., Burgett, D.M., Sugar, D., Loper, J.E., 1993. Dispersal of *Erwinia amylovora* and *Pseudomonas fluorescens* by honey bees from hives to apple and pear blossoms. Phytopathology 83, 478–484. https://doi.org/10.1094/Phyto-83-478.
Kabaluk, J.T., Svircev, A.M., Goettel, M.S., Woo, S.G., 2010. The use and regulation of microbial pesticides in representative jurisdictions worldwide. IOBC Global, 99pp.
Kanga, L.H.B., James, R.R., Boucias, D.G., 2002. *Hirsutella thompsonii* and *Metarhizium anisopliae* as potential microbial control agents of *Varroa destructor*, a honey bee parasite. J. Invertebr. Pathol. 81, 175–184. https://doi.org/10.1016/S0022-2011(02)00177-5.
Kanga, L.H.B., Jones, W.A., James, R.R., 2003. Field trials using the fungal pathogen, *Metarhizium anisopliae* (Deuteromycetes: hyphomycetes) to control the ectoparasitic mite, *Varroa destructor* (Acari: varroidae) in honey bee, *Apis mellifera* (Hymenoptera: apidae) colonies. J. Econ. Entomol. 96, 1091–1099. https://doi.org/10.1093/jee/96.4.1091.
Kanga, L.H.B., Jones, W.A., Gracia, C., 2006. Efficacy of strips coated with *Metarhizium anisopliae* for control of *Varroa destructor* (Acari: varroidae) in honey bee colonies in Texas and Florida. Exp. Appl. Acarol. 40, 249–258. https://doi.org/10.1007/s10493-006-9033-2.
Kanga, L.H.B., Adamczyk, J., Patt, J., Gracia, C., Cascino, J., 2010. Development of a user-friendly delivery method for the fungus *Metarhizium anisopliae* to control the ectoparasitic mite *Varroa destructor* in honey bee, *Apis mellifera*, colonies. Exp. Appl. Acarol. 52, 327–342. https://doi.org/10.1007/s10493-010-9369-5.
Kapongo, J.P., Shipp, L., Kevan, P., Broadbent, B., 2008a. Optimal concentration of *Beauveria bassiana* vectored by bumble bees in relation to pest and bee mortality in greenhouse tomato and sweet pepper. BioControl 53, 797–812. https://doi.org/10.1007/s10526-007-9142-9.
Kapongo, J.P., Shipp, L., Kevan, P., Sutton, J.C., 2008b. Co-vectoring of *Beauveria bassiana* and Clonostachys rosea by bumble bees (*Bombus impatiens*) for control of insect pests and suppression of grey mould in greenhouse tomato and sweet pepper. Biol. Control 46, 508–514. https://doi.org/10.1016/j.biocontrol.2008.05.008.
Karise, R., Muljar, R., Smagghe, G., Kaart, T., Kuusik, A., Dreyersdorff, G., Williams, I.C. H., Mänd, M., 2016. Sublethal effects of kaolin and the biopesticides Prestop-Mix and BotaniGard on metabolic rate, water loss and longevity in bumble bees (*Bombus terrestris*). J. Pest. Sci. 89, 171–178. https://doi.org/10.1007/s10340-015-0649-z.
Karise, R., Raimets, R., Dreyersdorff, G., Mänd, M., 2018. Using respiratory physiology techniques in assessments of pesticide effects on bees. Julius-Kühn-Arch. 462, 61–66. https://doi.org/10.5073/jka.2018.462.014.
Kaya, H.K., Marston, J.M., Lindegren, J.E., Peng, Y.S., 1982. Low susceptibility of the honey bee, *Apis mellifera* L. (Hymenoptera: apidae), to the entomogenous nematode, *Neoaplectana carpocapsae* Weiser. Environ. Entomol. 11, 920–924. https://doi.org/10.1093/ee/11.4.920.
Kerwin, J.L., Dritz, D.A., Washino, R.K., 1988. Nonmammalian safety tests for *Lagenidium giganteum* (Oömycetes: lagenidiales). J. Econ. Entomol. 81, 158–171. https://doi.org/10.1093/jee/81.1.158.
Kingsbury, P., McLeod, B., Mortensen, K., 1978. Impact of Applications of Nuclear Polyhedrosis Virus of the Red-Headed Pine Sayfly, Neodiprion Lecontei (Fitch), on Non-target Organisms in 1977. Report FRM-X-11, Canadian Forestry Service. Department of Fisheries and the Environment, Ontario.
Knox, D.A., 1970. Tests of certain insect viruses on colonies of honeybees. J. Invertebr. Pathol. 16, 152. https://doi.org/10.1016/0022-2011(70)90225-9.
Köhl, J., Kolnaar, R., Ravensberg, W.J., 2019a. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front. Plant Sci. 10, 845. https://doi.org/10.3389/fpls.2019.00845.
Köhl, J., Booij, K., Kolnaar, R., Ravensberg, W.J., 2019b. Ecological arguments to reconsider data requirements regarding the environmental fate of microbial biocontrol agents in the registration procedure in the European Union. BioControl 64, 469–487. https://doi.org/10.1007/s10526-019-09964-y.
Kovach, J., Petzoldt, R., Harman, G.E., 2000. Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. Biol. Control 18, 235–242. https://doi.org/10.1006/bcon.2000.0839.
Krieg, A., 1961. *Bacillus thuringiensis* Berliner. Über seine Biologie, Pathogenie und Anwendung in der biologischen Schädlingsbekämpfung. Mitt. Biol. Bundesanst. Land- Forstwirtsch. Berl.-Dahl. 103 https://doi.org/10.5073/20210622-112833.
Krieg, A., 1962. Neues über insektenpathogene, kristallbildende Bazillen. Anz. für Schädlingskd. 35, 182–188. https://doi.org/10.1007/BF01881891.
Krieg, A., 1964. Über die Bienenverträglichkeit verschiedener Industrie-Präparate des *Bacillus thuringiensis*. Anz. für Schädlingskd. 37, 39–40. https://doi.org/10.1007/BF01858122.
Krieg, A., 1973. About toxic effects of cultures of *Bacillus cereus* and *Bacillus thuringiensis* on honey bees (*Apis mellifera*). J. Plant Dis. Prot. 80, 483–486.
Krieg, A., Herfs, W., 1962. Nebenwirkungen von *Bacillus thuringiensis*. Einwirkungen auf Bienen (*Apis mellifera* L.). Coll. Int. Pathol. Insectes, Paris, 1962. Entomophaga Mem. Hors Ser. 2, 193–195.
Krieg, A., Herfs, W., 1963. Über die Wirkung von *Bacillus thuringiensis* auf Bienen. Entomol. Exp. Appl. 6, 1–9. https://doi.org/10.1111/j.1570-7458.1963.tb00598.x.
Krieg, A., Hassan, S., Pinsdorf, W., 1980. Wirkungsvergleich der Varietät *israelensis* mit anderen Varietäten des *Bacillus thuringiensis* an Nicht-Zielorganismen der Ordnung Hymenoptera: *Trichogramma cacoeciae* und *Apis mellifera*. Anz. Schädlingskd. Pfl. 53, 81–83. https://doi.org/10.1007/bf01958130.
Kwon, Y.J., Saeed, S., Duchateau, M.J., 2003. Control of *Plodia interpunctella* (Lepidoptera: Pyralidae), a pest in *Bombus terrestris* (Hymenoptera: apidae) colonies. Can. Entomol. 135, 893–902. https://doi.org/10.4039/n03-013.
Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? Biol. Control 21, 230–248. https://doi.org/10.1006/bcon.2001.0938.
Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., 2015. Insect pathogens as biological control agents: back to the future. J. Invertebr. Pathol. 132, 1–41. https://doi.org/10.1016/j.jip.2015.07.009.
LeComte, J., Martouret, D., 1959. Non toxicité pour les abeilles des traitements a base de *Bacillus thuringiensis*, souche anduze (Bactérie pathogène pour les larves de Lépidoptères). Ann. Abeille 2, 171–175. https://doi.org/10.1051/apido:19590205.
Lehmann, D.M., Camp, A.A., 2021. A systematic scoping review of the methodological approaches and effects of pesticide exposure on solitary bees. PLoS One 16, e0251197. https://doi.org/10.1371/journal.pone.0251197.
Libardoni, G., de Gouvea, A., Costa-Maia, F.M., Lozano, E.R., de Freitas, P.F., Colombo, F. C., Raulino, F., Maciel, R.M.A., Potrich, M., 2018. Effect of different *Bacillus thuringiensis* strains on the longevity of Africanized honey bee. Ciênc. Agrár. 39, 329–338. https://doi.org/10.5433/1679-0359.2018v39n1p329.
Libardoni, G., Neves, P.M.O.J., Abati, R., Sampaio, A.R., Costa-Maia, F.M., de Souza Vismara, E., Lozano, E.R., Potrich, M., 2021. Possible interference of *Bacillus thuringiensis* in the survival and behavior of Africanized honey bees (*Apis mellifera*). Sci. Rep. 11, 3482. https://doi.org/10.1038/s41598-021-82874-1.
Lipa, J.J., Hokkanen, H.M.T., 1992. Safety of *Nosema meligethi* I. And R. (Microsporida) to *Apis mellifera* L. And *Coccinella septempunctata* L. J. Invertebr. Pathol. 60, 310–311. https://doi.org/10.1016/0022-2011(92)90015-V.
Maccagnani, B., Giacomello, F., Fanti, M., Gobbin, D., Maini, S., Angeli, G., 2009. *Apis mellifera* and *Osmia cornuta* as carriers for the secondary spread of *Bacillus subtilis* on apple flowers. BioControl 54, 123–133. https://doi.org/10.1007/s10526-008-9163-z.
Maebe, K., Vanderhaegen, R., Pisman, M., Eeraerts, M., Cottyn, B., Vanhoutte, B., Smagghe, G., 2020. Distribution of a model biocontrol agent (Serenade® MAX) in apple and pear by mason bees and bumble bees. Agric. For. Entomol. 23, 97–103. https://doi.org/10.1111/afe.12396.
Malone, L.A., Pham-Delègue, M.-H., 2001. Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.). Apidologie 32, 287–304. https://doi.org/10.1051/apido:2001130.
Malone, L.A., Burgess, E.P.J., Stefanovic, D., 1999. Effects of a *Bacillus thuringiensis* toxin, two *Bacillus thuringiensis* biopesticide formulations, and a soybean trypsin inhibitor on honey bee (*Apis mellifera* L.) survival and food consumption. Apidologie 30, 465–473. https://doi.org/10.1051/apido:19990601.
Maramorosch, K., 1985. Viral Insecticides for Biological Control. Academic Press, Orlando, Fl, USA.
Medina, R.G., Paxton, R.J., Hernández-Sotomayor, S.M.T., Pech-Jiménez, C., Medina-Medina, L.A., Quezada-Euán, J.J.G., 2020. Heat stress during development affects immunocompetence in workers, queens and drones of Africanized honey bees (*Apis mellifera* L.) (Hymenoptera: apidae). J. Therm. Biol. 89, 102541. https://doi.org/10.1016/j.jtherbio.2020.102541.
Meikle, W.G., Mercadier, G., Girod, V., Derouané, F., Jones, W.A., 2006. Evaluation of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: hyphomycetes) strains isolated from varroa mites in southern France. J. Apicult. Res. 45, 219–220. https://doi.org/10.1080/00218839.2006.11101352.
Meikle, W.G., Mercadier, G., Holst, N., Nansen, C., Girod, V., 2007. Duration and spread of an entomopathogenic fungus, *Beauveria bassiana* (Deuteromycota: hyphomycetes), used to treat Varroa mites (Acari: varroidae) in honey bee (Hymenoptera: apidae) hives. J. Econ. Entomol. 100, 1–10. https://doi.org/10.1093/jee/100.1.1.
Meikle, W.G., Mercadier, G., Holst, N., Nansen, C., Girod, V., 2008a. Impact of a treatment of *Beauveria bassiana* (Deuteromycota: hyphomycetes) on honeybee (*Apis mellifera*) colony health and on *Varroa destructor* mites (Acari: varroidae). Apidologie 39, 247–259. https://doi.org/10.1051/apido:2007057.
Meikle, W.G., Mercadier, G., Holst, N., Girod, V., 2008b. Impact of two treatments of a formulation of *Beauveria bassiana* (Deuteromycota: hyphomycetes) conidia on Varroa mites (Acari: varroidae) and on honeybee (Hymenoptera: apidae) colony health. Exp. Appl. Acarol. 46, 105–117. https://doi.org/10.1007/s10493-008-9160-z.
Meikle, W.G., Mercadier, G., Annas, F., Holst, N., 2009. Effects of multiple applications of a *Beauveria* based biopesticide on *Varroa destructor* (Acari: varroidae) densities in honey bee (Hymenoptera: apidae) colonies. J. Apicult. Res. 48, 220–222. https://doi.org/10.3896/IBRA.1.48.3.13.
Meikle, W.G., Mercadier, G., Guermache, F., Bon, M.C., 2012a. *Pseudomonas* contamination of a fungus-based biopesticide: implications for honey bee (Hymenoptera: apidae) health and Varroa mite (Acari: varroidae) control. Biol. Control 60, 312–320. https://doi.org/10.1016/j.biocontrol.2011.12.004.
Meikle, W.G., Sammataro, D., Neumann, P., Pflugfelder, J., 2012b. Challenges for developing pathogen-based biopesticides against *Varroa destructor* (Mesostigmata: varroidae). Apidologie 43, 501–514. https://doi.org/10.1007/s13592-012-0118-0.
Meixner, M.D., Francis, R.M., Gajda, A., Kryger, P., Andonov, S., Uzunov, A., Topolska, G., Costa, C., Amiri, E., Berg, S., Bienkowska, M., Bouga, M., Büchler, R., Dyrba, W., Gurgulova, K., Hatjina, F., Ivanova, E., Janes, M., Kezic, N., Korpela, S., Le Conte, Y., Panasiuk, B., Pechhacker, H., Tsoktouridis, G., Vaccari, G., Wilde, J., 2014. Occurrence of parasites and pathogens in honey bee colonies used in a European genotype-environment interactions experiment. J. Apicult. Res. 53, 215–229. https://doi.org/10.3896/IBRA.1.53.2.04.
Menapace, D.M., Sackett, R., Wilson, W.T., 1978. Adult honey bees are not susceptible to infection by *Nosema locustae*. J. Econ. Entomol. 71, 304–306. https://doi.org/10.1093/jee/71.2.304.
Mommaerts, V., Smagghe, G., 2011. Entomovectoring in plant protection. Arthropod Plant Interact 5, 81–95. https://doi.org/10.1007/s11829-011-9123-x.

Mommaerts, V., Boulet, J., Sterk, G., Smagghe, G., 2007. Effects of biological control agents (BCAs) on the pollinator, *Bombus terrestris*. XVI International Plant Protection Congress: Congress Proceedings 2, 862–863.

Mommaerts, V., Platteau, G., Boulet, J., Sterk, G., Smagghe, G., 2008. *Trichoderma*-based biological control agents are compatible with the pollinator *Bombus terrestris*: a laboratory study. Biol. Control 46, 463–466. https://doi.org/10.1016/j.biocontrol.2008.05.007.

Mommaerts, V., Sterk, G., Hoffmann, L., Smagghe, G., 2009. A laboratory evaluation to determine the compatibility of microbiological control agents with the pollinator *Bombus terrestris*. Pest Manag. Sci. 65, 949–955. https://doi.org/10.1002/ps.1778.

Mommaerts, V., Jans, K., Smagghe, G., 2010. Impact of *Bacillus thuringiensis* strains on survival, reproduction and foraging behaviour in bumblebees (*Bombus terrestris*). Pest Manag. Sci. 66, 520–525. https://doi.org/10.1002/ps.1902.

Mommaerts, V., Put, K., Smagghe, G., 2011. *Bombus terrestris* as pollinator-and-vector to suppress *Botrytis cinerea* in greenhouse strawberry. Pest Manag. Sci. 67, 1069–1075. https://doi.org/10.1002/ps.2147.

Mommaerts, V., Put, K., Vandeven, J., Smagghe, G., 2012. Miniature-dispenser-based bioassay to evaluate the compatibility of powder formulations used in an entomovectoring approach. Pest Manag. Sci. 68, 922–927. https://doi.org/10.1002/ps.3251.

Montesinos, E., 2003. Development, registration and commercialization of microbial pesticides for plant protection. Int. Microbiol. 6, 245–252. https://doi.org/10.1007/s10123-003-0144-x.

Morton, H.L., Moffett, J.O., Stewart, F.D., 1975. Effect of alfalfa looper nuclear polyhedrosis virus on honeybees. J. Invertebr. Pathol. 26, 139–140. https://doi.org/10.1016/0022-2011(75)90184-6.

Mulimani, V., KulkaRni, K.A., 2004. Evaluation of safety of *Nomuraea rileyi* (Farlow) Samson to larval parasitoid, *Microplitis maculipennis* Szepl. and honeybee, *Apis cerena* indica Fabricius. J. Biol. Control 18, 85–86. https://doi.org/10.18311/jbc/2004/4068.

Nguyen, K.B., Smart, G.C., 1991. Pathogenicity of *Steinernema scapterisci* to selected invertebrates. J. Nematol. 23, 7–11.

Omuse, E.R., Niassy, S., Wagacha, J.M., Ong'amo, G.O., Lattorff, H.M.G., Kiatoko, N., Mohamed, S.A., Subramanian, S., Akutse, K.S., Dubois, T., 2022. Susceptibility of the Western honey bee *Apis mellifera* and the African stingless bee *Meliponula ferruginea* (Hymenoptera: apidae) to the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. J. Econ. Entomol. 115, 46–55. https://doi.org/10.1093/jee/toab211.

Peng, C.Y.S., Zhou, X., Kaya, H.K., 2002. Virulence and site of infection of the fungus, *Hirsutella thompsonii*, to the honey bee ectoparasitic mite, *Varroa destructor*. J. Invertebr. Pathol. 81, 185–195. https://doi.org/10.1016/S0022-2011(02)00188-X.

Peng, G., Tong, S.M., Zeng, D., Xia, Y., Feng, M.G., 2020. Colony heating protects honey bee populations from a risk of contact with wide-spectrum *Beauveria bassiana* insecticides applied in the field. Pest Manag. Sci. 76, 2627–2634. https://doi.org/10.1002/ps.5803.

Porcar, M., Gómez, F., Gruppe, A., Gómez-Pajuelo, A., Segura, I., Schröder, R., 2008. Hymenopteran specificity of *Bacillus thuringiensis* strain PS86Q3. Biol. Control 45, 427–432. https://doi.org/10.1016/j.biocontrol.2008.02.002.

Portilla, M., Luttrell, R., Snodgrass, G., Zhu, Y.C., Riddick, E., 2017. Lethality of the entomogenous fungus *Beauveria bassiana* strain NI8 on *Lygus lineolaris* (Hemiptera: Miridae) and its possible impact on beneficial arthropods. J. Entomol. Sci. 52, 352–369. https://doi.org/10.18474/JES17-15.1.

Potrich, M., da Silva, R.T.L., Maia, F.M.C., Lozano, E.R., Rossi, R.M., Colombo, F.C., Tedesco, F.G., de Gouvea, F., 2018. Effect of entomopathogens on africanized *Apis mellifera* L. (Hymenoptera: apidae). Rev. Bras. Entomol. 62, 23–28. https://doi.org/10.1016/j.rbe.2017.12.002.

Potrich, M., Colombo, F.C., Zanon, A.C., Lozano, E.R., Maciel, R.M.A., Tedesco, F.G., Rodrigues, M.C., Costa-Maia, F.M., 2020. Control agents on the quality of Africanized honeybee queens. Ciênc. Agrár. 41, 1773–1782. https://doi.org/10.5433/1679-0359.2020v41n5p1773.

Preininger, C., Sauer, U., Bejarano, A., Berninger, T., 2018. Concepts and applications of foliar spray for microbial inoculants. Appl. Microbiol. Biotechnol. 102, 7265–7282. https://doi.org/10.1007/s00253-018-9173-4.

Qu, S., Wang, S., 2018. Interaction of entomopathogenic fungi with the host immune system. Dev. Comp. Immunol. 83, 96–103. https://doi.org/10.1016/j.dci.2018.01.010.

Ramanaidu, K., Cutler, G.C., 2013. Different toxic and hormetic responses of *Bombus impatiens* to *Beauveria bassiana, Bacillus subtilis* and spirotetramat. Pest Manag. Sci. 69, 949–954. https://doi.org/10.1002/ps.3456.

Reeh, K.W., Hillier, N.K., Cutler, G.C., 2014. Potential of bumble bees as bio-vectors of *Clonostachys rosea* for Botrytis blight management in lowbush blueberry. J. Pest. Sci. 87, 543–550. https://doi.org/10.1007/s10340-014-0565-7.

Renzi, M.T., Amichot, M., Pauron, D., Tchamitchian, S., Brunet, J.L., Kretzschmar, A., Maini, S., Belzunces, L.B., 2016. Chronic toxicity and physiological changes induced in the honey bee by the exposure to fipronil and *Bacillus thuringiensis* spores alone or combined. Ecotoxicol. Environ. Saf. 127, 205–213. https://doi.org/10.1016/j.ecoenv.2016.01.028.

Richardson, P.N., 1996. British and European legislation regulating rhabditid nematodes. Biocontrol Sci. Technol. 6, 449–464. https://doi.org/10.1080/09583159631415.

Ricroch, A., Akkoyunlu, S., Martin-Laffon, J., Kuntz, M., 2018. Assessing the environmental safety of transgenic plants: honey bees as a case study. Adv. Bot. Res. 86, 111–167. https://doi.org/10.1016/bs.abr.2017.11.004.

Rodríguez, M., Gerding, M., France, A., Ceballos, R., 2009. Evaluation of *Metarhizium anisopliae* var. *anisopliae* Qu-M845 isolate to control Varroa destructor (Acari: varroidae) in laboratory and field trials. Chil. J. Agric. Res. 69, 541–547. https://doi.org/10.4067/S0718-58392009000400009.

Rosana, A.R.R., Pokorny, S., Klutsch, J.G., Ibarra-Romero, C., Sanichar, R., Engelhardt, D., van Belkum, M.J., Erbilgin, N., Bohlmann, J., Carroll, A.L., Vederas, J.C., 2021. Selection of entomopathogenic fungus *Beauveria bassiana* (*deuteromycotina: hyphomycetes*) for the biocontrol of *Dendroctonus ponderosae* (*Coleoptera: Curculionidae, scolytinae*) in western Canada. Appl. Microbiol. Biotechnol. 105, 2541–2557. https://doi.org/10.1007/s00253-021-11172-7.

Sabo, R., Kopčáková, A., Hamarová, L., Cingeľová Maruščáková, I., Mudroňová, D., Sabová, L., Javorský, P., Legáth, J., 2020. Sublethal effects of commercial plant protection product containing spores *Bacillus amyloliquefaciens* QST 713 (formerly *subtilis*) on winter adult honeybees. Apidologie 51, 226–239. https://doi.org/10.1007/s13592-019-00705-9.

Schisler, D.A., Slininger, P.J., Behle, R.W., Jackson, M.A., 2004. Formulation of *Bacillus* spp. for biological control of plant diseases. Phytopathology 94, 1267–1271. https://doi.org/10.1094/PHYTO.2004.94.11.1267.

Sewify, G.H., Ibrahim, Y.Y., El-Deen, M.S., 2015. *Beauveria bassiana* (Balsamo), a potential mycopesticide for efficient control of the honey bee ectoparasitic mite, *Varroa destructor* Anderson and Trueman. Egypt. J. Biol. Pest Control 25, 333–337. https://doi.org/10.1016/j.icheatmasstransfer.2011.12.002.

Shamseldean, M.M., El-Sadawy, E., Allam, S.F.M., 2004. Comparative safety of entomopathogenic nematodes on honeybee workers. Egypt. J. Biol. Pest Control 14, 147–153.

Shanks, J.L., Haigh, A.M., Riegler, M., Spooner-Hart, R.N., 2017. First confirmed report of a bacterial brood disease in stingless bees. J. Invertebr. Pathol. 144, 7–10. https://doi.org/10.1016/j.jip.2017.01.004.

Shaw, K.E., Davidson, G., Clark, S.J., Ball, B.V., Pell, J.K., Chandler, D., Sunderland, K.D., 2002. Laboratory bioassays to assess the pathogenicity of mitosporic fungi to *Varroa destructor* (Acari: mesostigmata), an ectoparasitic mite of the honeybee, *Apis mellifera*. Biol. Control 24, 266–276. https://doi.org/10.1016/S1049-9644(02)00029-4.

Shimanuki, H., Hartman, P.A., Rothenbuhler, W.C., 1963. Effect of *Bacillus thuringiensis* on honey bee populations. Bacteriol. Proc. 63, 10.

Shipp, L., Kapongo, J.P., Park, H.H., Kevan, P., 2012. Effect of bee-vectored *Beauveria bassiana* on greenhouse beneficials under greenhouse cage conditions. Biol. Control 63, 135–142. https://doi.org/10.1016/j.biocontrol.2012.07.008.

Sinia, A., Guzman-Novoa, E., 2018. Evaluation of the entomopathogenic fungi *Beauveria bassiana* GHA and *Metarhizium anisopliae* UAMH 9198 alone or in combination with thymol for the control of *Varroa destructor* in honey bee (*Apis mellifera*) colonies. J. Apicult. Res. 57, 308–316. https://doi.org/10.1080/00218839.2018.1430983.

Smagghe, G., De Meyer, L., Meeus, I., Mommaerts, V., 2013. Safety and acquisition potential of *Metarhizium anisopliae* in entomovectoring with bumble bees, *Bombus terrestris*. J. Econ. Entomol. 106, 277–282. https://doi.org/10.1603/EC12332.

Smagghe, G., Boecking, O., Maccagnani, B., Mänd, M., Kevan, P.G., 2020. Entomovectoring for Precision Biocontrol and Enhanced Pollination of Crops. Springer Nature, Cham, Switzerland. https://doi.org/10.1007/978-3-030-18917-4.

Soni, J., Thakur, M., 2011. Effect of biopathogens on honey bees. Pest Technol. 5, 86–90.

Steinigeweg, C., Alkassab, A.T., Beims, H., Eckert, J.H., Richter, D., Pistorius, J., 2021. Assessment of the impacts of microbial plant protection products containing *Bacillus thuringiensis* on the survival of adults and larvae of the honeybee (*Apis mellifera*). Environ. Sci. Pollut. Res. 28, 29773–29780. https://doi.org/10.1007/s11356-021-12446-3.

Steinigeweg, C., Alkassab, A.T., Erler, S., Beims, H., Wirtz, I.P., Richter, D., Pistorius, J., 2022. Impact of a microbial pest control product containing *Bacillus thuringiensis* on brood development and gut microbiota of *Apis mellifera* worker honey bees. Microb. Ecol.

Sterk, G., Heuts, F., Merck, N., Bock, J., 2002. Sensitivity of non-target arthropods and beneficial fungal species to chemical and biological plant protection products: results of laboratory and semi-field trials. 1st International Symposium on Biological Control of Arthropods 306–313.

Stone, L.B.L., Bidochka, M.J., 2020. The multifunctional lifestyles of *Metarhizium*: evolution and applications. Appl. Microbiol. Biotechnol. 104, 9935–9945. https://doi.org/10.1007/s00253-020-10968-3.

Straub, L., Strobl, V., Neumann, P., 2020. The need for an evolutionary approach to ecotoxicology. Nat. Ecol. Evol. 4, 895. https://doi.org/10.1038/s41559-020-1194-6.

Stute, K., 1963. Über die Wirkung von *Bacillus thuringiensis* auf die Honigbiene (*Apis mellifera* L.). Nachrichtenbl. Deut. Pflanzenschutzd. 15, 102–104.

Taha, E.H., Abdelmegeed, S.M., 2016. Effect of entomopathogenic nematodes, *Heterorhabditis bacteriophora* on *Galleria mellonella* in bee hives of *Apis mellifera*. J. Biol. Sci. 16, 197–201. https://doi.org/10.3923/jbs.2016.197.201.

Toledo-Hernández, R.A., Ruíz-Toledo, J., Toledo, J., Sánchez, D., 2016. Effect of three entomopathogenic fungi on three species of stingless bees (Hymenoptera: apidae) under laboratory conditions. J. Econ. Entomol. 109, 1015–1019. https://doi.org/10.1093/jee/tow064.

Tsutsumi, T., Sakaguchi, N., Totogawa, S., Yamanaka, M., 1998. Pathogenicity of entomogenous fungus, cerambycid-parasitic type of *Beauveria brongniartii*. Jpn. J. Appl. Entomol. Zool. 42, 163–165. https://doi.org/10.1303/jjaez.42.163.

Uzunov, A., Costa, C., Panasiuk, B., Meixner, M., Kryger, P., Hatjina, F., Bouga, M., Andonov, S., Bienkowska, M., Le Conte, Y., Wilde, J., Gerula, D., Kiprijanovska, H., Filipi, J., Petrov, P., Ruottinen, L., Pechhacker, H., Berg, S., Dyrba, W., Ivanova, E., Büchler, R., 2014. Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic originin a pan-European experiment. J. Apicult. Res. 53, 248–260. https://doi.org/10.3896/IBRA.1.53.2.06.

van der Steen, J.J.M., Langerak, C.J., van Tongeren, C.A.M., Dik, A.J., 2004. Aspects of the use of honeybees and bumblebees as vector of antagonistic micro-organisms in plant disease control. Proc. Neth. Entomol. Soc. 15, 41–46.

van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A., 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63, 39–59. https://doi.org/10.1007/s10526-017-9801-4.

Vandenberg, J.D., 1990. Safety of four entomopathogens for caged adult honey bees (Hymenoptera: apidae). J. Econ. Entomol. 83, 755–759. https://doi.org/10.1093/jee/83.3.755.

Vandenberg, J.D., Streett, D.A., Herbert, E.W., 1990. Safety of grasshopper entomopoxviruses for caged adult honey bees (Hymenoptera: apidae). J. Econ. Entomol. 83, 784–787. https://doi.org/10.1093/jee/83.3.784.

Verma, S.K., 1995. Studies on the control of greater wax moth, *Galleria mellonella* L. in *Apis ceranae* F. colonies with the biological insecticide, DIPEL. Indian Bee J. 57, 121–123.

Wallner, K., 1988. Gefahren für die Honigbiene durch den Maikäfer-Bekämpfungsversuch im Forstbezirk Karlsruhe-Hardt. Mitt. Forstl. Vers.- Forsch. anst. Baden-Württ., Freiburg/B. 132, 155–163.

Wilson, W.T., 1962. Observations on the effects of feeding large quantities of *Bacillus thuringiensis* Berliner to honey bees. J. Insect Pathol. 4, 269–270.

You, H., Wan, H., Li, J., Jin, B.R., 2010. Molecular cloning and characterization of a short peptidoglycan recognition protein (PGRP-S) with antibacterial activity from the bumblebee *Bombus ignitus*. Dev. Comp. Immunol. 34, 977–985. https://doi.org/10.1016/j.dci.2010.04.007.

Yun, D.C., Yang, S.Y., Kim, Y.C., Kim, I.S., Kim, Y.H., 2013. Identification of surfactin as an aphicidal metabolite produced by *Bacillus amyloliquefaciens* G1. Appl. Biol. Chem. 56, 751–753. https://doi.org/10.1007/s13765-013-3238-y.

Zimmermann, G., 2007a. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Sci. Technol. 17, 553–596. https://doi.org/10.1080/09583150701309006.

Zimmermann, G., 2007b. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Technol. 17, 879–920. https://doi.org/10.1080/09583150701593963.

Żółtowska, K., Lipiński, Z., Łopieńska, E., 2003a. Beneficial nematodes: a potential threat to honey bees? Bee World 84, 125–129. https://doi.org/10.1080/0005772X.2003.11099588.

Żółtowska, K., Lipiński, Z., Łopieńska, E., 2003b. The level of protein and activity of hydrolases after infection of honeybee larvae with entomopathogenic nematodes. J. Apicult. Sci. 47, 31–37.