Entomopathogenic fungi: unconventional applications

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Abstract Entomopathogenic fungi (EPF) are microorganisms that cause fatal diseases of arthropods. The infection process involves several stages that consist of direct contact of the fungus with the surface of the cuticle of the attacked insect. The factors that determine the effectiveness of the infection process include lytic enzymes, secondary metabolites, and adhesins produced by EPF. Because of their high insecticidal effectiveness, these fungi are commonly used as biopesticides in organic farming. As the environment and farmlands are contaminated with many compounds of anthropogenic origin (e.g., pesticides), the effects of these toxic compounds on EPF and the mechanisms that affect their survival in such a toxic environment have been studied in recent years. This review presents information on the capacity of EPF to remove toxic contaminants, including alkylphenols, organotin compounds, synthetic estrogens, pesticides and hydrocarbons. Moreover, these

fungi produce numerous secondary metabolites that can be potentially used in medicine or as antimicrobial agents. Despite their huge potential in biocontrol processes, the use of EPF has been underestimated due to a lack of knowledge on their abilities. In our work, we have presented the available data on the possibilities of the additional and unconventional use of these microorganisms.

Keywords Entomopathogenic fungi · Toxic compounds · Nanoparticle biosynthesis · Biodegradation

1 Introduction

Entomopathogenic fungi (EPF) are bioinsecticides with an ability to infect and kill arthropods. Although they are mainly isolated from arthropod carcasses, their natural habitat is soil (Behie and Bidochka 2014). These fungi are categorized in six classes: *Oomycetes*, *Chytridiomycota*, *Microsporidia*, *Entomophthoromycota*, *Basidiomycota*, and the most common *Ascomycota*. The main function of EPF in the environment is the biocontrol of the insect population. The literature data provide information on the infectious properties of EPF, the infectious process (Skinner et al. 2014; Lacey et al. 2015; Mascarín and Jaronski 2016), and the use of these microorganisms in commercial

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preparations as biopesticides (Jaihan et al. 2016; Ríos-Moreno et al. 2016).

In the last few decades, the constantly increasing environmental pollution is a serious concern. Every day substances are being introduced in the environment that have an adverse effect on the balance of the ecosystem and living organisms. Fungi that inhabit the soil are also sensitive to contaminants (e.g., herbicides, insecticides, fungicides and heavy metals) that cause decreased or delayed growth, abnormalities of cellular metabolic pathways, and damage of cellular structures (Róžalska et al. 2014; Gola et al. 2018; Szewczyk et al. 2018; Stolarek et al. 2019; Subbanna et al. 2019). Adverse changes in the cells caused by these compounds prevent these microorganisms from playing their proper ecological role. Some fungi have also developed mechanisms that allow them to survive in the presence of such contaminants (Szewczyk et al. 2018; Nowak et al. 2019).

Because of high enzymatic activity, the ability to produce secondary metabolites, and good growth in culture media, EPF are also being tested for use in other fields of biotechnology, e.g., nanoparticle biosynthesis and bioconversion of steroids or flavonoids, which are important from an economic point of view (Róžalska et al. 2016; Gonzalez et al. 2017; Yosri et al. 2018; Dou et al. 2019; Kozłowska et al. 2019).

2 Entomopathogenic fungi—short description

Entomopathogenic fungi are parasitic microorganisms with an ability to infect and kill arthropods. They are mainly used as biopesticides in ecologic farming as a safe alternative to toxic chemical insecticides, although some of them are also used in biotechnological processes or in Chinese medicine (Jaihan et al. 2016; Ríos-Moreno et al. 2016; Lovett and Leger 2017).

Entomopathogenic fungi do not form one monophyletic group. Thus far, 12 species of *Oomycetes*, 65 species of *Chytridiomycota*, 339 species of *Microsporidia*, 474 species of *Entomophthoromycota*, 238 species of *Basidiomycota*, and 476 species of *Ascomycota* have been reported (Araújo and Hughes 2016). Species belonging to *Ascomycota* and *Entomophthoromycota* occur most frequently in nature. In the literature, species belonging to *Ascomycota* to the genera *Metarhizium* (*M. anisopliae*, *M. robertsii*, *M.*

brunneum, *M. lepidiotae*, *M. globosum*, *M. acridum*, *M. majus*, *M. flavoviride*, *M. rileyi*, *M. pingshaense*, *M. lepidiotae* and *M. guizhouense*), *Beauveria* (*B. bassiana* and *B. brongniartii*), *Isaria* (*I. fumosorosea*—formerly *Paecilomyces fumosoroseus*, *I. farinosa* and *I. tenuipes*), *Ophiocordyceps* (*O. sinensis*—formerly *Cordyceps sinensis*, *O. unilateralis*), *Cordyceps* (*C. militaris*), *Torubiella* (*T. ratticaudata*), *Pochonia* (*P. chlamydosporia*), *Lecanicillium* (*L. lecani*—formerly *Verticillium lecanii*, *L. longisporum*), *Hirsutella* (*H. thompsonii*, *H. nodulosa*, *H. aphidis*), and the species *Paecilomyces variotii*, *Purpureocillium lilacinum* are described (Khan et al. 2012; Tkaczuk et al. 2015; Jaihan et al. 2016).

Among the known EPF, Entomophthorales (e.g., *Furia*, *Conidiobolus*, *Entomophaga*, or *Erynia*) show the highest insecticidal activity; however, because of technical difficulties in breeding in laboratory conditions, they are not used as components of biopreparations. The most commonly used EPF are easily cultured saprotrophic fungi belonging to *Ascomycota* (Mascarin and Jaronski 2016). Worldwide, several different biopesticides based on species belonging to the genera *Metarhizium*, *Beauveria*, *Paecilomyces*, *Isaria* and *Lecanicillium* have been used. These fungi have a wide spectrum of activity, and they can therefore infect a wide variety of arthropod species (Khan et al. 2012; Castro et al. 2016; Ríos-Moreno et al. 2016).

Entomopathogenic fungi are heterogeneous organisms that play various ecological roles. For example, species of the genus *Metarhizium* and *Beauveria*, which are commonly found in soil, not only control natural arthropod populations but also form complex relationships with plants. They are described as endophytes of plant roots, stems, and leaves (Jaber and Enkerli 2017). It has been shown that *M. robertsii* and *B. bassiana* (but not *L. lecani*) provide plants with nitrogen that is assimilated during the parasitization of insects (Behie and Bidochka 2014), thus supporting plant growth (Ríos-Moreno et al. 2016). *Beauveria bassiana* acts as an endophyte in about 25 plant species, contributing to the control of pests and fungal plant pathogens (McKinnon et al. 2017; Vega 2018). As a fungal endophyte and epiphyte, it colonizes leaves and shoots in addition to plant roots making plants more resistant to insects (Klieber and Reineke 2016; Ramakuwela et al. 2020), and also successfully protects plants from microbial pathogens by

suppressing disease-causing agents or increasing plant defense responses (Moonjely et al. 2016). Similarly, *Lecanicillium* also prevents fungal disease by growing on the surface of leaves, limiting available nutrients and producing antimicrobial compounds in addition to inducing plant responses while colonizing plant roots (Moonjely et al. 2016). Among EPF, *I. fumosorosea* seems not to fall into strong interactions with plants. This is probably due to the fact that *Isaria* spp. are sensitive to chemical substances secreted by plants and belonging to the defense system against plant pathogens (Lacey and Mercadier 1998; Zimmermann 2008). Interestingly, isolates of *I. fumosorosea* are reported to act against the root nematode, *Meloidogyne javanica*, although infection rates were found to be very low (below 40%) (Tigano-Milani et al. 1995). EPF are primarily used in biocontrol of insect pests in the laboratory, greenhouse or in the field.

2.1 The infectious process

Entomopathogenic fungi infect insects by direct penetration of the cuticle. Unlike bacteria or viruses, they do not have to be ingested by an insect (Bilgo et al. 2018). The infection process starts with the adhesion of spores to arthropod shells and has two stages: the first depends on the action of hydrophobic and electrostatic forces and the second requires the activity of enzymes and low-molecular-weight proteins called hydrophobins (Skinner et al. 2014). Spore germination occurs in the presence of carbon and energy sources on the insect's cuticle at sufficient humidity and temperature. The optimum temperature for the growth and germination of EPF is between 20 and 30 °C. Spores can also germinate at temperatures outside this range and this is a characteristic feature of the particular fungal strain (Skinner et al. 2014). Subsequently, appressoria emerge, causing strong mechanical pressure on the cuticle and the production of lytic enzymes (proteo-, lipo- and chitinolytic) that disintegrate the insect's body shells (Skinner et al. 2014; Lacey et al. 2015). After penetrating the arthropod's body cavity (hemocel), the fungal hyphae start to grow. Some EPF can produce blastospores that enter host's hemolymph and produce secondary hyphae that inhabit the host's tissues. At this stage, the fungi produce secondary metabolites that cause paralysis and disrupt the host's physiological processes, mainly its immune responses (Donzelli and

Krasnoff 2016). Because of the developing infection, the insect's body is destroyed by both mechanical damage to the internal organs by the developing hyphae and nutrient depletion (Donzelli and Krasnoff 2016; Mascarin and Jaronski 2016; Fan et al. 2017).

As a result of the progressive infection, the insect's body, initially soft, becomes stiff due to fluid absorption by the fungus. Cadavers of insects attacked by fungi of the genus *Beauveria* may initially take on a dark red color. The entire infection process is relatively long and takes approximately 14 days after infection, but first symptoms of infection usually occur about 7 days post infection (or even earlier, depending on fungal species). After killing the insect and using all nutrition, the hyphae of the fungus emerge from the cadaver of the host through holes in its body (mouth hole, anus) and through intersegmental areas. Then, resting or infective spores are produced, which allows the fungus to spread and infect other individuals (Skinner et al. 2014).

2.2 Infectious agents

The pathogenesis caused by EPF requires the involvement of several infectious agents, the most important of which are adhesins, lytic enzymes, and secondary metabolites.

2.2.1 Adhesins

The first stage of the fungal infection process is spore adhesion to the surface of the arthropod's body. In this stage, two types of proteins are produced: hydrophobins (whose layers disintegrate during the sporulation of spores) and adhesins (MAD1 and MAD2), which enable both close adhesion to the insect's cuticle and recognition of the host by the fungal pathogen (Wang and Leger 2007; Greenfield et al. 2014).

2.2.2 Lytic enzymes

Lytic enzymes play the most important role during the process of insect infection by EPF. Their action, structure, and types are relatively well described. Their main role is hydrolysis of the components of the insect cuticle, which allows appressoria to penetrate the outer covers of arthropods. Lytic enzymes are

produced as soon as the spore attaches to the cuticle and begins to form appressorium (Santi et al. 2010).

Lipases are produced first and hydrolyze the lipids and lipoproteins located in the insect epicuticle (outer cuticle) (Pedrini et al. 2007). These enzymes hydrolyze the ester bonds of triacylglycerols, which leads to the release of free fatty acids, diacylglycerols, monoacylglycerols, and glycerol (Silva et al. 2009). Moreover, lipases support the adhesion of germinating spores to insect cuticles by increasing hydrophobic interactions between the fungus and the cuticle surface (Santi et al. 2010). All EPF have been shown to contain these enzymes, most of which have been well described (Supakdamrongkul et al. 2010; Mondal et al. 2016). One of these enzymes is *M. brunneum* (formerly *M. anisopliae* var. *anisopliae*) phospholipase C, which hydrolyzes phosphodiester bonds and is responsible for degrading phospholipids of insect cell membranes, which allows the fungus to enter the insect's hemocel and infect its tissues (Santi et al. 2010).

A key factor in the virulence of EPF is proteolytic enzymes that hydrolyze peptide bonds of insect cuticles, leading to the exposure of chitin fibrils. One of the first comprehensively described proteases is subtilisin (Pr1, serine endoprotease), which degrades both cuticle proteins and modifies the surface of the cuticle, thereby facilitating spore adhesion. This endoprotease is present in *M. anisopliae* (Shah et al. 2005), *O. sinensis* (Zhang et al. 2008), and *B. bassiana* (Donatti et al. 2008). However, other proteases such as trypsin-like acid Pr2 protease, cysteine Pr4 proteinase, and metalloprotease in *M. anisopliae*; Pr1- and Pr2-like serine proteases in *B. brongniartii*, *L. lecanii*, *M. rileyi*, and *Aschersonia aleyrodidis*; and serine elastase in *Conidiobolus coronatus* and *B. bassiana* also participate in the pathogenesis process (Zhao et al. 2016). Some studies indicate that proteases of EPF play a dual role in the infectious process. Although their main function is hydrolysis of cuticular proteins, proteases can also participate in the inactivation of antifungal proteins produced in the epidermis of insects (Sotelo-Mundo et al. 2007).

The last group of enzymes are chitinases that are classified as follows depending on the place of their action on the chitin molecule: endochitinases (hydrolyze the β -1,4-glycosidic bond inside the chitin molecule) and exochitinases (hydrolyze *N*-acetylglucosamine oligomers formed during the action of

endochitinases). The combined action of endo- and exochitinases is required for the complete degradation of insect chitin. Chitinolytic enzymes have been found in numerous EPF (Duo-Chuan 2006).

Apart from proteases, lipases, and chitinases, EPF also produce other enzymes that are not directly involved in the digestion of the cuticle but play an important role in pathogenesis. One of them is acid trehalase (ATM1) that hydrolyzes trehalose, the main disaccharide of insect hemolymph, with the release of two glucose molecules, thus providing a nutrient for EPF. Inactivation of the ATM1 gene in *M. acridum* causes a significant reduction in the virulence of this strain, which is manifested as the inability of this species to grow inside the insect's body (Jin et al. 2015).

2.2.3 Secondary metabolites: role in infection and possible applications

Entomopathogenic fungi secrete abundant low-molecular-weight organic compounds called secondary metabolites, especially in response to environmental conditions. The number of produced compounds suggests that they are necessary both for maintaining the vital functions of the hosts and for effectively infecting pathogens by damaging the nervous system or reducing insect resistance (Donzelli and Krasnoff 2016). Secondary metabolites are determined using chromatographic methods combined with mass spectrometry and molecular biology techniques. On the basis of their chemical structure, they can be divided into the following groups: cyclic depsipeptides (cyclic tetradepsipeptides and cyclic hexadepsipeptides), peptides (octadepsipeptides, dipeptides, and depsipeptides), amino acid derivatives, polyketides, peptide hybrids, and terpenoids (Donzelli and Krasnoff 2016; Wang et al. 2018b) (Online resource 1). A large number of these compounds can be distinguished due to their wide occurrence and unique properties. Taking advantage of this fact, researchers keep making efforts to extend the knowledge of the mechanisms of the secondary metabolites biosynthesis and their impact on living organisms and the environment.

Destruxins (dtxs) are cyclic hexadepsipeptides isolated mainly from *Metarhizium* sp. More than 40 types of dtxs have been reported, of which dtxs A, B, and E are the most significant in the process of pathogenesis (Arroyo-Manzanares et al. 2017). Dtxs

are well known for their insecticidal activity and phytotoxicity. They show various biological properties such as antimicrobial, antiviral, antiproliferative, cytotoxic, and immunosuppressive properties. Dtxs inhibit the action of V-ATPase proton pumps, which allows them to be used in cancer therapies or osteoporosis treatment (Liu and Tzeng 2012). However, sublines of HCT116 colon carcinoma cells are resistant to dtxs A, B, and E because of changes in membrane properties and reduction in ionophoric activity during increased activation of cholesterol synthesis (Heilos et al. 2018). Dtxs are also helpful in the treatment of Alzheimer's disease as they can inhibit and prevent the generation of β -amyloid (A β) (Wang et al. 2012). On the other hand, their presence in food products is a concern as they can pose a threat to human health and disturb the balance of the ecosystem (Taibon et al. 2015). Dtxs have been found in strawberries, maize (Taibon et al. 2015), and potatoes (Carpio et al. 2016).

Beauvericins produced e.g., by *B. bassiana*, *I. tenuipes* or *I. fumosorosea* (Wang and Xu 2012; Weng et al. 2019) and bassianolide isolated from *B. bassiana* or *Lecanicillium* sp. are other cyclic hexadepsipeptides with interesting properties. On the one hand, beauvericins contribute to the contamination of cereal crops worldwide, bioaccumulate in different tissues, and enter the food chain by polluting eggs, milk, meat, and plant products, consequently leading to apoptosis, production of reactive oxygen species (ROS), or mitochondrial damage (Olleik et al. 2019). On the other hand, they have been shown to exert strong cytotoxic effect on tumor cells, inhibit the activity of *Mycobacterium tuberculosis* or *Plasmodium falciparum*, and restrict the activity of the cholesterol acyltransferase inhibitor (Urbaniak et al. 2019; Jajić et al. 2019). Bassianolide has activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and, like beauvericins, against *M. tuberculosis*.

It inhibits several cancer cell lines and the development of the human malaria parasite *Plasmodium falciparum* (Keswani et al. 2019). Additionally, it inhibits acetylcholine-induced smooth muscle contraction (Xu et al. 2009).

Cordycepin, which belongs to octadepsipeptides, is a product of secondary metabolism of, among others, *C. militaris* and *Isaria cicadae* (formerly *Cordyceps cicadae*). It has been used in Chinese

medicine for many years because of its antioxidant, anti-inflammatory, antitumor, and neuroprotective properties (Wang et al. 2019b). As cordycepin is similar to adenosine in structure, it can inhibit processes in which adenosine is involved (Xia et al. 2017). Cordycepin reduces osteoarthritis symptoms, i.e., synovial inflammation, joint pathology, and pain behavior (Ashraf et al. 2019). It has been proved that cordycepin inhibits senescence of cells in rodents through the NRF2 (nuclear factor erythroid 2-related factor 2) and AMPK (AMP-activated protein kinase) pathways in the inflammatory process. This application gives hope for the future for obviating cells aging and radiation ulcer (Wang et al. 2019b).

Oosporein isolated mainly from *B. bassiana* or *B. brongniartii*, a highly reactive oktadepsipeptide with insecticidal and antiviral activity, is an important secondary metabolite that can also be used as an antibiotic against gram-positive bacteria (Feng et al. 2015). The insecticidal activity of oosporein is based on the stimulation of infection rather than the direct elimination of insects. Oosporein produced by *B. caledonica* has immunosuppressive properties and contributes to greater and faster mortality of insects (Mc Namara et al. 2019). Oosporein from *Cochliobolus kusanoi* exerts cytotoxic effects on Madin–Darby canine kidney (MDCK) and mouse macrophage (RAW 264.7), which is mediated by ROS through oxidative stress that increases lipid peroxidation and glutathione (GSH) level (Ramesha et al. 2015).

A research study on oosporein as a siderophore in the root endophyte *Chaetomium cupreum* revealed that the compound enhances aluminum (Al) tolerance in the plant *Miscanthus sinensis* Andersson. Furthermore, it has been proved that oosporein detoxifies Al similar to that by the phytosiderophore chlorogenic acid (Haruma et al. 2019).

M. robertsii produces polyketides that are yellow compounds named as NG-391 and NG-393 (Donzelli and Krasnoff 2016). They have structure and properties similar to those of fusarins. Like fusarin C, NG-391 exerts a strong mutagenic effect on the S9 fraction from rat liver in the Ames test (Donzelli et al. 2010). Further studies on these polyketides are required as they show antiproliferative effects on K-562 human leukemia cells, resulting in the impairment of the integrity of nucleic acid biosynthesis; on the other hand, their bioactivity may be related to binding to a protein involved in nucleoside phosphate biosynthesis

or polymerization or cell death independent of caspase (Bohnert et al. 2013).

Because the secondary metabolites of EPF are a pool of compounds with diverse activity, researchers are interested in further exploring their structures, searching for new compounds, understanding their biosynthesis pathways, and conducting in-depth studies on their properties.

3 Effect of toxic compounds on entomopathogenic fungi

The widespread use of synthetic, anthropogenic substances has increased environmental pollution through accumulation of their residues. These toxic substances cause an adverse effect on almost all living organisms to a greater or lesser extent. Pesticides are one of the basic groups of harmful substances whose presence has been detected in soil and water. These compounds enter the soil through human activities intended to improve the growth of crops. These diverse chemical compounds are usually categorized according to their mechanism of action. Among these chemicals, herbicides (substances used to control unwanted plants), fungicides and insecticides (substances used for pest control) are the most predominant ones, and both of them have harmful effects on EPF.

There are several reports on the effects of toxic compounds related to insecticides, which are often used interchangeably with EPF (such as *B. bassiana* or *M. anisopliae*) to control pests. Organophosphate (profenophos and methyl demeton) and chloroorganic insecticides (indoxacarb) showed the highest detrimental effect on *B. bassiana*. Profenophos and methyl demeton inhibited *B. bassiana* growth by 93.72% and 95.17%, respectively, after 14 days of inoculation and by 83.55% and 93.86%, respectively, after 30 days of inoculation. Indoxacarb inhibited the growth of *B. bassiana* by 100% after 14 and 30 days of inoculation (Amutha et al. 2010). The organophosphate Lorsban showed the highest toxicity to *M. anisopliae* and *I. fumosorosea*. This compound caused more than 70% mycelial growth reduction and more than 80% conidial germination reduction in both the tested fungi (Asi et al. 2010). Insecticides belonging to carbamates can also adversely affect the growth of EPF. Carbamates (Lannate, Larvin) and a pyrrole insecticide (chlorfenvinpyr; Pirate) caused 40–60% mycelia growth

reduction and 50–70% conidial germination reduction in *M. anisopliae* and *I. fumosorosea* (Asi et al. 2010). The effect of the insecticide λ -cyhalothrin, belonging to pyrethroids, is less severe, and this compound at the recommended field dose caused 13.6% and 20.6% growth inhibition in *H. nodulosa* and *B. bassiana*, respectively (Tkaczuk et al. 2015). Among the pyrethroid insecticides, the effect of deltamethrin on *B. bassiana* was studied in detail (Forlani et al. 2014). Deltamethrin at $40 \mu\text{g cm}^{-2}$ concentration caused a significant decrease in the colony-forming unit of *B. bassiana*, but the mean inhibitory concentration (IC_{50}) was $50 \mu\text{g cm}^{-2}$. These studies also investigated the possible mechanisms of the xenobiotic's detoxification. It was observed that exposure to the xenobiotic induced two genes of cytochrome P450 (belonging to phase I detoxification) and genes of the antioxidant system such as superoxide dismutase, catalase, and glutathione S-transferase. These findings suggest that *B. bassiana* activates defense mechanisms in response to the harmful effects of deltamethrin.

Neonicotinoids, another group of insecticides, also have toxic effects on *B. bassiana*. Acetamiprid and thiamethoxam applied at average doses recommended for field application caused a significant reduction in the vegetative growth of *B. bassiana*, while the growth of *M. anisopliae* was reduced only in the presence of acetamiprid. Interestingly, among all the tested neonicotinoids, imidacloprid (highly toxic for bees and banned in many countries) did not affect germination, vegetative growth, and conidia production of the tested EPF (Neves et al. 2001). Natural insecticides are less toxic to EPF, and one of such insecticide, Spinosad (isolated from *Saccharopolyspora spinosa*), caused only approximately 7–8% mycelial growth reduction and approximately 20% conidial germination reduction in *M. anisopliae* and *I. fumosorosea* (Asi et al. 2010).

Another natural insecticide, neem oil (derived from plants) was found to have a negative effect on EPF. It was revealed that it reduced the germination ratio, vegetative growth, and conidia production in *M. anisopliae* and *B. bassiana* (Hirose et al. 2001).

Research studies on the effects of insecticides on EPF are ongoing, and their main aim is to use lower doses of insecticides together with EPF for pest control. This task is quite important because determining a dose of an insecticide lower than that commonly applied in the field and supplementing its

action with EPF will reduce the use of toxic compounds and significantly reduce global environmental pollution. Many studies have indicated the possibility of this synergistic action, but recently, some studies have criticized this approach (Subbanna et al. 2019).

It seems that interactions between insect pathogenic fungi and insecticides are more complicated and can have both positive and negative effects on pest control. The interaction of two botanical insecticides tondexir and palizin with *B. bassiana* and *L. lecanii* was shown. These compounds at field applied doses did not cause significant inhibition of fungal germination, but significantly reduced the susceptibility of *Galleria mellonella* larvae to *B. bassiana* infection. Moreover, palizin reduced the effectiveness of *L. lecanii* against *G. mellonella* larvae (Sohrabi et al. 2019).

The appropriate use of insecticides and fungi seems to be another important aspect. Studies conducted by Meyling et al. (2018) revealed that the synergistic effect between α -cypermethrin and *B. bassiana* was observed only when the EPF was applied first (with an interval of > 48 h between applications). No synergy was observed when the insecticide was applied prior to fungal exposure within 24 h (Meyling et al. 2018).

A different approach was shown in other studies where only the effect of herbicides on EPF was tested and no attention was given to the effect of these compounds on the infectious potential of the fungi. The conducted research indicated that the pesticides quizalofop-p-ethyl and glyphosate, applied at the recommended field dose, caused growth inhibition of *H. nodulosa* by 63.4% and 52.1%, respectively, and of *B. bassiana* by 43.9% and 6.5%, respectively, during 20 days of cultivation (Tkaczuk et al. 2015). Among the tested herbicides, the effect of ametryn (an s-triazine herbicide) on EPF was studied most thoroughly. This compound not only inhibited growth and slowed down the degradation of glucose by *M. brunneum* but also disrupted its carbon and nitrogen metabolism. Ametryn also induced oxidative stress in the mycelia of *M. brunneum* (Szewczyk et al. 2018).

Another problem is the widespread use of fungicides that adversely affect all soil-borne fungi due to their mode of action. For several years, intensive research has been conducted to determine side effects of fungicides on beneficial organisms used in the Integrated Pest Management programs. Fungicides (similarly to insecticides) vary in compatibility

depending on the concentration of the fungicide and the entomopathogenic fungi applied.

I. fumosorosea is sensitive to copper-based fungicides containing a higher (more than 40%) percentage of equivalent metallic copper (Avery et al. 2013). The fungicides containing lower copper doses had no apparent impact on the growth of *I. fumosorosea* and *I. farinosa* but affected the infection capacity of the fungal EPF (D'Alessandro et al. 2011; Demirci et al. 2011; Avery et al. 2013). Toxic effect of copper oxide (containing 53% and 46% metallic copper) was also confirmed for *B. bassiana* and *L. muscarium* (Kouassi et al. 2003; Ali et al. 2013).

Dithiocarbamate fungicides, harbouring metals (Zn, Mn) in their molecules also have strong inhibitory effect on EPF. Mancozeb at low concentrations ($5 \mu\text{g mL}^{-1}$) was found to inhibit *I. farinosa* conidia germination. This fungicide applied at average doses recommended for field application caused growth inhibition of *B. bassiana* and *I. farinosa* (Todorova et al. 1998; Kouassi et al. 2003). Mancozeb and propineb in low doses inhibited the sporulation of *B. bassiana* (Celar and Kos 2016). Unfortunately, mancozeb also decreased mortality of insects inoculated with EPF (Kouassi et al. 2003; Celar and Kos 2016).

Fungicide chlorothalonil (a broad-spectrum of fungicide, widely used, nitro compound) seems to have little effect on *B. bassiana* growth. However, it was shown that this fungicide severely inhibited conidial germination of *I. farinosa* and *B. bassiana* even at a low concentration of $5 \mu\text{g mL}^{-1}$ (Demirci et al. 2011; Celar and Kos 2016). In other studies chlorothalonil in field doses demonstrated fungicidal activity against *B. bassiana* and *M. anisopliae* by the inhibition or reduction of growth and sporulation (Fiedler and Sosnowska 2017). This fungicide also inhibited germination of *L. muscarium* spores (at concentration $10 \mu\text{g mL}^{-1}$) but unexpectedly it did not suppress chitinase, Pr1, Pr2 and lipase activity suggesting its possible compatibility with the fungus (Bagga et al. 2004). Similarly to chlorothalonil, another widely used fungicide captan (dicarboximide) only slightly hindered mycelial growth but severely inhibited conidial germination *B. bassiana*, *I. fumosorosea*, *L. longisporum* (Shah et al. 2009; Dara 2017). Captan applied in field doses with *B. bassiana* severely affected the mortality in *T. molitor* larvae (Dara 2017).

Triazole fungicides have a more diverse effect on EPF and among them the most toxic is tebuconazole.

This compound suppressed the growth of *B. bassiana* and *M. anisopliae* at concentration 1000 ppm and *L. lecanii* at concentration 100 ppm. Other triazole fungicides, difenaconazole, tricyclazole and myclobutanil were also toxic to *L. lecanii* and *L. muscarium* (Demirci et al. 2011; Reddy et al. 2018). Tebuconazole was highly toxic to *I. farinosa* (at concentration $1 \mu\text{g mL}^{-1}$) and to *P. lilacinus* (at $3 \mu\text{g mL}^{-1}$) causing both conidial germination and mycelial growth inhibition (Demirci et al. 2011). This fungicide also hindered the entomopathogenic activity of *I. farinosa* in all three life stages of the mealybug (Demirci et al. 2011) and completely inhibited the entomopathogenic activity of the fungus *Erynia neoaphidis* on pea aphid (*Acyrtosiphon pisum*) (Latteur and Jansen 2002).

Azoxystrobin (strobilurin fungicide) was found to have a negative impact on growth and sporulation of *B. bassiana* and *M. anisopliae* and to be highly deleterious to conidial production and growth of *I. fumosorosea* (D'Alessandro et al. 2011; Fiedler and Sosnowska 2017). It exhibited moderate toxicity toward *M. majus* (growth inhibition by 40%, sporulation reduction by 60%) compared to other common fungicides used in banana agro-system (Sivakumar et al. 2019). However, the influence of azoxystrobin on EPF virulence varied. This compound did not affect the insect infection by *B. bassiana*, whereas it caused a significant inhibition of insect infection by *I. fumosorosea* (Shah et al. 2009).

Recent scientific research suggests that EPF sensitivity to fungicides can be reduced by using microsclerotia instead of spores alone. Chlorothalonil and iprodione, seem to have a limited impact on the ability of microsclerotia of *M. brunneum* to grow out and produce viable conidia, compared to their impact on germination of conidia and growth of mycelium (Wu et al. 2020). Some fungicides may need to be applied some time before or after the application of EPF to minimize any suppressive effects. However, the benefit of separating the applications in time needs to be investigated in future studies.

The effects of other anthropogenic compounds such as endocrine disrupting compounds (EDCs) on EPF are relatively rarely studied. It was found that nonylphenols are toxic to *Metarhizium*. These compounds at high concentrations (50 and 100 mg L^{-1}) caused severe changes in the pellet morphology of *M. robertsii* and reduced its metabolic activity. These

disruptions were associated with changes in the hyphae structure that were manifested in terms of increased vacuolization, an increase in the size and change in the shape of mitochondria, and destruction of the plasma membrane (that leads to autolysis). Changes were also noted in the thickness and composition of the cell wall (Różalska et al. 2014). Another EDC—dibutyltin—and the by-product of its dealkylation, monobutyltin, caused nitrosative and oxidative stress and changes in the lipid profiles of *M. robertsii* (Siewiera et al. 2017a; Stolarek et al. 2019).

Industrial dyes can also adversely affect the growth of EPF. Reactive remazol red, Indanthrene blue, Yellow 3RS, and Vat Novatic Grey caused a reduction in mycelium biomass. Yellow 3RS showed the lowest adverse effect on the growth of *B. bassiana*. In the presence of this dye, the amount of biomass obtained was 5.3 g L^{-1} . Reactive remazol red and Indanthrene blue were more harmful to *B. bassiana* than Yellow 3RS. Among the tested dyes, Vat Novatic Grey was the most toxic to *B. bassiana* MTCC 4580 strain (Gola et al. 2018).

Other anthropogenic substances such as biofertilizers, which seem to be harmless to the environment, may also affect the growth of soil-inhabiting EPF. Biofertilizers are products applied on the surface of a plant or in soil and contain live microorganisms that promote plant growth and development. These products may include bacterial species such as *Rhizobium*, *Azotobacter*, and *Azospirillum* as well as blue green algae (BGA) (Kumar et al. 2017). Hirose et al. (2001) determined the effect of three biofertilizers (E.M.-4, MultibionTM, and Supermagro) on two species of EPF: *M. anisopliae* and *B. bassiana*. E.M.-4 and MultibionTM reduced the germination ratio, vegetative growth, and conidia production in *M. anisopliae*. MultibionTM was found to be moderately toxic to *B. bassiana* (Hirose et al. 2001).

4 Removal of toxic pollutants by entomopathogenic fungi

Entomopathogenic fungi are commonly found in natural environment, where they come in contact with various anthropogenic pollutants. Although the mechanisms regulating the pathogenesis of insects by EPF have been relatively well understood, only a few studies have reported the interaction of toxic

impurities with these microorganisms. One of the most harmful groups of anthropogenic compounds are EDCs. They are natural or synthetic substances which, because of their structural similarity and ability to bind to hormone receptors in the cell, can disrupt the functioning of the human and animal endocrine system. This group includes, among others, alkylphenols (e.g., nonylphenol), organotin compounds (tributyl tin [TBT]), synthetic estrogens (e.g., 17 α -ethinylestradiol [EE2]), and pesticides (e.g., ametryn) (Różalska et al. 2015; Szewczyk et al. 2018; Nowak et al. 2019; Stolarek et al. 2019).

4.1 Removal of 4-*n*-nonylphenol by entomopathogenic fungi

Nonylphenols are xenoestrogens widely used in the manufacture of many household products. The most commonly used nonylphenols are para-nonylphenols (4-nonylphenols [4-NP]), which occur in the form of several dozen isomers differing in the degree of branching of the alkyl fragment of the molecule. These compounds are extremely harmful for the natural environment because of their high toxicity to aquatic organisms and the ability to accumulate. They enter the environment primarily as biodegradable products of surfactants and detergents. 4-NPs also disrupt the functioning of the human and animal endocrine system by imitating 17 β -estradiol and binding to estrogen receptors. These compounds have a negative effect on microorganisms and soil invertebrates and restrict plant growth (Acir and Guenther 2018).

Strains of the genus *Metarhizium* can degrade the unbranched isomer of nonylphenol (4-*n*-NP). *M. robertsii* and *M. brunneum* showed the highest activity in cultures after 24 h incubation with the compound, with more than 90% loss of substrate (Różalska et al. 2013). The extracts of *M. robertsii* culture showed 35 derivatives that were formed after the degradation of this xenobiotic (Różalska et al. 2015; Nowak et al. 2019). The proposed degradation pathway indicates that the fungi of the genus *Metarhizium* have unique capabilities to perform numerous, simultaneous mono- and dihydroxylation reactions both in the aliphatic chain and the 4-*n*-NP aromatic ring (Nowak et al. 2019). In addition, these fungi completely degraded nonylphenol in the culture environment to form CO₂ and H₂O (Różalska et al. 2015; Nowak et al. 2019).

4.2 Triazine removal by entomopathogenic fungi

Triazines are herbicides that possess a heterocyclic ring containing three unsaturated nitrogen atoms. These compounds have a carcinogenic effect and disrupt the functioning of the endocrine system in living organisms; therefore, their use is prohibited in the European Union, although their presence is still found in the natural environment. The conducted studies showed the ability of *M. brunneum* to partially decompose ametryn, a triazine, at an initial concentration of 100 mg L⁻¹ to generate four derivatives (Szewczyk et al. 2018).

4.3 Dibutyltin removal by entomopathogenic fungi

Dibutyltin (DBT) is used as a curing agent for silicone rubbers and as a catalyst in esterification reactions. It enters the environment through polyvinyl chloride (PVC) materials and as a degradation product of TBT. DBT exhibits immunotoxic, neurotoxic, and pro-oxidative properties. Aerated cultures of *M. robertsii* can completely biodegrade DBT (added at an initial concentration of 20 mg L⁻¹) to monobutyltin (MBT) and hydroxylated MBT. The addition of vitamin C caused a decrease in oxidative stress associated with the presence of tin compounds in the medium, and the tested strain in the presence of this vitamin degraded MBT threefold faster (Siewiera et al. 2017a). The presence of natural estrogens—estrone (E1) and 17 β -estradiol (E2)—accelerated the degradation of DBT. Moreover, both the tested estrogens protected EPF against oxidative stress caused by the presence of MBT in the culture medium (Siewiera et al. 2017b).

The cell membrane fluidity of *M. robertsii* raises the possibility of this EPF to degrade toxic substances. A membrane with a less compact structure, and thus with higher permeability, allows the transport of toxic compounds into the cell, where they are degraded (Stolarek et al. 2019).

4.4 Synthetic estrogen removal by entomopathogenic fungi

EE2 is a synthetic estrogen ubiquitously present in the environment (soil, groundwater, rivers, lakes, and seas) because of its widespread use and resistance to biodegradation. It is a derivative of E2 and is used in

hormonal contraception. Various strains of *M. robertsii* can partially remove EE2 from the growth medium with significant efficiency, which reaches up to 85% (Różalska et al. 2015).

4.5 Removal of hydrocarbons (*n*-alkanes) by entomopathogenic fungi

Entomopathogenic fungi can assimilate *n*-alkanes. The metabolic pathways of the decomposition of these compounds and the enzymes involved in this process are relatively well known (Keyhani 2018). These hydrocarbons are xenobiotics commonly found in the environment; they are also found in small amounts in the insect epicuticle, which, together with the wax layer, is the first important barrier to overcome for EPF. Lipids containing long hydrocarbon chains are present in all external insect layers. The fungus *B. bassiana* can use *n*-alkanes (extracted from insect surfaces) as a carbon source. These compounds are first hydroxylated by cytochrome P450 present in the microsomal fraction and then catabolized by β -oxidation. This fungus can also grow and metabolize synthetic straight-chain alkanes (up to *n*-C33) (Huarte-Bonnet et al. 2015). Other EPF, e.g., *M. robertsii*, are also capable of using these linear hydrocarbons (e.g., *n*-hexadecane or *n*-octacosan) as the only source of carbon and energy, and one of the first stages of degradation is carried out by cytochrome P450 (Huarte-Bonnet et al. 2018).

4.6 Removal of industrial dyes by entomopathogenic fungi

Gola et al. (2018) tested the ability of the EPF *B. bassiana* MTCC 4580 to remove four industrial dyes (Reactive remazol red, Indanthrene blue, Yellow 3RS, and Vat Novatic Grey) from wastewater. The strain *B. bassiana* MTCC 4580 could eliminate the dyes (added to the cultures at a high initial concentration of 500 mg L⁻¹) with high efficiency, which reached even 97%. Maximum removal was observed for Indanthrene blue (97%), while the removal of Vat Novatic Grey and Reactive remazol red was 91% and 84%, respectively. The mechanism of synthetic dye removal by *B. bassiana* is based on bioaccumulation and not on biodegradation (Gola et al. 2018).

5 Influence of heavy metals on entomopathogenic fungi

Entomopathogenic fungi can be used for heavy metal removal from the environment by improving the phytoremediation properties of plants (Farias et al. 2019). Even trace amounts of metals in the environment can have an adverse effect on soil, the ecosystem, water circulation, and elements in the environment as well as on human and animal health. The presence of heavy metals in the human body can contribute to the development of autoimmune diseases, digestive system disorders, and diseases of many important internal organs, including the heart, liver, kidneys, and lungs. An increase in heavy metal occurrence in the environment is due to the development of mining and electroplating industries, vehicular traffic, and waste (Hussein et al. 2011; Gola et al. 2016). The removal of heavy metal residues from the environment poses problems because of their tendency to accumulate in soil. The use of physicochemical methods of metal removal is expensive and unfavorable for the environment and uneconomic when the metal concentration is low.

The EPF *B. bassiana* has confirmed ability to remove heavy metals from contaminated water. This fungal species can remove several heavy metals such as Cu (II), Ni (II), Cd (II), Zn (II), Cr (VI), and Pb (II) added at the initial concentration of 30 mg L⁻¹ with good efficiency ranging from 58% to 75%. This study also revealed the bioaccumulation of Pb (II) inside *B. bassiana* cells, although the exact mechanism of this phenomenon has not yet been proposed (Gola et al. 2018). Lead was also found to cause severe morphological alterations such as an increase in surface roughness and hyphae density and aggregation. It was also found that heavy metals are not very toxic to *B. bassiana* and the minimum inhibitory concentrations (MIC) were as follows: Zn (II): 250 mg L⁻¹; Cd (II): 200 mg L⁻¹; Cu (II), Ni (II) and Cr (VI): 100 mg L⁻¹ (Gola et al. 2018).

The mechanism of *B. bassiana* susceptibility to Cr (III) has been partly explained by the studies on the role of the aldo-keto reductase Bdakr1 (Wang et al. 2018b). Like other heavy metals, chromium causes oxidative stress in fungal cells and intracellular lipid peroxidation, which leads to the accumulation of malonaldehyde (MDA). Under the normal condition, intracellular MDA levels were similar between the

wild type and Δ Bbakr1. The presence of the heavy metal (5 mM Cr (NO₃)₃) in the culture medium caused a significant increase in MDA levels in both the wild type and Δ Bbakr1 cells, but in the case of the mutant, the levels were significantly higher than those in the wild type. A growth reduction of Δ Bbakr1 strain was also noted. These data suggest an impaired ability of the mutant cells to detoxify the heavy metal by catalyzing reduction reactions of aldehydes or ketones to primary and secondary alcohols (Wang et al. 2018a).

The biomass of two EPF (*B. bassiana* AUMC 3263 and *M. anisopliae* AUMC 3085) can also serve as an efficient biosorbent for Pb (II) and Cd (II) from aqueous metal solutions. The maximum lead adsorption capacities of *B. bassiana* and *M. anisopliae* were 83.33 and 66 mg g⁻¹, respectively, while for cadmium, these values reached 46 and 44 mg g⁻¹, respectively. The higher adsorption capacity of *B. bassiana* biomass than that for *M. anisopliae* could be due to the differences in the cell wall structure. The highest rate of metal absorption was observed during the first 5 min of the experiment and reached a constant value after 30 min (Hussein et al. 2011).

Heavy metals can also negatively affect the growth of EPF. Tkaczuk et al. (2019) determined the effect of five heavy metals (Zn (II), Ni (II), Cu (II), Cd (II), and Pb (II)) on the growth of *I. farinosa*, *I. fumosorosea*, and *I. tenuipes*. The experiments were conducted on Sabouraud solid medium supplemented with heavy metals at concentrations that normally occur in soil (i.e., Zn (II): 33.0; Pb(II): 13.8; Cu (II), Ni (II): 6.5; Cd (II): 0.22 mg L⁻¹) and at concentrations that were 10 and 100 times higher. The obtained data revealed that the greatest toxic effect of heavy metal ions on the EPF was observed when their concentration was 100 times higher than the natural content (on average, approximately 50.5% inhibition of growth). The greatest inhibitory effect on the growth of the fungal colonies was showed by Ni (II), while Pb (II) showed the least effects. Among all the tested species, *I. fumosorosea* had the highest tolerance to the presence of heavy metals.

Plants are widely used for bioremediation of heavy metal-contaminated areas. They interact closely with microorganisms, which can enhance plant growth and health by increasing nutrient uptake and improving plant resistance to pathogens and stress (Kidd et al. 2017). EPF are not only insect pathogens, but they are

also associated with plant rhizosphere, thereby promoting plant growth. Farias et al. (2019) investigated the effect of fungal consortium containing four species of EPF (*B. bassiana*, *M. anisopliae*, *P. chlamydosporia*, and *P. lilacinum*) on the ability of *Jacaranda mimosifolia* to absorb Cu (II), Zn (II), and Mn (II). After 45 days of experiments, it was found that the use of the fungal preparation in combination with biochar caused an increase in the mass of roots and shoots of *J. mimosifolia*, intensified the translocation of metals from roots to shoots, and inhibited the leaching of metals from plant tissues, which resulted in an increased concentration of these metals in *J. mimosifolia* tissues. The application of the consortium of fungal isolates and biochar improved the phytoremediation potential of *J. mimosifolia*.

6 Nanoparticle biosynthesis by entomopathogenic fungi

During the last decade, metal nanoparticles have received considerable attention due to their wide range of applications in many different fields (e.g., pest management). They have also been used as antimicrobial agents and biomarkers. The production of nanoparticles by fungi is an interesting and ecological alternative to chemical and physical syntheses. Extracts from EPF or their culture media containing numerous enzymes can also serve as catalysts for the production of nanoparticles.

The waste mycelium after biodegradation processes is a valuable source of both enzymes and many other substances that can be reused in various processes. An example is the use of *M. robertsii* waste biomass (obtained after nonylphenol degradation) for the production of silver nanoparticles (AgNPs). Aqueous extracts from the waste mycelium *M. robertsii*, in dilutions of 25% and 50%, allowed the synthesis of silver nanoparticles from silver nitrate, and the obtained nanoparticles were small in size (from 2 to 29 nm) and relatively monodisperse, which is an important factor in their later use. The obtained colloids also exhibited antimicrobial activity, and the addition of 30 ppm AgNPs caused growth inhibition of *S. aureus* and *E. coli* (Różalska et al. 2016). These nanoparticles were also found to have fungicidal properties against *Candida albicans*, *C. glabrata*, and *C. parapsilosis* minimum inhibitory concentration

(MIC) values ranged from 1.56 to 6.25 $\mu\text{g mL}^{-1}$. An 85% decrease in viability of the tested yeast was observed after 6 h of incubation with AgNPs (added at a concentration of 6.25 $\mu\text{g mL}^{-1}$); it was also observed that this concentration of nanoparticles was not toxic to mammalian cells. The main mechanism of action of AgNPs against pathogenic yeast is probably based on changes in membrane permeability associated with a decrease in the amount of C18:2 phospholipids (Róźalska et al. 2018).

In developing countries, the major public health concerns are vector-borne diseases. EPF are frequently used in protection against mosquitoes. Moreover, nanoparticles produced by these microorganisms were also found to be effective in biocontrol programs.

Myco-synthesized AgNPs are also a potential larvicidal and eco-friendly agent for controlling mosquito population. AgNPs obtained from *M. anisopliae* culture extracts may be relevant as biocontrol agents for the malaria vector *Anopheles culicifacies* mosquitos. The LC_{50} (median lethal concentration) of the studied nanoparticles ranged from 32 to 51 mg L^{-1} for various larval stages and 60 mg L^{-1} for pupae, although the low doses of silver nanoparticles limited the development of larvae and pupae (Amerasan et al. 2016). AgNPs synthesized by *I. fumosorosea* are even more effective against mosquitos. These nanoparticles were found to be more susceptible against all larval instars of *Culex quinquefasciatus* and *Aedes aegypti*. The mortality of all larval instars in the presence of 1 ppm of AgNPs ranged from 92% to 100%. The LC_{50} and LC_{90} values were 0.240–0.652 and 1.219–2.916, respectively, for all larval instars (I–IV) of *C. quinquefasciatus* and 0.065–0.137 and 0.558–1.278 ppm for *A. aegypti* (Banu and Balasubramanian 2014). The filtrates of *I. fumosorosea* can also produce iron nanoparticles that can serve as an insecticide against sweet potato whitefly *Bemisia tabaci*. These nanoparticles showed high pathogenicity against nymphs (second and third instar) and pupae, with LC_{50} values ranging from 19.17 to 37.71 ppm. LT_{50} (lethal time) at the iron nanoparticle concentration of 50 ppm was shorter for second instar nymphs (3.15 days) and longer for pupae (4.22 days) (Wang et al. 2019a).

Metarhizium anisopliae-synthesized titanium nanoparticles (TiNPs) also have insecticidal potential. The larvicidal activity of TiNPs against *G. mellonella* was 22%, but the synergistic effect of these

nanoparticles and gamma-irradiated *M. anisopliae* (with higher lipase, protease, amylase, and nitrate reductase activities) caused 82% larval mortality (Yosri et al. 2018).

7 Biotransformation performed using entomopathogenic fungi

Entomopathogenic fungi can perform biotransformation, leading to the generation of derivatives with higher activity. These processes are favorable, because they improve the absorbability of products (Yang et al. 2018; Dou et al. 2019; Kozłowska et al. 2019).

7.1 Steroid biotransformation

Steroids are compounds with high economic importance, and they are widely used in medicine as hormones and active drugs. Compounds of pharmaceutical significance are obtained by modification and transformation. Because chemical methods of steroid modification are very complicated and involve even several stages, transformations are most often performed using microorganisms. Microbiological transformations of steroids include hydroxylation, Baeyer–Villiger oxidation, reduction, and isomerization. Among them, 11α -, 11β -, and 17α -hydroxylation reactions carried out by filamentous fungi are the key reactions to obtain molecules with high biological activity (Xiong et al. 2006; Gonzalez et al. 2017; Kozłowska et al. 2019).

Many filamentous fungi are used in steroid transformations, and EPF can also be efficiently used for transformations. Recent studies have shown that 12 *I. farinosa* strains could effectively transform dehydroepiandrosterone (DHEA) to 7α - and 7β -hydroxy derivatives. One of these strains, *I. farinosa* KCh KW1.1, transformed androstenediol, androstenedione, adrenosterone, 17α -methyltestosterone, 17β -hydroxyandrost-1,4,6-triene-3-one, and progesterone to mono or dihydroxy derivatives with a high yield and stereoselectivity (Kozłowska et al. 2019).

In another study, biotransformation of estrogens (E1, E2, and EE2) by *I. fumosorosea* was confirmed. Most of the estrogens were hydroxylated at C-6 position and glycosylated at C-3 position. The presence of a methyl group at the C-3 steroid ring

completely inhibited the glycosylation of estrogens by *I. fumosorosea*, indicating that a free hydroxyl group at this position is necessary for glycosylation (Kozłowska et al. 2019).

Entomopathogenic fungi can also transform steroids on a large scale, which can be used for industrial purpose in the future. 11 α -Hydroxy was transformed to 13-ethyl-gon-4-ene-3,17-dione (GD, a key step in the synthesis of oral contraceptive desogestrel) by *M. anisopliae* and nano-liposome technique with a high yield. The main reaction product 11 α -hydroxy-13-ethyl-gon-4-ene-3,17-dione (HGD) was also produced at high concentration of GD as 6 g L⁻¹ with no reduction of transformation efficiency. The main byproduct of the reaction 6 β ,11 α -dihydroxy derivative was found to benefit the purification of HGD (Feng et al. 2014).

Beauveria bassiana may also find applications in the pharmaceutical industry because it gives high yields of valuable 11 α -hydroxy intermediates of steroids (Gonzalez et al. 2017). This process, however, seems to require optimization. In *B. bassiana* culture media with androst-4-ene-3,17-dione at pH = 6.0, only two hydroxylated metabolites were found, while in samples at pH = 7.0, four hydroxylated and/or reduced metabolites were detected. These data show that *B. bassiana* is clearly an efficient biocatalyst for 11 α -hydroxylation and reduction of the 17-carbonyl group (Xiong et al. 2006). *B. bassiana* strain ATCC7159 transforms DHEA to 3 β ,11 α ,17 β -trihydroxyandrost-5-ene in the presence of a moderate DHEA concentration (0.83–1.0 g L⁻¹) and in a mild environment (26 °C, pH 7) (Gonzalez et al. 2017). The addition of various compounds can positively affect the biosynthesis of these pharmaceutical metabolites. An example is the enhanced hydroxylation capacity of *B. bassiana* with *n*-alkane solvents as a carbon source. The addition of *n*-dodecane (*n*-C12) and *n*-hexadecane (*n*-C16) was found to enhance the conversion of DHEA to 3 β ,11 α ,17 β -trihydroxyandrost-5-ene, resulting in the appearance 22.8% mM hydroxylated product in the culture medium. An unusual feature of *Beauveria* is the mode of conducting the reaction, which consists of the reduction of the C-17 ketone of DHEA preceded by 11 α -hydroxylation. This phenomenon differs from the reaction carried out by other microorganisms, which first activate an unfunctionalized carbon to 11 α -hydroxy-17-oxo derivative before

obtaining a 3 β ,11 α ,17 β -triol product (Gonzalez et al. 2015).

7.2 Flavonoid biotransformation

Flavonoids are derivatives of plant polyphenols. These are glycosides with O- and C-glycosidic bonds connecting the base with sugar residues (Dymarska et al. 2018a, b; Dou et al. 2019), including most often glucose, rhamnose, galactose, xylose, and arabinose (Yang et al. 2018). It is believed that the sugar group of flavonoids is the main factor affecting the absorption of these compounds by the human body (Sordon et al. 2017). There are six classes of flavonoids: flavones, isoflavones, flavanones, flavanols, flavanonols, and flavan-3-ols. Because of their antioxidant, anti-inflammatory, and antibacterial properties, they exert beneficial effects on human health. In addition, flavonoids have anti-tumor and immunomodulatory activity and protective effects on the human nervous system. The consumption of foods containing flavonoids can prevent the development of heart disease, diabetes, and depression (Dymarska et al. 2018a; Yang et al. 2018; Dou et al. 2019). They occur naturally in vegetables, fruits, cereals, wine, and tea (Yang et al. 2018; Dou et al. 2019).

Microorganisms can efficiently biotransform flavonoids to flavonoid glycosides, which are better absorbed than flavonoids themselves. Among microorganisms, EPF seem to have great potential in these processes; however, currently, *Isaria* and *Beauveria* are studied frequently, and the potential of *Metarhizium* remains undiscovered. The relevant data are presented in Table 1.

8 Conclusions

A review of literature showed that in addition to the use of EPF in biocontrol, these fungi can also be used in different ways that have not yet been described in previous reviews. EPF can also be used for removing harmful substances from the environment, because they possess enzymes that enable the removal of toxic compounds of anthropogenic origin. For example, *M. robertsii* and *M. brunneum* species can degrade nonylphenols, triazines, tin compounds, synthetic estrogen, hydrocarbons, and even industrial dyes. These microorganisms also interact with heavy

Table 1 Flavonoid transformation by entomopathogenic fungi

The group of flavonoids	Source—flavonoid	Product of transformation	Entomopathogenic fungi strain	Bibliography
Flavones	Flavone	Flavone 2-O- β -D-(4-O-methyl)-glucopyranoside 4'-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2	Dymarska et al. (2018a)
	3-hydroxyflavone	Flavone 4'-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.6	
		3-O- β -D-(4''-O-methyl)-glucopyranoside flavone	<i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.4, <i>I. farinosa</i> J1.6	Dymarska et al. (2018b)
		3-O- β -D-glucopyranoside flavone	<i>I. fumosorosea</i> KCH J2	
		3-O-[[β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl]o]-4'-hydroxyflavone	<i>I. fumosorosea</i> KCH J2	Dymarska et al. (2018a)
	5-hydroxyflavone	5-hydroxyflavone 4'-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.4	Dymarska et al. (2018a)
	6-hydroxyflavone	6-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.6, <i>I. farinosa</i> KW1.2	Dymarska et al. (2018a)
	7-hydroxyflavone	7-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.4, <i>I. farinosa</i> J1.6	Dymarska et al. (2018a)
	5,6,7-trihydroxyflavone (Baicalein)	5,7-dihydroxyflavone 6-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2	Dymarska et al. (2018b)
		3-methoxyflavone 4'-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.4, <i>I. farinosa</i> J1.6	Dymarska et al. (2018b)
Flavonols	7-aminoflavone	7-acetamidoflavone	<i>I. fumosorosea</i> KCH J2	Dymarska et al. (2018a)
	Luteolin	4'-hydroxy-7-aminoflavone	<i>I. fumosorosea</i> KCH J2	Dou et al. (2019)
		4',5,7-trihydroxyflavone 3'-O- β -D-(4-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> ACCC 37814	
		3',5,7-trihydroxyflavone 4'-O- β -D-(4-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> ACCC 37814	
	Diosmetin	5,7-dihydroxy-4'-methoxyflavone 3'-O- β -D-(4-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> ACCC 37814	Dou et al. (2019)
	Apigenin	Mono-(4-O-methyl)-methylglucoside	<i>I. fumosorosea</i> ACCC 37814	Dou et al. (2019)
	Quercetin	3',4',5,7-tetrahydroxyflavone 3-O- β -D-(4''-O-methyl)-glucopyranoside	<i>I. fumosorosea</i> KCH J2	Dymarska et al. (2018b)
		3',4',5,7-tetrahydroxyflavone 3-O- β -D-glucopyranoside (isoquercetin)		
	Kaempferol	Mono-(4-O-methyl)-methylglucoside, monoglucoside	<i>I. fumosorosea</i> ACCC 37814	Dou et al. (2019)

Table 1 continued

The group of flavonoids	Source—flavonoid	Product of transformation	Entomopathogenic fungi strain	Bibliography
Flavanones	Naringenin	4',5-dihydroxyflavanone	<i>I. fumosorosea</i> ACCC 37814	Dou et al. (2019)
		glucopyranoside		
		5,7-dihydroxyflavanone		
		4'-O-β-D-(4-O-methyl)-glucopyranoside		
Isoflavones	Hesperetin Daidzein	5,7-dihydroxyflavanone	<i>I. fumosorosea</i> ACCC 37814 <i>I. fumosorosea</i> KCH J2, <i>I. farinosa</i> J1.6, <i>I. farinosa</i> KW1.2 <i>B. bassiana</i> AM278 <i>I. fumosorosea</i> ACCC 37814	Dou et al. (2019) Dymarska et al. (2018a) Sordon et al. (2017) Dou et al. (2019)
		4'-O-β-D-glucopyranoside		
		5-hydroxyflavanone		
		4',7-di-O-β-D-(4-O-methyl)-glucopyranoside		
		Mono-(4-O-methyl)-methylglucoside, monoglucoside		
		4''-O-methyl/daidzin		
	Formononetin Genistein Biochanine A	4-methoxyisoflavone	<i>I. fumosorosea</i> ACCC 37814 <i>B. bassiana</i> AM278 <i>I. fumosorosea</i> ACCC 37814 <i>I. fumosorosea</i> ACCC 37814 <i>B. bassiana</i> AM278 <i>B. bassiana</i> AM278	Dou et al. (2019) Sordon et al. (2017) Sordon et al. (2017) Sordon et al. (2017)
		glucopyranoside		
		Mono-(4-O-methyl)-methylglucoside		
		4''-O-methyl/genistin		
		4''-O-methyl/biochanin A		

metals, which sometimes adversely affect their growth or hyphae morphology; however, these fungi possess the ability to remove heavy metals from the environment, for example, from contaminated waters (zinc, copper, cadmium, nickel, and chromium) and indirectly by improving the phytoremediation properties of plants (copper, manganese, and zinc). On the basis of this current review article, the authors conclude that EPF have great potential in the biosynthesis of biologically active compounds used in medicine, for example, hydroxylated steroids and glycosylated flavonoids or for bioproduction of silver nanoparticles with antibacterial and antifungal properties. Because of the high potential shown by EPF and the many different functions that they can perform, they are being increasingly researched.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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