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REVIEW

Filamentous fungi in biological control: current status and future perspectives

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ABSTRACT

Agriculture is the largest economic sector in the world. The awareness of the current environmental degradation caused by conventional farming practices has allowed the use of entomopathogenic fungi as a biological control technique to become increasingly widespread. Several studies from the laboratory bench to field trials show that fungi can be directly applied or, more recently, can be carried to the target by other biological vectors (i.e., insects), which increases their potential for dispersal and transmission. In addition, studies on the development of formulations have intensified, with the aim to enable their commercialization and reduce costs for the more sustainable management of crops. This review discusses the positive aspects of the use of filamentous fungi in the biological control of pests, specifically in terms of the use of antagonistic fungal plant pathogens and nematophagous fungi.

Key words: Biological control, environmental degradation, filamentous fungi, sustainable agriculture.

INTRODUCTION

Currently, more than two billion tons of pesticides are used every year all over the world. These pesticides include fungicides, bactericides, herbicides, insecticides and others that are used to eliminate undesirable agents, mainly fungi, weeds and insects, which are considered crop pests, with the aim to guarantee a high yield.

The cost of this practice is not only financially high, but also environmentally, because the excessive use of pesticides leads to water bodies, groundwater and soil contamination in addition to affecting human and other animals' health due the toxicity, recalcitrance and the carcinogenic potential of many of these compounds.

The biological control of pests has been recognized as an alternative to the use of pesticides. Nonetheless, even though this is a cheaper technology and less harmful to the environment, biological control practices are currently very little used in relation to pesticides. This fact mainly occurs because biological management is more specific and takes a longer time to achieve the desired results.

Therefore, due to the worsening of deleterious environmental effects promoted by the excessive use of pesticides, biological control became a target of in-depth researches and its application has been increased worldwide. This review aims to describe the main current uses of filamentous fungi in biological control, including new techniques that have been developed in addition to discussing about new possible applications of these fungi, specifically for plant growth promotion, opening novel and real possibilities for the search for cleaner and healthy agricultural practices.

Taxonomy and the application of fungi in biological control

Among the wide variety of microorganisms that have reported potential and are used in biocontrol practices, fungi are the most studied and applied (Schrank and Vainstein, 2010). According to Thomas and Read (2007), the main reasons for their broad use are their efficiency in eliminating their hosts, their huge metabolic diversity, which increases the chances of finding appropriate isolates for biocontrol, and their relative environmental safety, as they are primarily decomposers.

Fungal species that are used in biological control include both basal and higher fungi (Table 1). Among the basal fungi, there are representatives of the phylum *Blastocladiomycota*, such as *Coelomomyces*, and of the subphylum *Entomophthoromycotina*, such as species of the genera *Conidiobolus*, *Entomophthora*, *Erynia* and *Entomophaga*. Among the higher fungi, several species of the phylum *Ascomycota* (Subkingdom *Dikarya*) have reported potential and are commercialized and applied in the field, such as *Purpureocillium*, *Metarhizium*, *Beauveria*, *Cordyceps*, *Fusarium*, *Trichoderma*, and others.

Before defining the use of a fungus as a commercial product, many characteristics must be observed, including its virulence, capacity to resist environmental stressors (ultraviolet [UV] resistance, temperature tolerance), mass production potential, sporulation capacity on low-cost alternative substrates, ability to cause infection under low humidity conditions and specificity to the parasites of interest (Pourseyed et al., 2010).

Biological control: one step toward the development of a more sustainable agriculture

Biological control can be defined as the introduction of an exogenous biological agent into an environment with an aim toward its permanent establishment to control the pests present therein over the long term (Kenis et al., 2017). The biological agent applied can be a parasitoid, a pathogen or a predator of the organism that is causing economic losses (Hajek and Delalibera, 2010).

Despite the advantages of biological control, the adoption of its practices was slow around the world. This was because the supply of chemicals, which are of low specificity and high efficiency but have rapid action against pests, was ample. Instead, biological approaches were based on the principle of using natural enemies, which are often specific to one or a few pests and require a longer time to produce significant results.

However, the growing problem related to the indiscriminate use of agricultural chemical inputs has been changing, which has led several organizations to begin debates and efforts to improve awareness regarding the need to reduce pesticide and fertilizer consumption and prohibit the use of products that have been shown to threaten food and occupational safety (Carneiro et al., 2015), resulting in greater visibility of biological control, which has led to the application of all the accumulated knowledge on this subject in the field.

Biological control was proposed for the first time in the 19th century, aiming to control either populations of disease-causing species or insect-dispersed pests. It is a detailed and specific methodology that involves the isolation and

Table 1. Overview of the current taxonomic position of the main entomopathogenic fungi genera.

Basal fungi	Phylum *Microsporidia* Phylum *Glomeromycota* Phylum *Neocallimastigomycota* Phylum *Chytridiomycota* Phylum *Blastocladiomycota*		Class *Blastocladiomycetes*	Order *Blastocladiales*	*Coelomomyces*
		Subphylum *Entomophthoromycotina* Subphylum *Zoopagomycotina* Subphylum *Mucoromycotina*		Order *Entomophthorales*	*Conidiobolus* *Entomophthora* *Entomophaga* *Erynia*
Higher fungi (Subkingdom Dikarya)	Phylum *Ascomycota*	Subphytum *Pezizomycotina*	Class *Sordariomycetes*	Order *Hypocreales*	*Metarrhizium* *Beauveria* *Paecilomyces* *Purpureocillium* *Cordyceps* *Nomurea* *Trichoderma* *Fusarium* *Verticillium*
	Phylum *Basidiomycota*				

These data are based on the fungal kingdom classification proposed by Hibbett et al. (2007).

identification of microorganisms, the identification of their potential for this purpose and their formulation to make their application in the field feasible. Innovative results have been generated from the utilization of biological control, actualizing the possibility of the development of a cleaner and sustainable agriculture and bringing increasing investments for research and the application of natural products.

Biological control of arthropods by fungi

Insects are the main class of the *Arthropoda* phylum and are among the most diverse living beings on the planet. Even only a small portion of this group contains species known as agricultural pests; they are responsible for causing considerable damage to crops, devastating approximately 20% of the global annual production (Schrank and Vainstein, 2010).

In this context, it arise the practices of biological control using entomopathogenic fungi, a group formed by several species that are able to infect and cause disease in insects and other arthropods (Vega et al., 2009; Pell et al., 2010). Most entomopathogenic fungi belong to the orders *Hypocreales* (phylum *Ascomycota*) and *Entomophthorales* (subphylum *Entomophthoromycotina*) (Hibbett et al., 2007) (Table 1). Generally, entomophthoralean fungi are specific to their hosts and therefore present a relatively low risk of infecting beneficial insects, such as pollinators, while fungi of the *Hypocreales* order are less selective and infect a wider range of hosts (Roy and Pell, 2000).

The spores that originate from asexual reproduction (aplanospores in *Entomophthorales* and conidia in *Hypocreales*) are the main infective structures involved in the process of host invasion by entomopathogenic fungi. Once in contact with the cuticle of a susceptible host, the conidium germinates (germinative tube formation), forms an appressorium and a penetration clamp and secrets hydrolytic enzymes that disrupt the cuticle and allow the colonization of internal tissues by the elongation of the hyphae (Shah and Pell, 2003; Kurtti and Keyhani, 2008). When the hyphae reach the hemolymph, unicellular blastospores, which are able to evade the host's immune cells, are formed and colonize the inside of the body (Wanchoo et al., 2009; Ortiz-Urquiza et al., 2015). Upon the death of the host, the fungus emerges, starts producing hyphae and begins the process of sporulation, usually on the outside of the corpse's body (Shah and Pell, 2003) (Figure 1).

Figure 1. Entomopathogenic fungi *Beauveria bassiana* (A), *Metarhizium anisopliae* (B) and *Isaria fumosorosea* (C) grown on potato dextrose agar (PDA) plates. Figures D-H represent the sugarcane borer *Diatraea saccharalis* colonized by *B. bassiana* (D and G), *M. anisopliae* (E and H) and *I. fumosorosea* (F).

The best characterized and most employed entomopathogenic fungi in biological control programs are *Metarhizium anisopliae* and *Beauveria bassiana*. They are two cosmopolitan and saprophytic species that can be found in almost all ecosystems, i.e., they are facultative pathogens that do not require an arthropod as a host to complete their life cycles (Schrank and Vainstein, 2010; Ortiz-Urquiza et al., 2015).

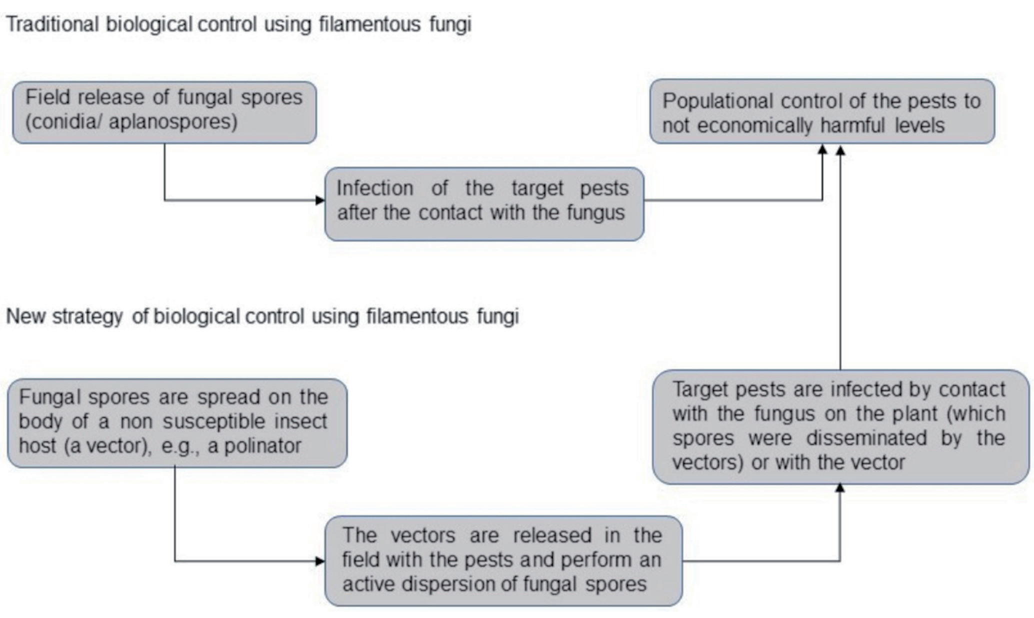
Pourseyed et al. (2010) showed the efficiency of *M. anisopliae* in the control of the fowl tick, *Argas persicus*, which, in addition to being a hematophagous parasite of fowls, can act as a vector of other parasites, bacteria and viruses. The authors indicate that, in addition to the problems of environmental contamination related to the use of acaricides, including mainly organophosphates and carbamate compounds, their application led to selection for resistance. In their study, they demonstrated that the V245 isolate of *M. anisopliae* was able to act on tick eggs, larvae and adults (already fed or not).

Entomopathogenic fungi can also be useful in the biological control of mosquitoes. These insects are of great concern to public health, especially in tropical and subtropical regions, because they are vectors of several diseases, such as malaria, yellow fever, filariasis, dengue, chikungunya, and Zika virus, among others. Several studies report the potential of fungi of the genera *Lagenidium*, *Coelomyces*, *Conidiobolus*, *Entomophthora*, *Culicinomyces*, *Erynia*, *Beauveria*, *Metarhizium*, *Entomophthora* and others in the control of many species of mosquitoes, including representatives of the genera *Anopheles*, *Culex*, *Aedes* and *Ochlerotatus* (Scholte et al., 2004).

Lee et al. (2015) demonstrated the use of entomopathogenic fungi in the control of *Aedes albopictus* mosquitoes, which are known as transmitters of several kinds of viral encephalitis and filariasis-causing nematodes. In this study, 12 isolated and identified species belonging to the entomopathogenic genera *Beauveria*, *Metarhizium*, *Cordyceps*, *Paecilomyces*, *Purpureocillium* and *Verticillium* were tested for their pathogenic potential against *A. albopictus* larvae. One strain of *Beauveria brongniartii* and two of *M. anisopliae* (isolates JEF-003 and JEF-004) were able to cause 100%, 93.3% and 86.7% larval mortality, respectively, highlighting *M. anisopliae* strain JEF-003, which killed 73% of the larvae in the first two days.

Beauveria bassiana is an opportunistic pathogen, and its use against many arthropods is widespread. New studies exploring alternative methods of dissemination of this fungus in the environment have been conducted with the aim to maximize the efficiency of its transmission to target arthropods. According to Lin et al. (2017), a promising strategy for biological control programs is the utilization of other invertebrates, such as mites and insects, as vectors of entomopathogenic fungi, increasing the chances of contact between a microorganism and its host. This strategy is particularly promising when biopesticides cannot be effectively released against their targets (Figure 2).

Figure. 2. Traditional and novel way of entomopathogenic fungi application in biological control. The new strategy guarantees a more efficient dispersion of the fungal agent, increasing the chances of contact with the target pest host.



Several examples of this kind of application have been described in the literature. Al Mazra'awi et al. (2006) effectively dispersed *B. bassiana* conidia using honey bees, *Apis mellifera*, as vectors. The contaminated bees were released in canola (*Brassica napus*) fields with the aim to transfer the fungus to the tarnished plant bug, *Lygus lineolaris*, a pest that severely affects canola fields. The bees are the main pollinators of this plant, and their great movement among the plants ensures wide dissemination of the fungus, carrying it to the bugs on the plants. By increasing fungus dispersal, the authors recorded mortality rates of up to five times higher when compared to the control.

Zhang et al. (2015) described a similar method of spreading *B. bassiana* among *Diaphorina citri* psyllids in *Murraya paniculata* (Rutaceae) plants through fungal inoculation in two different species of mites, *Neoseiulus cucumeris* and *Amblyseius swirskii*, which are natural predators of the psyllid. The mites present some resistance to fungal attack, with low mortality rates (10%-15%) observed in their nymphs after 7 d of exposure to the conidia. Meanwhile, mortality rates of 100% were observed in *in vitro* assays for *D. citri* when exposed to concentrations above 1×10^4 conidia mL^{-1}. The majority of *D. citri* individuals were killed by *B. bassiana* a few days after contact with the mites that were exposed to the fungus in assays with plants.

The use of fungi in the biological control of plant pathogens

In many cases, the control of pathogens can involve direct interaction between fungi and plants. In this case, the fungi are able to act as plant pathogen antagonists, i.e., they can use several different mechanisms, such as the production of metabolites (antibiotics, volatile compounds - ammonia, cyanide, alcohols, esters, ketones, etc. - or enzymes), competition (for space, C, N or mineral sources), parasitism, or the induction of systemic resistance in the plant or an increase in its growth response, resulting in a reduction in the pathogen's activities (Vega et al., 2009).

The genus *Trichoderma* (*Hypocreales*) is one of the best known because of its activities against plant pathogens. It includes cosmopolitan species commonly found in the soil. Members of this genus present rapid growth, and their major role in nature is as primary decomposers. In addition, *Trichoderma* spp. have been targets of studies and are used for commercial exploitation due their ability to produce antibiotics and several enzymes of interest and their potential as biocontrol agents (Anees et al., 2010). These fungi are able to inhibit the growth of phytopathogenic fungi by inducing plant resistance or by acting directly against the pathogen as an antagonist, mycoparasite or competitor (Howell, 2003; Verma et al., 2007). Knowing how the fungus behaves in the presence of other plant parasitic fungi is essential to improve the application of this microorganism.

As mycoparasites, *Trichoderma* species produce helical hyphae around the hyphae of a pathogenic fungus, forming appressoria, where degrading lytic enzymes are produced and released, allowing the penetration of the pathogen's hyphae (Anees et al., 2010). As antagonists, they produce several compounds with antimicrobial activity (Vinale et al., 2008), and they are also great competitors for space and nutrients (Hjeljord and Tronsmo, 1998; Anees et al., 2010).

One of the main uses of *Trichoderma* spp. is in the fight against the basidiomycete *Rhizoctonia solani*, a known phytopathogen that causes disease in the roots of several plants. Anees et al. (2010) reported the antagonistic potential of *Trichoderma gamsii* T30 against *R. solani* AG 2-2 *in vitro* and *in vivo*, demonstrating that, for this strain, antibiosis is the best way to combat this pathogen, since no helical hyphae were observed on the pathogen's hyphae during morphological analysis. Moreover, in the same study, when testing *Trichoderma* strains taken from the soil of a sugar beet crop in which healthy and symptomatic areas in terms of the presence of *R. solani* could be observed, the authors noticed that strains obtained from diseased areas presented higher activities of antagonism and plant resistance induction compared to those isolated from healthy areas.

Currently, new studies are being developed to explore strains that have already been used extensively in biocontrol strategies. For example, studies involving *Metarhizium* and *Beauveria* species have generally focused on their application in the control of insects and other arthropods. However, new studies have suggested the existence and importance of complex relationships that these fungi may have with plants (Behie and Bidochka, 2014), pointing to the possibility that these interactions have been predominant in the past and that entomopathogenicity is an opportunistic evolutionary adaptation in these genera (Ortiz-Urquiza et al., 2015).

The well-known entomopathogenic species *B. bassiana* has also demonstrated an expressive dual character in scenarios when it directly interacts with plants. Ownley et al. (2008) described how *B. bassiana* protects tomato seedlings against attack by *R. solani* and *Pythium myriotylum*, which cause root rot and damping-off. The seeds were treated with conidia of the strain *B. bassiana* 11-98 and were then sown in soil artificially contaminated with the pathogens. The seedlings from the germination of seeds treated with the fungus presented better growth and a significant reduction in the symptoms caused by *R. solani*.

Positive results for the biological control of the damping-off caused by *R. solani* using *B. bassiana* have also been described in cotton (Griffin et al., 2005). The addition of fungal conidia (1×10^9 conidia per seed) resulted in healthier seedlings, with higher survival rates: 31% in non-treated seeds against 69% in those that received the fungal conidia. It is believed that *B. bassiana* is able to endophytically colonize plant tissues and that, by using mechanisms such as competition for space, parasitism or systemic resistance induction, it is able to act in the biocontrol of pathogens (Ownley et al., 2008).

Nematophagous fungi and the biological control of nematodes

Among the more than 26 000 known nematode species, approximately 4100 are plant parasites that are commonly found in soil and impair plant roots (Nicol et al., 2011); among these, nearly 100 species are considered to be economically relevant due to the annual losses of the more than US$100 billion they cause around the world (Degenkolb and Vilcinskas, 2016).

The lack of specificity of the symptoms caused by nematode parasitism is a great challenge in diagnosing their presence in crops. A wide range of infestation symptoms can be observed, such as wilting, discoloration, reduced vigor, nutrient deficiency, root lesions, reduced flowering, fruit loss, low productivity and even death. Only some of these are evident: in the aerial part, there may be chlorotic or necrotic spots that spread rapidly from the infection site and, in the roots, the formation of galls, cysts and agglomerates of worms may occur (Degenkolb and Vilcinskas, 2016) (Figure 3A).

Figure 3. (A) Galls of nematodes in guava (*Psidium guajava* var. Paluma) roots. (B) Photomicroscopy image of *Purpureocillium lilacinum* microculture stained with lactophenol blue and (C) macroscopic overview of *P. lilacinum* grown on potato dextrose agar (PDA).

The efficiency of nematicides is a point of disagreement because the mechanisms of action of these compounds are not specific, they are expensive and non-selective, and they present toxicity to harmless vertebrate and invertebrate species, including humans (Li et al., 2015; Degenkolb and Vilcinskas, 2016). In addition, another disadvantage of using chemical nematicides is that they do not affect the development of the eggs. The eggshell of nematodes is composed of several layers of chitin, a barrier that makes them resistant to nematicides. Therefore, the use of biological agents is a more economic and environmentally friendly way to combat nematodes.

Nematophagous fungi are specific parasites of these helminths, and their use has been explored in terms of nematode control. To be considered nematophagous, the fungal conidia must germinate on the body of their host, and the hyphae must penetrate the nematode and make it sick (Sexton and Howlett, 2006).

Purpureocillium (= *Paecilomyces*) *lilacinum* (Figure 3B-C) is one of the most studied and tested fungi in the biological control of nematodes due to its ability to parasitize even nematode eggs (Atkins et al., 2005). Many biological nematicides that have been registered up to now are composed of *P. lilacinum* (Dong and Zhang, 2006). Species of the genus *Verticillium* have also had their potential for biological control described, with *V.* (= *Pochonia*/*Metacordyceps*) *chlamydosporium* being the best known representative for this purpose (Hajji et al., 2017) besides other species as *V. lecanii*.

Another positive aspect related to *Purpureocillium* representatives is the production of secondary metabolites that are able to promote plant growth, such as phytohormones (gibberellins and auxins), and substances that facilitate their defense against the harmful effects of biotic and abiotic stresses (pH and salinity, for example) (Khan et al., 2012). Moreover, it has been reported that these fungi can promote plant growth by performing P solubilization in soil. Phosphorus can be found in great amounts in soil; however it is not in an assimilable form. Insoluble forms are not available for plant uptake, so fungal action on these substrates modify them, allowing to provide this essential element to plants (Rinu and Pandey, 2011; Lima-Rivera et al., 2016).

CONCLUSIONS

Due to the economic and environmental advantages of biological control, its improvement and expansion of use will certainly be seen in the future. In-depth studies focused on the metabolic and genetic abilities of fungi related to the biocontrol process reveal that they present multiple potential applications. Entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* can be more than arthropod parasites, associating with the roots and tissues of plants and helping in the fight against pathogens. Antagonistic fungi, such as *Trichoderma* spp. and the well-known nematophagous fungus *Purpureocillium lilacinum*, are able to promote plant growth by solubilizing nutrients and providing them to the plants. Therefore, studies on the identification and development of biopesticides are in an ascending phase that meets with plant growth-promoting microorganisms, a promising partnership that has the potential to undoubtedly revolutionize agricultural practices in the context of their evolution toward being less harmful to the environment.

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