



Biosafety Assessment and Residual Pathogenic Effects of Entomopathogenic Fungi

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Abstract

Entomopathogenic fungi (EPF) have emerged as promising biological alternatives to chemical pesticides for integrated pest management (IPM). However, their widespread application necessitates a thorough understanding of their safety and residual pathogenic effects. This review critically evaluates the current knowledge on EPF toxicity to non-target organisms, including pollinators, beneficial arthropods, vertebrates, and humans. The implications of secondary metabolite production, endophytic behavior, and environmental persistence are discussed in relation to ecological and human health safety. Regulatory frameworks from major regions, including the USA, EU, Canada, Australia, Brazil, India, China, and Japan, are compared to highlight global standards in EPF risk assessment. Even with strict rules in place, there are still important areas we do not fully understand, especially about how long these substances last, which plants they affect, how toxic the metabolites are, and the risks to humans. The review underscores the urgent need for harmonized safety protocols, robust field data, and genomic tools to enhance risk evaluation and ensure the responsible deployment of EPF in sustainable agriculture.

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1 Introduction

Pests are responsible for the loss of 20% to 40% of global crop production annually. Invasive insect species alone result in an estimated economic cost of approximately \$70 billion worldwide (Gula 2023). Crop protection makes up just 5–8% of total farming costs, yet its effectiveness varies by pest type. Managing pests leads to up to 32% yield enhancements. Protection is generally more effective in cash crops, preventing 53–68% of losses, versus 43–50% in food crops. With climate change and global trade accelerating, pest impacts are expected to rise—yield losses may increase by 10–25% for every 1 °C rise in temperature (Gordon 2023).

Since pests cause significant damage to agriculture, farmers often use pesticides as a quick and effective method to protect their crops and reduce losses. In the USA, nearly 1.3 billion USD of pesticides are used annually to combat weeds, insects, fungi, and other pests threatening crops (TraceXtech Technologies 2024). Pesticides, including herbicides, insecticides, and fungicides, are formulated to target pests, offering immediate relief from infestations that can otherwise devastate harvests. However, the benefits of pesticides come with considerable environmental costs. These chemicals do not always remain confined to the area where they are applied. Through runoff and leaching, pesticides can contaminate nearby rivers, lakes, and groundwater. Aquatic organisms, including fish, amphibians, and insect larvae, are particularly vulnerable to pesticide exposure, which can lead to population declines and biodiversity loss. Furthermore, pesticides often affect non-target organisms, such as pollinators and natural pest predators and parasitosis, disrupting ecological balance. Many synthetic pesticides are persistent, leading to bioaccumulation—a process where toxic substances build up in organisms over time and transfer up the food chain, impacting predators and top consumers. The case of DDT is a powerful reminder of these dangers: the pesticide weakened eggshells in birds of prey like bald eagles, pushing them toward near extinction before its ban (US EPA 2025). Similarly, over 120 countries, under the Stockholm Convention, agreed to phase out the toxic pesticide endosulfan, an organochlorine-based insecticide, by 2012, permitting limited use on specific crop-pest combinations, such as cotton for bollworm control, until 2017 (Hogue 2011).

The Directorate of Plant Protection, Quarantine and Storage, India, has circulated a document titled ‘List of Banned, Refused Registration, and Restricted Pesticides in India (as of 31.03.2024)’, which outlines key regulatory measures taken by the Indian government to ensure pesticide safety. A total of 49 pesticides have been completely banned for manufacture, import, and use due to their high toxicity and environmental persistence; these include chemicals such as Endosulfan, Aldicarb, Carbaryl, Paraquat, Phorate, and Triazophos. Additionally, some pesticide

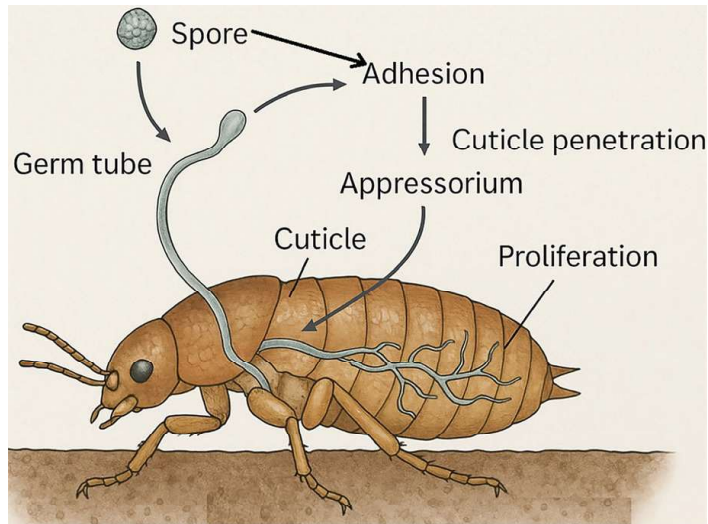
formulations like Dichlorvos and Captafol 80% powder are banned for domestic use but still permitted for export. The list also includes eight pesticides that have been withdrawn from use until the required safety data is submitted and approved, such as Simazine and Warfarin. Furthermore, 18 pesticides have been refused registration altogether, including 2,4,5-T, lead arsenate, and azinphos methyl, meaning they cannot be produced, sold, or used in the country. Several pesticides are also restricted in their usage; for instance, monocrotophos is banned on vegetables, DDT is limited to public health applications, and aluminum phosphide use is restricted to trained experts under government supervision. These actions reflect India's commitment to safeguarding human health and the environment and aligning with global standards for pesticide management (Directorate of Plant Protection, Quarantine and Storage 2024).

To mitigate these impacts, sustainable pest management approaches are increasingly being promoted, with a focus on reducing chemical inputs and restoring ecological balance (Stellarix 2024). One promising solution is the use of EPF—beneficial microorganisms that naturally infect and kill insect pests (Ghorui et al. 2024). EPF, such as *Beauveria bassiana* and *Metarhizium anisopliae*, are environmentally friendly biocontrol agents that offer targeted pest suppression without harming beneficial insects or the surrounding ecosystem. These fungi can be applied to crops much like conventional pesticides, but with significantly lower ecological risks.

Incorporating EPF into IPM strategies can help reduce dependency on chemical pesticides, making agriculture more resilient, sustainable, and aligned with environmental conservation goals. Various companies produce EPN-based products targeting pests like whiteflies, aphids, and thrips, among others. Bioworks Inc., USA, offers PRINCIPLE WP, which contains *B. bassiana* Strain BW149, effective against thrips, aphids, whiteflies, mealybugs, and other pests (Digital 2025). Koppert Biological Systems (Netherlands) markets Mycotal, which is based on *Akanthomyces muscarius* Ve6 strain (formerly known as *Lecanicillium lecanii*), targeting pests such as white fly, thrips, and aphids (Mycotal 2025). Lallemand Plant Care (USA) provides LALGUARD M52 OD (formerly Met52), which includes *Metarhizium brunneum* strain F52 (formerly *M. anisopliae*) and is used for controlling whiteflies, thrips, mites, and aphids (Lallemand Plant Care 2025). BASF (Germany) produces Velifer, which contains *B. bassiana* strain PPRI 5339 and is aimed at managing whiteflies and thrips (BioProtection Portal 2023).

EPF are soil-dwelling organisms that infect and kill insect pests by breaching their outer cuticle and invading their internal tissues, where they grow and consume the host (Dara 2017). These fungi belong to several divisions, including Ascomycota, Zygomycota, Basidiomycota, Glomeromycota, and Chytridiomycota (Mora et al. 2018). EPF play a significant role in regulating insect pest populations within soil ecosystems, employing multiple mechanisms to infect and kill pests, including starvation, toxin production, and enzymatic degradation of the insect cuticle (Dara 2017). These fungi secrete hydrolytic enzymes such as chitinases, proteases, and lipases, which help breach the cuticle—the insect's first line of defense (Vega et al. 2008). Spores or conidia adhere to the insect surface through

Fig. 17.1 Infection of entomopathogenic fungus on insects



the interaction of surface proteins and host cuticle lipids, sometimes aided by sticky secretions or hydrophobic proteins like hydrophobins (Holder and Keyhani 2005). The overview of infection mechanisms of EPF on insects was illustrated in Fig. 17.1.

Following adhesion, conidia germinate and may form specialized structures like appressoria to physically and enzymatically penetrate the host (Télliez-Jurado et al. 2009). Fungal entry is typically through the cuticle but can also occur via spiracles or sensory organs (St Leger 1991). Once inside the hemocoel, fungal hyphae proliferate, disrupting internal organs and leading to host death (Locke 2001). Virulence is strongly associated with enzyme production, such as PR1 proteases in *M. anisopliae* and chitinases in *B. bassiana* (St Leger 1991; Fan et al. 2007). These traits highlight EPF's potential as biological control agents.

The presence of EPF in natural ecosystems has been widely recognized and documented across the globe (Zimmermann 2007). Over the years, several EPF species have emerged as eco-friendly alternatives to synthetic chemical insecticides, many of which have been phased out due to their environmental hazards, health risks, and the development of resistance among target pest populations (Butt et al. 2001). Some EPF species that have shown commercial potential or are already in use include *M. anisopliae*, *B. bassiana*, *Beauveria brongniartii*, *Lecanicillium* spp. (previously known as *Verticillium* spp.), and *Isaria fumosorosea* (formerly, *Paecilomyces fumosoroseus*). A range of registered biopesticide products based on these fungi has been catalogued, reflecting their practical application in sustainable pest management (de Faria and Wraight 2007). These developments underscore the growing significance of EPF as integral components of IPM strategies, offering sustainable, biologically based solutions for long-term pest management.

The prime objective in testing the applicability and efficacy of chemicals and/or microbiological agents used for plant protection and environmental safety is crucial (Brühl and Zaller 2019; Scheepmaker et al. 2019). Regulatory frameworks aim to distinguish products with acceptable risk profiles, particularly regarding their effects on non-target organisms (Gwynn 2017). In the EU, this is assessed using a tiered

system that begins with basic laboratory toxicity tests. If risks appear significant, more advanced tests that simulate real-world conditions are required (Schäfer et al. 2019). Historically, assessment protocols did not differentiate between chemical and microbial agents, overlooking key differences such as specificity, persistence, and the ability of microbes to reproduce (Chandler et al. 2008; OECD 2019). This mismatch often led to unmet data requirements for microbial products, delaying their registration and increasing development costs (Köhl et al. 2019). To address this, separate assessment frameworks have been recommended (Sundh and Goettel 2012). While general guidance on microbial safety has been issued by the OECD (2014), specific protocols, especially for non-target organism testing, are still lacking (Arora et al. 2016). Consequently, there remains a pressing need for the development and harmonization of tailored regulatory protocols that accurately reflect the unique characteristics of microbial agents, thereby facilitating their safe and efficient integration into sustainable agricultural practices.

Biosafety and risk assessment are essential for the responsible use of EPF in pest management. As living organisms, EPF may interact with non-target species or ecosystems in unpredictable ways. Assessing their safety ensures they do not pose risks to human health, beneficial organisms, or the environment. This is particularly important since EPF products differ significantly from chemical pesticides in behavior, persistence, and by-products. Therefore, a thorough and scientifically grounded biosafety and risk assessment framework is crucial to support the safe deployment of EPF, ensuring their benefits in pest management are realized without compromising ecological integrity and/or public health.

This review will critically evaluate the safety profile of EPF, encompassing their effects on non-target organisms, environmental persistence, and risks associated with secondary metabolites, while identifying key knowledge gaps in host specificity, long-term ecological impacts, and the need for harmonized global risk assessment protocols.

2 Safety Assessment

The environmental and animal safety of biological agents has been a recurring concern. Many biochemical compounds produced by fungal endophytes are known to cause adverse effects in livestock. The environmental biosafety of the secondary metabolites and mycotoxins of *Isaria*-based mycopesticides has been the main source of concern (Weng et al. 2019). *B. bassiana*, *B. brongniartii*, and *M. anisopliae* have all been extensively reviewed for their negative effects on non-target organisms, such as earthworms, silkworms, bees, other pollinators, predators, and parasitoids (Goettel et al. 1989; Danfa and Van Der Valk 1999; Hokkanen and Hajek 2003; Zimmermann 2007). Hence, continuous evaluation of these biological agents, with a focus on their metabolites and impacts on non-target species, remains essential to ensure their safe integration into sustainable pest management practices (Table 17.1).

Table 17.1 Toxicity of entomopathogenic fungi to non-target organisms

Category	Finding	Species affected	Remark	Reference
Pollinators	<i>Metarhizium</i> , <i>Beauveria</i> , and <i>Clonostachys</i> reduced Varroa mites but also reduced adult bee weight. Immune gene expression was triggered.	<i>Apis mellifera</i> (honeybee)	EPF may aid mite control but could stress bee physiology	Hamiduzzaman et al. (2012)
	Exposure to <i>B. bassiana</i> altered bee recognition, leading to social exclusion.	<i>Tetragonisca angustula</i>	Rejected bees at colony entrance, impaired foraging	Almeida et al. (2022)
	Various fungi caused bee mortality; oral exposure more hazardous than contact.	<i>Scaptotrigona depilis</i> , <i>T. angustula</i> , <i>A. mellifera</i> , and <i>Bombus terrestris</i>	Strain- and species-specific susceptibility	Leite et al. (2022)
	BoverilWP and MetarrilWP were low to moderately toxic; OctaneSC highly toxic.	<i>Melipona quadrifasciata</i> , <i>P. droryana</i> and <i>S. bipunctata</i>	Risk levels varied by product and bee species	Faita et al. (2023)
Beneficial arthropods	<i>M. brunneum</i> showed species-specific toxicity.	<i>Orius majusculus</i> (high mortality), <i>Dalotia coriaria</i> , <i>Aphidoletes aphidimyza</i> (moderate); <i>Gaeolaelaps aculeifer</i> (safe)	Importance of species-specific evaluation	De Azevedo et al. (2019)
	Exposure to <i>Lecanicillium muscarium</i> and <i>B. bassiana</i> reduced aphid longevity and lacewing emergence.	<i>Aphis gossypii</i> , <i>Chrysopa carnea</i>	Sublethal and transgenerational effects noted	Pavlyushin (2020)
	Early larval and pupal stages of ladybird beetles highly sensitive.	<i>Coccinella undecimpunctata</i> and <i>Hippodamia variegata</i>	Spray/contact methods more hazardous	Sayed et al. (2021)
	<i>B. bassiana</i> ATCC 74040 showed slight but measurable impacts.	<i>Chrysoperla lucasina</i>	Minor sublethal effects; strain mostly compatible	Morda et al. (2024)

(continued)

Table 17.1 (continued)

Category	Finding	Species affected	Remark	Reference
Vertebrates	No toxicity/pathogenicity from <i>M. anisopliae</i> via injection, inhalation, oral exposure.	White rats	Early safety confirmation	Schaerffenberg (1968)
	No histological abnormalities; no weight loss or behavior change.	White mice and Guinea pigs	28-day oral exposure	Latch (1976)
	No ocular irritation or tissue infection from spores; fungi cleared by day 21.	Rats, mice, and Guinea pigs	Non-pathogenic	Shaddock et al. (1982)
	<i>M. anisopliae</i> cleared subcutaneously after ~1 month; spores excreted.	Mice and Guinea pigs	Subcutaneous and ingestion routes	El-Kadi et al. (1983)
	<i>B. bassiana</i> detectable for 3 days post-injection; no pathogenicity observed.	Mice	Short-lived presence	Semalulu et al. (1992)
	<i>B. bassiana</i> and <i>M. anisopliae</i> considered non-toxic across vertebrate studies.	Rats, birds, and other mammals		Goettel and Jaronski (1997) and Toriello et al. (2005)
	No histological effects in birds consuming spores/infected insects.	Sparrows, pheasants, and hens	No differences in body weight or survival	Althouse et al. (1997), Johnson et al. (2002), and Zimmermann (2007)
	No clinical symptoms or inflammation; minimal germination observed.	CD-1 mice	High-dose oral exposure (10^8 conidia)	Toriello et al. (2005)
	No dermal toxicity of <i>Isaria fumosorosea</i> observed over 14 days.	Rats	Confirmed dermal safety	Brunner-Mendoza et al. (2017)

(continued)

Table 17.1 (continued)

Category	Finding	Species affected	Remark	Reference
	Pulmonary exposure to two <i>M. anisopliae</i> strains caused high toxicity.	Mice	Strain-specific risk	Goettel et al. (1989)
	Weak sensitization, no acute toxicity, or irritation of <i>Isaria fumosorosea</i> .	Rats, rabbits		Weng et al. (2019)
	Occasional human infections (e.g., keratitis) linked to <i>B. bassiana</i> .	Humans	Rare but documented	Sachs et al. (1985), Low et al. (1997), and Kisla et al. (2000)

2.1 Toxicity to Non-Target Invertebrates

While EPF are widely regarded as eco-friendly alternatives to chemical pesticides, their environmental safety, especially concerning non-target organisms, requires thorough evaluation. Among the most sensitive and ecologically important non-target groups are pollinators and beneficial arthropods, which play crucial roles in agroecosystem stability and productivity. Therefore, it is imperative to conduct comprehensive toxicity assessments to evaluate the potential risks EPF pose to these vital non-target species, ensuring that their use does not disrupt ecological balance or threaten ecosystem services.

2.1.1 Effects on Pollinators

Pollinators such as honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) are essential for the pollination of many food crops. Although EPF generally has a high degree of host specificity, some strains may affect pollinators through direct contact or exposure to fungal spores on treated plants or in the environment. EPF can influence pollinators in both beneficial and harmful ways. Three isolates of *M. anisopliae*, *B. bassiana*, and *Clonostachys rosea* effectively reduced *Varroa destructor* mites, a major parasite affecting honeybee brood. However, direct exposure of brood to *M. anisopliae* or *B. bassiana* reduced adult bee weight, indicating possible harm. Notably, infected mites triggered immune gene expression in bees, countering mite-induced suppression. While EPF can help control *Varroa*, their effects on bee health require careful evaluation (Hamiduzzaman et al. 2012). Exposure of *B. bassiana* to *Tetragonisca angustula*, a native stingless bee, affected their key social behaviors. Bees exposed to fungal conidia were chemically altered, leading nest guards to reject them at the colony entrance. This behavioral response may help prevent the spread of pathogens within the colony but could also reduce foraging efficiency.

These findings suggest EPF can impact bee behavior, highlighting the need for cautious application in agroecosystems (Almeida et al. 2022). Fungal biopesticides are considered low-risk options for pest control, but their impact on pollinators needs evaluation. This study assessed the effects of five concentrations of *B. bassiana*, *M. anisopliae*, and *Cordyceps fumosorosea* on four bee species—*Scaptotrigona depilis*, *Tetragonisca angustula*, *Apis mellifera*, and *Bombus terrestris*—through direct contact and ingestion. All fungi caused varying levels of mortality, with *S. depilis* and *B. terrestris* being more sensitive to *B. bassiana*, and *B. terrestris* and *A. mellifera* showing higher susceptibility to *M. anisopliae* orally. Results highlight potential risks to bees at the individual level under lab conditions (Leite et al. 2022). Stingless bees are key pollinators with environmental and agricultural importance but face risks from insecticide exposure. This study evaluated the impact of three fungal bioinsecticides—*B. bassiana* (Boveril WP), *M. anisopliae* (Metarril WP), and *C. fumosorosea* (Octane SC)—on *Melipona quadrifasciata*, *Plebeia droryana*, and *Scaptotrigona bipunctata*. Mortality varied among species, with *M. quadrifasciata* being most sensitive. While Boveril WP and Metarril WP were considered harmless to moderately harmful, Octane SC was highly toxic. Results highlight that EPF selectivity varies, and careful application is essential to protect pollinators in sustainable farming (Faita et al. 2023).

Laboratory assays often show low acute toxicity, but chronic effects and sublethal impacts such as reduced foraging efficiency or compromised immune responses need further investigation under field conditions. As such, strain selection and formulation refinement are crucial for minimizing unintended effects on pollinator health. Understanding species-specific sensitivity, chronic effects, and behavioral changes is essential to ensuring that EPF applications do not harm essential pollinator populations. Further research on field conditions and the development of selective strains will be key to optimizing the use of EPF in a way that promotes both effective pest control and pollinator conservation.

2.1.2 Impact on Beneficial Arthropods

EPF, though effective in managing insect pests, may also negatively affect beneficial arthropods. Certain fungal species can infect helpful insects such as lacewings and other natural predators, potentially causing their death and disturbing ecological balance, which can undermine integrated biological control efforts. The study assessed the non-target effects of the microbial biological control agent *M. brunneum* applied in soil on four beneficial arthropods: the predatory mite *Gaeolaelaps aculeifer* (Canestrini), the rove beetle *Dalotia coriaria* (Kraatz), the gall midge *Aphidoletes aphidimyza* (Rondani), and the predatory insect *Orius majusculus* (Reuter). *Gaeolaelaps aculeifer* showed no adverse effects, while *Orius majusculus* experienced high mortality and significantly reduced longevity and fecundity. *Dalotia coriaria* and *Aphidoletes aphidimyza* showed intermediate negative responses, including increased mortality and reduced reproductive output. These findings emphasize the importance of evaluating species-specific risks when using entomopathogenic fungi in integrated pest management to prevent unintended harm to beneficial arthropods (De Azevedo et al. 2019).

Studies have shown that EPF, such as *Lecanicillium muscarium* and *Beauveria bassiana*, can exert negative impacts on beneficial arthropods. In aphids (*Aphis gossypii*), commonly used as hosts in biological control systems, exposure to fungal conidia reduced longevity, fertility, and progeny production, with transgenerational effects observed up to the fifth generation. Similarly, lacewings (*Chrysopa carnea*), important predators in agroecosystems, exhibited reduced adult emergence and toxigenic effects in their progeny after exposure to fungal conidia. These effects occurred without visible fungal infection, suggesting the role of fungal metabolites. Such findings highlight the potential for unintended non-target effects and underscore the need for careful integration of EPF in multi-agent biological control strategies (Pavlyushin 2020). The study assessed the impact of an indigenous *Beauveria bassiana* isolate on various life stages of two beneficial predatory beetles, *Coccinella undecimpunctata* and *Hippodamia variegata*. Results revealed that the early developmental stages, particularly the first larval instars of both species and the pupal stage of *C. undecimpunctata*, were negatively affected, with significantly higher mortality observed, especially under the spray and contact methods. These findings suggest that *B. bassiana*, though generally considered safe, can pose risks to vulnerable life stages of beneficial arthropods like predatory ladybird beetles, which are important for biological pest control (Sayed et al. 2021).

The study evaluated the effects of *B. bassiana* strain ATCC 74040 on the predatory insect *Chrysoperla lucasina*, both in laboratory-reared and wild populations. While the results generally indicated compatibility between the fungus and the predator, some slight negative effects were observed on survival, development, or reproduction. Thus, it is important to evaluate case-by-case safety issues prior to field application, even if this *B. bassiana* strain is generally not harmful. It may nevertheless have slight negative effects on beneficial predatory insects (Morda et al. 2024). The unintended consequences on natural predators and other non-target beneficial species, such as reduced survival, fecundity, or developmental delays, underscore the need for species-specific assessments. As such, integrating EPF into integrated pest management strategies requires a nuanced approach to balance pest control efficacy with the preservation of ecosystem services provided by beneficial arthropods. Further research is crucial to fine-tune EPF formulations and application techniques to minimize risks to non-target organisms.

2.2 Toxicity to Vertebrates

Early mammalian safety evaluations of *M. anisopliae* in adult white rats through injection, inhalation, and oral administration revealed no signs of toxicity or pathogenicity (Schaerffenberg 1968). No tissue abnormalities in white mice and guinea pigs fed *M. anisopliae* conidia over 28 days, with no weight loss or abnormal behavior detected during histological analysis (Latch 1976). Research data suggested by demonstrating that rats, mice, and guinea pigs exposed to *M. anisopliae* spores either by injection or exposure did not experience ocular irritation or spore germination in their tissues (Shadduck et al. 1982). Preliminary

safety tests of *M. anisopliae* showed no signs of illness, death, or ocular irritation in exposed animals. Tissue reactions were limited to injection sites, with no histological evidence of spore germination. Although fungi were temporarily recovered from certain organs, they were absent by day 21. The results indicated no pathogenicity of *M. anisopliae* in mammals (Shadduck et al. 1982).

Further investigations confirmed that *M. anisopliae* remains non-pathogenic and non-toxic following ingestion, inhalation, and subcutaneous exposure in white mice and guinea pigs, with viable spores being excreted and persisting subcutaneously for about a month (El-Kadi et al. 1983). Mice given an intramuscular injection of *B. bassiana* showed that the fungus was only present in their bodies for 3 days (Semalulu et al. 1992). *Beauveria bassiana* was found to be non-toxic in toxicity tests conducted on rats and other vertebrates (Goettel and Jaronski 1997). Additional studies have consistently reported similar non-toxic outcomes across different animal models (Toriello et al. 2005). Similarly, many bird species have undergone toxicity tests with different strains of EPF, where birds were either given fungal spores blended into their food or insects infected with EPF. For instance, ring-necked pheasants (*Phasianus colchicus Linnaeus*) were exposed to *B. bassiana* conidia, whereas American sparrowhawks (*Falco sparverius Linnaeus*) were fed *B. bassiana* spores (Althouse et al. 1997; Johnson et al. 2002), and hens ate white grubs contaminated with *B. brongniartii*. According to these studies, the birds exposed to EPF did not exhibit any histological abnormalities. Additionally, survival, body weight, and growth indicators did not significantly differ between the treatment and control groups (Zimmermann 2007). The oral toxicity and pathogenicity of *M. anisopliae* var. *anisopliae* were assessed in CD-1 mice. Mice received a single intragastric dose (10^8 conidia/animal) of either viable or nonviable conidia and were monitored through clinical, mycological, and histological assessments. No clinical symptoms, weight loss, or inflammatory reactions were observed. Minimal fungal germination was detected in isolated cases among mice treated with viable conidia. The study concluded that EH-479/2 is nonpathogenic and nontoxic under these conditions (Toriello et al. 2005). An acute cutaneous toxicity test was performed on *Isaria fumosorosea*. Over a 14-day observation period, no clinical signs of disease, skin inflammation, or abnormal weight changes were observed, confirming the dermal safety of this fungal strain (Brunner-Mendoza et al. 2017).

However, contrasting results were reported by Mycotech, a biopesticide company, where two strains of *M. anisopliae* (var. *anisopliae* and var. *acridum*) showed high toxicity in mice following pulmonary exposure (Goettel et al. 1989). Similarly, *I. fumosorosea* was classified as a weak sensitizer, with minimal acute oral, dermal, and inhalation toxicities reported in rats; no eye irritation or dermal sensitization was observed on treated rabbit skin (Weng et al. 2019). On rare occasions, *B. bassiana* has been linked to illnesses in humans, including mycotic keratitis (Sachs et al. 1985; Low et al. 1997; Kisla et al. 2000). Biopesticides based on *Metarhizium* pose a low risk to aquatic organisms. Most EPF, including *M. anisopliae*, *B. bassiana*, and *I. fumosorosea*, are generally non-toxic and non-pathogenic to vertebrates under typical exposure conditions. However, certain strains may show toxicity in specific exposure methods or species. While the overall risk to vertebrates is low, there are

reports of toxicity under specific conditions, such as pulmonary exposure or individual sensitivity. Further research is needed to ensure the safe application of EPF-based biopesticides.

2.3 Occupational and Human Health Risks

There is currently no proof that EPF in air or water has harmed human health (Weng et al. 2019). A rare case of human deep tissue infection caused by a *Beauveria* species highlights possible health risks from EPF. While *B. bassiana* is classified as biosafety level 1 and widely used in biocontrol, this clinical strain showed high virulence in insect bioassays. Though it did not grow at 37 °C in vitro, which has caused infections in immunocompromised individuals, it may still pose a risk under certain conditions. Strain-specific differences in virulence suggest that not all EPF strains are equally safe for humans (Henke et al. 2002). While EPF generally pose no health risks, rare infections, such as with *B. bassiana* in immunocompromised individuals, suggest certain strains may pose risks. Strain selection is crucial for ensuring safety.

2.4 Risk Assessment Implications of Secondary Metabolites

Degradation and Stability Secondary metabolites (SMs) of EPF degrade rapidly in submerged media, insect hemolymph (both live and post-mortem), and crops (not detected or below detection limits). They are only stable under specific preserved conditions, which are irrelevant for environmental risk assessment. In natural settings, temperature and UV light cause rapid degradation (OECD 2018).

Endophytic Concerns Some EPF (e.g., *Beauveria*, *Metarhizium*) can occur as endophytes and may theoretically produce SMs within plants. Though this raises potential food chain concerns (Vega 2018), current evidence does not confirm complete fungal life cycles inside plants or arthropod death due to endophytically produced SMs (Gurulingappa et al. 2011). Endophytism may enhance plant productivity via nitrogen translocation (Behie and Bidochka 2013).

Field-Level SM Impact Using *Plutella xylostella* LC₅₀ of 17 µg/mL for destruxin E, field application requires 17 mg/L in spray solution. However, destruxin E levels in Microbial Pest Control Agent (MPCA) formulations (97 ± 146 mg/L) diluted for field use result in concentrations ~175 times lower than LC₅₀, indicating negligible risk to target insects (OECD 2018).

Off-Field Risk Drift to off-field areas results in further dilution, especially in vegetated zones. While NTOs (non-target organisms) closely related to targets may be sensitive, the actual exposure remains low. Risk assessment includes consideration of assessment factors and potential additive or synergistic effects of multiple destruxins (e.g., from *Metarhizium* spp.) (European Commission 2002).

While secondary metabolites (SMs) from EPF degrade rapidly in natural conditions and are not a significant environmental risk, the potential impact of endophytic production of SMs and off-field drift should be considered in risk assessments. These factors, along with potential synergistic effects, require careful evaluation to ensure safe use.

3 Residual Pathogenic Effects

EPF are valued for their minimal residual effects on animal health and the environment (Sharma et al. 2023). The effectiveness of these fungal species as biocontrol agents relies not only on their ability to selectively and efficiently eliminate insect pests but also on minimizing or avoiding harmful impacts on beneficial non-target insects in the field (Rizwan et al. 2021). Ongoing evaluation of their residual effects and careful management of their application are essential to ensure minimal environmental and health impacts (Table 17.2).

Table 17.2 Residual effects of entomopathogenic fungi

Category	Key Point	Reference
Crops	<i>B. bassiana</i> and <i>M. anisopliae</i> have persistent effects on maize through endophytic activity, with residual effects promoting pest resistance and altering plant nutrient composition	Liu et al. (2022) and Krell et al. (2018)
Non-target organisms	EPF can affect non-target insects, including beneficial predators like <i>Dicyphus tamaninii</i> and <i>Chrysoperla carnea</i> . They may also impact bees and other beneficial species	Broza et al. (2001), James and Lighthart (1994), Ginsberg et al. (2002), and Leite et al. (2022)
	<i>M. brunneum</i> caused high mortality in predatory bugs like <i>Orius majusculus</i> and reduced reproductive success in other beneficial arthropods	De Azevedo et al. (2019)
Soil	Soil characteristics influence EPF persistence. Favorable conditions, such as moisture-rich orchard soils, support EPF survival. Soil also protects EPF from UV and temperature fluctuations	Majchrowska-Safaryan and Tkaczuk (2021) and Qayyum et al. (2021)
Water	EPF spores have minimal mobility in water and are unlikely to contaminate groundwater. They can survive in water bodies but have limited proliferation in air over time	Zimmermann (2007) and Shah and Pell (2003)
Air	EPF spores have limited ability to survive or proliferate in the air for extended periods. They are vulnerable to UV radiation and other environmental stressors	Shah and Pell (2003) and Milner et al. (2002)

3.1 Persistence on Crop Surfaces

A study examined the growth-promoting effects of *B. bassiana* and *M. anisopliae* in maize, focusing on whether their action is primarily rhizospheric or endophytic. Fungal populations in hydroponic solution declined rapidly, with less than 10% (*M. anisopliae*) and 1% (*B. bassiana*) remaining after 1 week, and both were nearly undetectable by day 28. In contrast, conidia remained viable in plant-free controls, suggesting that the decline was due to endophytic colonization. This highlights that the residual effects of these fungi on crops are mainly due to their endophytic activity rather than rhizospheric presence (Liu et al. 2022).

EPF can induce lasting changes in plant nutrient composition and defensive metabolites, creating a less favorable environment for herbivores. Such residual pathogenic effects—through sustained production of deterrent compounds—reduce the plant's suitability as a food source and enhance its ability to repel pests over time (Krell et al. 2018). The persistence of EPF on crop surfaces, primarily through endophytic activity, plays a crucial role in enhancing plant defenses and reducing pest suitability, offering sustained pest management benefits with minimal environmental impact.

3.2 Risk of Exposure to Non-Target Insects

The use of EPF in pest control carries the potential risk of adversely affecting non-target insects, including beneficial natural enemies, thereby raising concerns about unintended ecological impacts. *M. anisopliae* caused mortality in *Dicyphus tamaninii* (10%), a predatory mirid, and *Chrysoperla carnea* (4%), a green lacewing (Broza et al. 2001). These findings highlight the risk of EPF affecting non-target insects, as different fungal species show varying pathogenicity. Previous studies confirm that EPF, like *B. bassiana*, can impact non-target species such as collembolans (springtails) and coccinellids (ladybugs), with some fungi causing significant mortality and others having minimal effects (James and Lighthart 1994; Dromph and Vestergaard 2002). *M. anisopliae* showed significant mortality in *Hippodamia convergens* (ladybird beetles) and *Acheta domesticus* (house crickets), with marginal effects on *Oncopeltus fasciatus* (milkweed bugs). Infected insects transmitted the fungus to untreated beetles and crickets, highlighting the risk of non-target effects. While *M. anisopliae* is widespread in North America, field trials are needed to confirm these lab results and assess methods to minimize non-target exposure (Ginsberg et al. 2002).

Beauveria bassiana, *Metarhizium anisopliae*, and *Cordyceps fumosorosea* pose a potential risk to non-target bees. *B. bassiana* was more likely to affect *Bombus terrestris* and *Scaptotrigona depilis*, but *M. anisopliae* was more likely to affect *Apis mellifera* and *B. terrestris* through oral exposure. Mortality did not always increase with higher fungal concentrations, suggesting the risk may not always increase with dosage. While this study indicates that EPFs can impact bee survival, further field studies are needed to fully assess the ecological risks to non-target organisms like

bees (Leite et al. 2022). *M. brunneum* (BIPESCO 5/F52), though effective for pest control, may affect non-target beneficial arthropods. Soil applications led to high mortality in the predatory bug *Orius majusculus* (96%) and moderate mortality in the rove beetle *Dalotia coriaria* (7.3%), with reduced longevity and fecundity in both. The gall midge *Aphidoletes aphidimyza* showed increased larval mortality (60%) but unaffected fecundity, while the predatory mite *Gaeolaelaps aculeifer* remained unaffected. These findings highlight the need for multi-parameter and multi-species assessments to gauge non-target risks of biocontrol agents (De Azevedo et al. 2019). Bioassays on non-target insects (honeybees and cockroaches) with local *Metarhizium* strains showed Less than 5% mortality was recorded, and no mycosis was observed, indicating minimal non-target effects (Bilgo et al. 2018).

3.3 Environmental Fate and Persistence

The effectiveness of EPF under field conditions is greatly influenced by environmental parameters such as temperature, humidity, and UV radiation. Cyclobutane pyrimidine dimers and other photoproducts are DNA damages brought on by UV radiation that can impair the viability and function of fungal cells. Even though filamentous fungi have photolyase enzymes that can repair DNA in the presence of visible light, their effectiveness decreases in the presence of bright light (Yasui et al. 1994; de Laat et al. 1999; Sancar 2003). The ideal temperature range for fungal germination and growth is usually 23–28 °C (Jaronski 2009). Faster conidial germination is also facilitated by high humidity. *B. bassiana* took 72 h at 90% relative humidity to germinate, but just 20 h at 25 °C and 95.5% RH (Luz and Fargues 1997).

The biodiversity or uneven localization of EPF in soils due to climatic and geographic factors significantly impacts fungal endophytes and EPF use. Fungal spore mobility and dissemination in soil are influenced by soil moisture content, soil type, soil organisms, and plant roots (Cabrera-Mora et al. 2019). The possibility that mycopesticide formulations and EPF will float into water bodies or the atmosphere is a common concern when using them in biological control. The downward movement of both wet and dry spores of *M. anisopliae* concluded that there is little chance of the fungus contaminating groundwater (Zimmermann 2007). Their longevity is aided by the production of resting structures or by adopting a saprophytic lifestyle, allowing them to survive extended periods in the absence of hosts. EPF is known to exist in a variety of habitats, including aquatic and terrestrial ecosystems, as well as arctic and tropical climates (Milner et al. 2002). This is mostly because the spores have a limited capacity to survive or proliferate under air conditions over an extended period (Shah and Pell 2003).

The persistence and dispersal of EPF are influenced by a variety of biotic and abiotic factors. Fungal development and infection processes are influenced by biotic factors, such as the characteristics of the insect host and plant-mediated effects (Cory and Ericsson 2009). Abiotic factors such as temperature (Bayissa et al. 2017), solar radiation (Fernández-Marín et al. 2006), humidity and rainfall (Inglis et al. 2001),

and soil properties (Quesada Moraga et al. 2007) directly affect the viability and infectivity of fungal spores. These factors collectively determine the ability of the fungus to persist, spread, and cause epizootics under field conditions. Soil serves as both a refuge and a challenge for EPF. It offers protection from harmful UV radiation and buffers against fluctuations in temperature and moisture (Inglis et al. 2001; Rangel et al. 2005), thereby supporting the long-term survival of EPF. Additionally, soil hosts a wide range of potential insect hosts, often in high densities, which promotes the persistence and evolutionary adaptation of EPF to their environment (Humber 2008).

However, soil also contains antimicrobial compounds produced by resident microbes, which can inhibit fungal infectivity. For instance, higher levels of soil fungi stasis have been linked to reduced virulence of *B. bassiana* against the Colorado potato beetle (Grodén and Lockwood 1991). Despite these antagonistic pressures, many EPF species persist by producing secondary metabolites within their insect hosts, aiding in competition against opportunistic microbes during the saprotrophic phase (Strasser et al. 2000). These adaptations highlight the ecological resilience and persistence of EPF within the complex soil environment. A large-scale comparative study assessed EPF occurrence in leaf litter and underlying soil across three forest types. *Beauveria*, *Cordyceps*, *Metarhizium*, and *Lecanicillium* spp. were detected in both habitats, with *Beauveria* being the most frequent. Even though leaf litter typically had greater CFUs, the steady recovery of EPF from soil layers shows how persistent they are in forest soils. *Lecanicillium* was the second most abundant genus in Polish forests (Majchrowska-Safaryan and Tkaczuk 2021).

Studies from southern Punjab, based on soil and insect cadaver samples from both natural and cultivated areas, indicate that soil characteristics greatly influence EPF presence and diversity. EPF were consistently isolated from orchard soils, suggesting these less-disturbed and moisture-rich environments support their persistence. Among 225 insect cadavers collected, EPF were most frequently associated with Coleoptera and Lepidoptera. Although *Aspergillus niger* and *Fusarium oxysporum* were the most common fungal isolates overall, the findings underscore that orchard soils and specific insect hosts are more favorable for isolating persistent EPF populations (Qayyum et al. 2021).

4 Genetic Stability and Horizontal Transmission

Metarhizium robertsii gained entomopathogenic traits through horizontal gene transfer (HGT), acquiring 18 genes essential for infecting insects. These genes, especially those involved in cuticle degradation and lipid utilization, enabled the fungus to expand its host range. This highlights how HGT enhances EPF adaptability, potentially impacting gene stability and altering ecological interactions in the environment. Insect cadavers can spontaneously produce entomopathogenic fungal spores that can travel up to 1000–1500 times their own size or, in the case of *Entomophthora muscae*, roughly 5–10 mm. These spores can then spread out through the air and land on adjacent plant surfaces (Małagocka et al. 2015; Ebani

and Mancianti 2021; Naundrup et al. 2022). While not immediately infectious, resting spores produced by many fungi in Hypocreales and Entomophthorales can survive in the environment for lengthy periods without a host (Jaronski 2013). These dormant spores either germinate to release infectious spores or directly infect new hosts when the right conditions are met.

4.1 Regulatory Guidelines and Risk Assessment Frameworks

Regulatory approval of microbial pesticides, including EPF, typically demands comprehensive data on strain identity, purity, and production methods, along with detailed safety evaluations. Key jurisdictions such as the USA, EU, Canada, and Australia have established rigorous guidelines addressing environmental behavior, non-target effects, exposure risks, metabolite production, persistence, and host specificity. Table 17.3 below outlines country-specific requirements across these categories, referencing applicable regulatory frameworks.

4.2 United States Environmental Protection Agency

Environmental Protection Agency (EPA) as microbial pesticides fall under the biopesticide category regulated by the EPA. The relevant guidelines are outlined in 40 CFR Part 158 Subpart V, which addresses microbial pesticide data requirements (EPA 2002).

Environmental Fate All EPF uses must include data on viability and persistence in soil and water. If Tier I (initial non-target toxicity) tests raise concerns, Tier II studies such as soil persistence, mobility, and photolysis may be required.

Toxicity Testing Tier I acute toxicity/pathogenicity tests are generally required for outdoor use on non-target organisms—such as birds, fish, aquatic invertebrates, bees, and beneficial arthropods. Higher-tier tests (Tier II–IV) are only triggered if adverse effects are noted. Protocols are detailed under EPA’s OPPTS (*Office of Prevention, Pesticides, and Toxic Substances*) 885 series (EPA 2025).

Exposure Scenarios EPA requires evaluation of exposure under typical outdoor use, including spray drift and runoff risks. Standard exposure models and spray-drift studies (e.g., nozzle characterization) are mandated when outdoor applications are proposed.

Metabolites and Toxins Any known or potential secondary metabolites or toxins produced by the EPF must be identified and assessed. If the fungal strain is associated with toxin-producing species (e.g., destruxins from *Metarhizium* or beauvericin from *Beauveria*), sensitive analytical methods and toxicological data must be submitted.

Table 17.3 Regulatory frameworks of entomopathogenic fungi

Country	USA (EPA)	EU (EFSA)	Canada (PMRA)	Australia (APVMA)
Environmental fate data	Required under 40 CFR Part 158 Subpart V. Tier II triggered by Tier I outcomes. Must assess soil and water persistence, viability	Reg. 283/2013 Part 7 requires persistence/multiplication studies in soil and water. PECs modeled using FOCUS	Tiered: Tier II/III triggered by Tier I outcomes. Persistence in soil/water assessed via microcosms	Tailored requirements. Persistence and movement assessed via lab and field studies as needed
Non-target toxicity testing	Tier I test required for birds, fish, aquatic invertebrates, bees, earthworms and others. Higher-tier tests if needed	Required for birds, bees, soil organisms and aquatic organisms. Based on OECD guidelines	Tier I: Acute tests for birds, fish, bees, earthworms. Higher-tier if effects noted	Required for birds, fish, aquatic invertebrates, bees. Based on same taxa as chemical pesticides
Exposure scenarios	Outdoor use requires drift/runoff exposure modeling. Subpart L mandates spray-drift studies	PECs based on intended use. Standard FOCUS exposure models used	Exposure calculated for spray/runoff under agricultural/forestry scenarios	Use-based exposure modeling; typically aligned with proposed agricultural uses
Metabolites/toxins	Must assess production of toxins (e.g., destruxins, beauvericin); toxicological data required if present	Must identify and quantify toxic metabolites (e.g., destruxins, cyclosporins). Residue definitions include toxins	Metabolite analysis mandatory. Sensitive tests for known toxins (e.g., destruxins) required	Identification and toxicology data required for any secondary metabolites.
Persistence evaluation	Tier II fate testing to assess viability/survival. Field decline data if needed	Persistence/multiplication assessed through lab and field studies. Required for non-indigenous EPF	Tier II includes studies on viability decline. Additional field data if persistence >30 days	Data required if persistence beyond typical microbial decay. Decline demonstrated in lab/field.
Host range evaluation	Must evaluate impacts on beneficial and related insects. Host range inferred from non-target tests	Host specificity inferred from non-target arthropod tests and insect order susceptibility data	Host specificity inferred from effects on ecologically/taxonomically related species	Host spectrum inferred from effects on beneficials and related pests

(continued)

Table 17.3 (continued)

Country	USA (EPA)	EU (EFSA)	Canada (PMRA)	Australia (APVMA)
Reference(s)	40 CFR 158.2150 US EPA 2002; Series 885—Microbial Pesticide Test Guidelines US EPA 2025, EPA 2011	Regulation—1107/2009—EN—EUR-LEX 2009; European Commission and Barroso 2013; EFSA 2025	Submission Management and Information Division, PMRA 2001	<i>Guideline for the Regulation of Biological Agricultural Products</i> , 2022

Persistence EPA assesses how long the EPF remains viable and whether it replicates in natural media like soil and water. Assessments of environmental risk factors should be supported by field persistence data.

Host Range Host specificity is indirectly evaluated through non-target arthropod testing. A broader-than-expected host range in lab tests may prompt higher-tier specificity studies to ensure minimal impact on non-target and beneficial species.

4.3 European Union—European Food Safety Authority

EPF, categorized as microbial active substances, are regulated under Regulation (EC) No 1107/2009 and its implementing acts (Regulations 283/2013 and 284/2013). Applicants must provide comprehensive data on the microorganism's identity, strain, purity, and biological characteristics.

Environmental Fate According to Annex II of Regulation 283/2013, studies must assess EPF persistence and multiplication in soil and water, including photolysis potential. If the organism is native to the environment, field studies may be needed to confirm natural decline. EFSA guidance and OECD protocols are used to interpret these findings (EFSA 2025).

Toxicity Testing Regulation 283/2013 requires acute and chronic toxicity/pathogenicity testing on a wide range of non-target organisms, like birds, aquatic life, bees, soil invertebrates, and soil microbes. These tests must follow OECD guidelines tailored for microbial agents.

Exposure Scenarios Risk assessments use standard FOCUS models to estimate environmental exposure concentrations (PECs) based on application rates and use patterns. These PECs, combined with toxicity data, help evaluate risks to non-target species.

Metabolites and Toxins EPF capable of producing bioactive metabolites (e.g., destruxins, beauvericin, cyclosporins) must be assessed for their presence in the product and environment. The residue definition includes the microorganism and any pertinent metabolites for regulatory purposes.

Persistence EU regulations require data on the microorganism's ability to multiply and persist in the environment. If lab data suggest prolonged survival, field studies may be requested to confirm that the organism will not spread uncontrollably in non-target habitats.

Host Range While a dedicated host range test is not specified, data on infectivity across insect groups and effects on beneficial arthropods (pollinators, predators, parasitoids) are used to assess specificity and ecological safety.

4.4 Canada—Pest Management Regulatory Agency

EPF are classified as Microbial Pest Control Agents (MPCAs) under Health Canada's Pest Management Regulatory Agency (PMRA) Directive DIR2001-02, which aligns closely with US EPA standards (PMRA 2001).

Environmental Fate Under Part 8 of the Directive, fate studies are tiered based on toxicity findings. If Tier I toxicity data show potential risks, Tier II (lab-based) or Tier III (field-based) tests assess survival, reproduction, and persistence under simulated environmental conditions (soil and water). Non-native or genetically modified strains face stricter scrutiny.

Toxicity Testing A tiered system is used. Acute toxicity/pathogenicity testing on fish, birds, mammals, aquatic invertebrates, bees, and earthworms is required by Tier I. If risks are indicated, Tier II introduces chronic tests, and higher-tier (Tier III/IV) field studies may follow. Protocols cover oral/inhalation studies for birds, 28-day fish tests, and bee contact/oral toxicity.

Exposure Scenarios Risk assessments consider typical use patterns and include modeling of spray drift, runoff, and exposure across soil, water, and air. Applicants must provide realistic environmental concentration estimates based on application rates.

Metabolites and Toxins Applicants must disclose and test for any toxic metabolites. If the EPF is related to known mycotoxin producers (e.g., fungi producing fumonisins or ophiobolins), sensitive assays must prove such toxins are absent or within acceptable levels.

Persistence Persistence data (soil and plant) assess whether the EPF can survive and multiply in the environment. A long half-life (e.g., >30 days) or ongoing reproduction triggers further field studies to ensure environmental safety.

Host Range Testing must include non-target arthropods that are ecologically or taxonomically close to the target pest. This data supports defining the EPF's host specificity. If Tier I tests show broad effects, additional higher-tier specificity testing is required.

4.5 Australia—Australian Pesticides and Veterinary Medicines Authority

In Australia, EPF are regulated as microbial agricultural products under the Australian Pesticides and Veterinary Medicines Authority (APVMA) Guideline for the Regulation of Biological Agricultural Products (Sect. 3.2). EPF are considered microbial pesticides and subject to a tailored risk assessment approach that aligns with, but is distinct from, chemical pesticide evaluations (APVMA 2022).

Environmental Fate Environmental fate data focus on persistence and movement in soil and water. If lab results suggest the EPF may persist, field or glasshouse dissipation studies are required to track decline under realistic use conditions.

Toxicity Testing Toxicity assessments are required for birds, fish, aquatic invertebrates, bees, and beneficial insects, particularly for outdoor applications. Section 4.4 of the guideline provides ecotoxicology test recommendations. If early tests show no adverse effects, additional testing may be waived.

Exposure Scenarios Applicants must provide exposure estimates under worst-case scenarios, especially for spray applications. APVMA requires spray drift analysis (as per Item 40 of their guideline) and exposure assessments for bystanders and aquatic systems in public health or non-agricultural uses.

Metabolites and Toxins Any secondary metabolites or toxins must be identified and characterized. If the fungal species is associated with known toxins, applicants must provide toxicological data or proof of absence.

Persistence Evaluation APVMA evaluates both shelf life (product stability) and environmental persistence. Though microbial products often degrade faster than chemicals, any potential for EPF reproduction and persistence in the environment must be documented and appropriately managed.

Host Range Applicants must submit host specificity data, usually drawn from efficacy studies and non-target insect testing. If the EPF affects non-target insects (e.g., native beetles, parasitoids), labeling restrictions or further testing may be required.

4.6 Brazil

Regulating Bodies Ministério da Agricultura, Pecuária e Abastecimento—Ministry of Agriculture (MAPA), Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)—Brazilian Institute of Environment and Renewable Natural Resources) and Agência Nacional de Vigilância Sanitária (ANVISA)—National Health Surveillance Agency (MAPA [2025](#); IBAMA [2025](#); ANVISA [2025](#)).

EPF Category Biological Pesticides (a subset of microbial pesticides).

Key Requirements Strain identity, non-target toxicity (birds, bees, fish, beneficials), environmental fate (soil persistence), and known mycotoxins.

Regulatory Basis National guidelines mirror OECD (*Organisation for Economic Co-operation and Development*) and FAO (*Food and Agriculture Organization*) principles.

4.7 India

Regulating Body Central Insecticides Board and Registration Committee (CIBRC) under the Insecticides Act (1968) (CIBRC [2025](#)).

EPF Category Microbial Biopesticide

Key Requirements Physicochemical characterization, pathogenicity/toxicity (target and non-target insects, mammals, fish, bees, and soil microbes), toxin analysis (demonstrate absence of known toxic metabolites).

Guidance Document Annex D of Biopesticide Guidelines (2011)

4.8 China

Regulating Body Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA) (ICAMA [2025](#)).

EPF Category Microbial Biopesticide

Key Requirements Strain ID, viability, growth conditions, non-target safety (birds, fish, mammals, bees), and environmental fate (soil and water).

Approach Harmonized with OECD and EPA standards.

4.9 Japan

Regulating Body Ministry of Agriculture, Forestry and Fisheries (MAFF) (MAFF 2014).

EPF Category Microbial Pesticide.

Key Requirements Acute oral/contact toxicity, pathogenicity in mammals, birds, and bees; soil and water persistence; host-range testing (minimum two insect orders).

Approach Based on OECD guidelines; covered in MAFF's Plant Protection Guidebook.

4.10 Other Countries (South Africa, New Zealand, ASEAN)

EPF Category Generally regulated as Microbial Biopesticides

Key Requirements Identity, non-target toxicity (bees, aquatic organisms, natural enemies), environmental fate (soil/water), and metabolite/toxin assessment.

Approach Aligned with OECD consensus; data from the USA/EU accepted in many cases.

5 Knowledge Gaps and Future Research Directions

Despite the growing application of EPF as environmentally friendly alternatives to chemical pesticides, several critical knowledge gaps remain regarding their safety assessment and potential residual pathogenic effects. One major area of concern is the long-term persistence of EPF spores in different environmental compartments such as soil, water, and plant surfaces, as current data are insufficient to predict their ecological longevity or potential accumulation. There is also a notable lack of long-term field safety data, which limits our ability to understand the real-world ecological impacts of repeated EPF applications over time. Additionally, there is limited understanding of their effects on non-target organisms, including beneficial insects like pollinators, soil fauna, aquatic invertebrates, and vertebrates such as birds and amphibians.

Human health risk assessments require more comprehensive data, particularly concerning exposure through dermal contact, inhalation, or ingestion—especially among agricultural workers and consumers. Another emerging issue is the lack of studies exploring the genetic stability of EPF strains and the possibility of horizontal gene transfer, which could alter their host range or pathogenicity. Furthermore, the toxicological profiles of secondary metabolites produced by EPF are not fully understood, raising concerns about unintended impacts on the environment and

non-target species. There is also a significant gap in assessing fungal residues on edible crops, with no standardized protocols currently available to evaluate the viability or safety of these residues at harvest.

To address these issues, future research should prioritize the development of harmonized and standardized testing protocols for EPF safety and efficacy, both in laboratory and field settings. Genomic approaches hold great promise for identifying markers of virulence and safety, enabling more targeted screening and monitoring of EPF strains. Additionally, the development of safer formulations and delivery systems can help minimize non-target exposure and enhance field performance. Establishing long-term field monitoring systems and investigating interactions between EPF and native microbiota are also essential to ensure the responsible and sustainable use of these biological control agents.

6 Conclusion

EPF represent a valuable tool in sustainable pest management strategies. However, ensuring their environmental and human safety requires a comprehensive understanding of their ecological interactions, genetic stability, and toxicological profiles. While global regulatory frameworks provide a structured approach for EPF assessment, notable gaps persist in field-based safety data, secondary metabolite risk evaluation, and long-term persistence in various ecosystems. Addressing these challenges through standardized protocols, molecular characterization, and long-term monitoring will be essential to harness the full potential of EPF while safeguarding environmental and public health. A balanced, science-based regulatory approach—integrating risk-benefit analyses and international cooperation—will be key to fostering responsible innovation in biopesticide development.

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