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THE GREAT POTENTIAL OF ENTOMOPHTHORALEAN FUNGI FOR BIOLOGICAL CONTROL: A REVIEW

Mihaela Monica DINU[1, 2], Ana-Cristina FĂTU[2], Narcisa BĂBEANU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., District 1, Bucharest, Romania
[2]Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., District 1, Bucharest, Romania

Corresponding author email: mihaela.dinu@icdpp.ro

Abstract

Entomopathogenic fungi are well known for their role in the biological control of pests. The manipulation techniques of filamentous entomopathogenic fungi belonging to order Hypocreales (Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Lecanicillium longisporum, etc.) are already well developed in the biotechnological industry. At the moment, these types of fungi are the only ones authorized for inundative biological control of pest. However, numerous studies from recent years draw attention to some ecological attributes of order Entomophthorales as being more advantageous than order Hypocreales. In this review, we discuss the general characteristics of the Entomophthorales, the differences between Hypocreales and Entomophthorales, and the advances and challenges of using entomophthoralean fungi as myco-insecticides.

***Key words**: entomopathogenic fungi, Entomophthorales, entomophthoralean fungi, biological control.*

INTRODUCTION

Entomophthoralean fungi

The order Entomophthorales is currently classified as part of the subphylum Entomophthoromycotina. The majority of extant species in this subphylum are soil living and saprotrophic, but most studied species are obligate or facultative parasites of insects (Möckel et al., 2022). In the present review, the entomopathogenic fungi we name entomophthoralean fungi to refer only to species of the ord. Entomophthorales.

Many species within Entomophthorales are pathogenic for insects and few species attack nematodes and mites (Jaronski, 2014). Entomophthoralean fungi are, from this point of view, considered important candidates for pest management with biological control agents (BCA). Even the name of ord. Entomophthorales is suggestive, the etymology of the word revealing that it comes from ancient Greek and it translates mot-a-mot "insect destroyer" (gr. entomo-= referring to insects - actually meaning “notched”, refers to the segmented body plan of the insect, and phthorá = “destruction”) (Britannica, 2019).

Biological control

The term “microbial control” was first used by Steinhaus in 1949: “that phase of biological control concerned with the employment by man of microorganisms for the control and reduction of the number of animals (or plants) in particular area or a given population” (Ravensberg, 2010). A comprehensive study of the history of biological control was conducted in the early twentieth century by Steinhaus (1956). However, this idea has its roots in entomology studies and cannot be associated with a single researcher (Stern et al., 1959; DeBach, 1964; van den Bosch, 1971).

Nowadays, biological control (or biocontrol) is defined in the plant protection discipline as an ecological alternative to chemical crop protection and a component of the integrated pest management (IPM) strategy. Biological control strategies use BCA as natural enemies (viruses, bacteria, fungi, arthropods, etc.) named beneficial organisms, to control crops, pests, and diseases. Eilenberg et al. (2001) suggested that the term “biological control should be restricted to the use of living organisms.” However, other authors consider botanical insecticides (plant extracts such as essential oils, alkaloids, flavonoids, glycosides, esters, and fatty acids) are also biological control tools (Hikal et al., 2017; Tembo et al., 2018). For example, The International Biocontrol Manufacturers Association (IBMA) defines biological control as: “pest and disease control for plant protection based on living organisms and naturally-sourced compounds”. Also, IBMA considers three types of biocontrol: conservation biological control, augmentative biological control, and classical biological control.

According to Eilenberg et al. (2001), biological control can be carried out following four strategies: (1) Classical biological control (“the intentional introduction of an exotic, usually co-evolved, biological control agent for the permanent establishment and long-term pest control”); (2) Inoculation biological control (“the intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently”); (3) Inundation biological control (“the use of living organisms to control pests when control is achieved exclusively by the released organisms themselves”); (4) Conservation biological control (“modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests”). Inundation biological control use bioinsecticides based on entomopathogenic fungi (mycoinsecticides) and it is the most widely used microbial control strategy. The application of mycoinsecticides in pest management is similar to chemical pesticides.

The term "biological control" has had many definitions and approaches over time, but the four strategies are now generally accepted by most authors.

European legislation on BCA

European Union legislation on the sustainable use of pesticides, EU Directive 2009/128/EC, encourages the development and introduction of IPM and alternative approaches or techniques to reduce dependence on pesticides. However, the implementation of this directive at the Member State level has not been as successful, and “very little progress has been made in promoting the uptake of alternative techniques, which are the key to ensuring real pesticide dependency reductions” (Committee on the Environment, Public Health, and Food Safety, 2019). At present, the approval of pesticides based on microorganisms follows the exact requirements as for chemicals. Research on biological low-risk pest control products should be encouraged, and legislation on the authorization of micro-organisms for use in plant protection should be adapted to the specific properties and hazards of micro-organisms and not clone chemical pesticide regulations (Sundh & Eilenberg, 2021; Reinbacher et al., 2021). European legislation has approved for plant protection 449 active substances, safeners, and synergists, of which only 66 are microbials (strains of fungi, bacteria, and viruses). The approval expiration is set on 30.04.2022 for 29 of these strains (source EU Pesticides database, accessed 01.03.2022). Currently, no Entomophthorales-based mycoinsecticides are available (Litwin et al., 2020). Member States of the European Union endorsed four legal acts on February 10, 2022, which will shorten the authorization process of biological plant protection products based on micro-organisms. These acts reflect the latest scientific developments and could become applicable in Q4 2022 (European Commission, 2022). Relaxing the regulations governing the approval of microorganism-based plant protection products will reduce costs and speed up the development of new items, such as entomophthoralean-based mycoinsecticides. In recent years, progress has been made in the study of these fungi formulation. This review aims to provide an overview of entomophthoralean fungi and their potential as microbial control agents.

MATERIALS AND METHODS

We systematically searched several scientific databases such as ScienceDirect and Wiley Online Library, for literature from 1980 to 2022 using the following search terms: “entomophthoralean”, “Entomophthorales” and “Entomophthoromycotina”. We retrieved 1456 results, to which we added publications from Academia, Research Gate, and Google Scholar. Duplicates and publications that were not relevant to entomopathogens or biological control of pests were eliminated after a thorough screening. The exclusion criteria selected was the field of application. A comprehensive search of the European Commission website and EUR-Lex database for legislation relating to Plant Protection Products based on microorganisms was also done, yielding three pertinent legal acts. Following the suitability evaluation, a total of 75 papers were selected for this review.

RESULTS AND DISCUSSIONS

Entomopathogenic fungi

Entomopathogenic microorganisms are currently used worldwide in microbial control (the use of micro-organisms in biological control) for controlling pests in agriculture, forestry, horticulture, etc., being a healthier alternative, environmentally friendly, and more sustainable to conventional chemical insecticides. Unlike other insect pathogenic microorganisms, most entomopathogenic fungi have the unique property of infecting through the cuticle, therefore they do not need to be ingested. (Roberts & Hajek, 1992). Only a few taxa (e.g. Culicinomyces) infect the host by invading through the alimentary canal (Inglis et al., 2001).

One of the earliest known accounts of insect diseases was found in the writings of Aristotle in Historia Animalium (probably written between 335 and 322 B.C.) (Steinhaus, 1956). The first known studies on entomopathogenic fungi were conducted in the 1800s to find a solution for managing silkworm disease, later called white muscardine, which severely affected the silk industry (Steinhaus, 1975). Following this discovery, studies of the interaction between arthropods and entomopathogenic fungi have been of particular interest due to their potential use of fungal entomopathogens for pest control (Keller, 1998; Hajek & Goettel, 2008.).

Not all arthropod species are necessarily closely associated with entomopathogenic fungi (Humber 2012a), but the number of insect-associated fungi is very high (Blackwell, 2011). Currently, arthropod species are estimated to be between 5-10 million (Ødegaard, 2000). The entomopathogenic property of about 700 fungal species belonging to 90 genera is already known, but only a few have been studied (Khachatourians & Qazi, 2008). Many researchers have studied entomopathogenic fungi (EPF) belonging to ord. Hypocreales (formerly Deuteromycetes) for use in the biological control of pests, especially fungi of the genera *Beauveria*, *Metarhizium*, *Isaria* (*Paecilomyces*), and *Lecanicillium* (*Verticillium*) (Inglis et al., 2001). For example, McCoy et al. (1988), Evans (1997), Ferron et al. (1991), Roberts & Hajek (1992), Tanada & Kaya (1993), Hajek & St. Leger (1994), Boucias & Pendland (1998), Wraight & Carruthers (1999), Zimmermann (2007), Butt et al. (2016), Islam et al. (2021), Rajula et al. (2021) to name a few, reviewed the main information about hyphomycetes and their use as microbial insecticides. Accordingly, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin, *Isaria fumosorosea* Wize, *Metarhizium anisopliae* (Metchnikoff) Sorokin, and *Lecanicillium lecanii* (Zimmerman) Viegas were mainly studied (Bamisile et al., 2021). These species have a wide range of hosts and are easy to produce on an industrial scale. Epizootics usually occur only in insect populations in soil (Keller & Zimmerman, 1989).

On the other hand, entomophthoralean fungi have a high host specificity (Jaronski, 2014) and could be combined with useful arthropods in pest control which is one of the significant advantages of using these fungi in biological control programs. Furthermore, these entomopathogens occur in temperate, subtropical, and equatorial climates, and they are natural enemies of many harmful insects of agricultural interest such as thrips, aphids, lepidopterous adults, and larvae. They have great potential in triggering epizootics in foliar insect or mite populations (Evans, 1989) and could remain active for years as resistant spores. In an experiment performed in America, the fungus caused an epizootic five years after its artificial introduction, demonstrating the advantages of a specific trait of these fungi, that could generate resistant spores. The identification and confirmation of the strain have been made using enzyme and restriction fragment length polymorphism analyses (Hajek et al., 1990).

Entomophthoralean fungi have been less studied, mainly because their use in biological control has proved to be more difficult due to the limitations on mass production, which seems to be the most critical bottleneck (Ravensberg, 2010). However, their great biological control potential has long been known (Pell et al., 2001).

Augmentation strategies followed by conservation, i.e., use of irrigation, an increase of humidity, and providing banker plants with alternative hosts (reservoirs of entomopathogens) have been shown to have very good results (Wilding et al., 1986; Shah et al., 2004; Gonzalez et al., 2016; Dinu et al., 2017). But any microorganism used to control an insect must be registered with the appropriate regulatory body. The approval procedure is both costly and lengthy. A commercial motivation of inoculation biological control is insufficient (Jaronski, 2014). Formulation and mass production of entomophthoralean fungi for inoculation biological control has been and continues to be a major challenge.

Entomophthorales taxonomy

The subphylum Entomophthoromycotina has been the subject of discussion among taxonomists for decades (Möckel et al., 2022). It originated from the oldest known lineages of terrestrial fungi, most likely appearing in the Silurian, more than 400 mya (Humber, 2012b; Gryganskyi et al., 2013). It does not appear to have co-evolved with insects, which occurred 300 million years ago, yet they have high degree of specialization to their hosts. The appearance and the radiation of Pterygota (winged insects) have been shown to contribute to the dispersal of the entomopathogenic lines of this phylum. Currently, Pterygota constitute the most parasitized host group (Möckel et al., 2022).

One of the newest phylogenetic classifications is proposed by Spatafora et al. (2016) and includes two phyla, Mucoromycota and Zoopagomycota, with Entomophtoromycotina classified as a subphylum of Zoopagomycota. Also, in the most recent phylogenomic studies that reassessed the phylogeny of this group, respectively based on conserved genes encoding ribosomal RNA and RNA polymerase II subunits, the authors taxonomically classify these fungi in the subphylum Entomophthoromycotina (Li et al., 2021; Möckel et al., 2022), within three classes, three orders and 6 families. The study on entomophthoromycotan genome characteristics has lately grown and it will also reveal key evolutionary mechanisms behind selection adaptation (Hajek, 2004). A novel isolation unit of entomophthoralean fungi, has been developed lately (Hu et al., 2018). It is operational in the field, making it easier to collect conidia, preserve them, and identify new species and fungal strains.

Currently, order Entomophthorales follows the same classification as the one proposed by Humber (2012b):

Order Entomophthorales G. Winter, Rabenh. Krypt.-Fl.
- Family Ancylistaceae J. Schröt.
- Family Completoriaceae Humber
- Family Entomophthoraceae Nowak.
  - Subfamily Entomophthoroideae S. Keller
  - Subfamily Erynioideae S. Keller
- Family Meristacraceae Humber

High potential of entomophthoralean fungi as naturally occurring biological control agents

Entomophthoralean fungi have unique physiological characteristics which are important factors for biological control effectiveness.

Host specialization evolved genetically in response to the challenge of utilizing resources and dealing with the immune systems of different hosts. Genomic and transcriptome techniques have the potential to help researchers better understand the molecular processes of entomophthoralean pathogenesis (Licht et al., 2016).

The behavior of diseased insects inside the colony has an impact on pathogen transmission. Arthropods infected with entomophthoralean fungi have been observed to exhibit behavioral patterns that facilitate fungal dissemination. (Roy et al., 2006). For example, some entomophoralean diseases drive infected insects to migrate to the plant's top before dying. (summit disease syndrome). Consequently, the conidia ejected by the insect's cadaver have a higher chance of landing on possible hosts, nearby or on the same plant (Figure 1).

Figure 1. Epizooty in an aphids' colony ©MMD

This is probably the most successful evolutionary adaptation of these pathogens (Inglis et al., 2001). Insect behavior is even more altered: once at the top of the plant, they fix themselves to the plant with their mandible or feet, so that when they die, they remain immovable to the plant. This type of behavior generally comes across solitary insects. The gregarious insects (as aphids) stay in the colony and become reservoirs of infectious spores. Rhizoids, which are specific to some fungal species, grow from the insect's abdomen and anchor it to the substrate. (Bałazy, 1993).

On the other hand, entomophtoralean fungal spores are actively discharged (forcibly ejected), so they have greater chances to come in contact with another host (Figure 2).

Figure 2. Forcibly discharged conidia ("halo" of conidia) in a colony of aphids from the corpse of *Sitobion* sp. infected by an entomophthoralean fungus ©MMD

More than that, if the ejected conidia fall on a non-host surface, it will produce higher-order conidia, named secondary conidia. A secondary conidium may germinate to produce a tertiary conidium, also actively discharged (Pell et al., 2001). When the spores reach a potential host, the mechanism specific to entomopathogenic fungi is triggered, respectively invasion of the host by germ hyphae produced by conidia. It multiplies inside insect hosts as hyphae, hyphal bodies, or protoplasts.

The central hypothesis as entomophthoralean grows as protoplasts in the hemolymph of insects is to advantage the fungus in escaping host immune recognition (Boomsma et al., 2014). Because protoplasts lack a sugar-rich cell wall, they are not recognized as invaders by the hemocytes that normally protect insects. The insect does not die immediately but slow down, feed less, stop laying eggs, or deposit eggs in unsuitable spots (Roy et al., 2016). For example, the average survival time of insects infected with the entomopathogen Pandora is 5-6 days. This feature encourages the rapid spread of the disease in pest populations (Görg et al., 2021).

Solitary insects seek cooler places, such as the top of the plant, during the last 1-2 days of infection. These locations are favorable for pathogen dissemination. Before the host dies, protoplasts acquire cell walls and are ready to resume their life cycle. Shortly after the insect dies, the fungus sporulates from its body. Conidia are produced externally on cadavers and are relatively short-lived (Licht et al., 2016).

Numerous authors have studied how dissemination is done by beneficial organisms used in biological control and have observed that they carry conidia in the foraging activity (Baverstock et al., 2008; Wells et al., 2011). It has also been observed that the attack of these entomopathogens stimulates transgenerational wing induction in some insects, thus contributing to the pathogen's spread (Hatano et al., 2012).

When the cold season approaches, the absence of the host insects triggers other physiological processes in entomophthoralean fungi. Winter survival is essential because the method for overwintering can be a key role in triggering epizootics during the seasons. Entomophthoralean fungi have four known winter survival strategies.: (1) as hyphal bodies in dead hosts (Keller, 1987); (2) as hyphal bodies in hibernating (living) hosts; (3) through a slow disease development and a slow disease transmission among hibernating hosts (Eilenberg et al., 2013); (4) as resting spores in soil (Hajek et al., 2018). Some *Conidiobolus* spp. and other less specialized entomophthoralean pathogens can survive and grow in the soil (Gryganskyi et al., 2017).

Production and formulation as biological control products

The inundation biological control requires large quantities of mycoinsecticide and mass production is the most critical bottleneck of entomophthoralean fungi (Ravensberg, 2010). Entomophthorales species have specific nutritional requirements for growth and sporulation *in vitro* (Latgé, 1981) They can be classified into four broad groups: (1) *Conidiobolus* spp. (family Ancylistaceae), which can be grown on standard media; (2) *Batkoa* (subfamily Entomophthoroideae), *Erynia,* and *Zoophthora* spp. (subfamily Erynioideae), which need supplements; (3) *Entomophthora* and *Entomophaga* spp. (subfamily Entomophthoroideae), which need special media; (4) *Strongwellsea* (subfamily Erynioideae) and *Neozygites* (order Neozygitales, family Neozygitaceae), which need tissue culture media (Keller, 1997; Pell et al., 2001). A synthesis of the formulation of fungi belonging to the order Entomophthorales was made by Pell et al. (2001). The author describes experiments in which various stages of the biological cycle of entomophthoralean fungi were exploited: production of the hyphal stage, formulation of hyphal material, and production of resting spores. By 2001, 46 species that produced resting spore *in vitro* had been described (Pell et al., 2001). The fragile nature of the mycelium and conidia makes these fungi more difficult to formulate than Hypocreales, which has led to their lack of commercial success.

Several other entomophthoralean formulations with fungal mycelium have been tested in recent years, some of them including broomcorn pellets (Hua & Feng, 2003), granules of broomcorn millet and polymer gel (Zhou & Feng, 2009), alginate pellets (Zhou & Feng, 2010), secondary conidia in inverted emulsion (water-in-oil formulation) (Batta et al., 2011), mycelium-encapsulated alginate pellets that float and sporulate continuously for utilization in watery fields (Zhou et al., 2015), encapsulation in calcium alginate beads (Muskat et al., 2022, a), to name a few. A complex nutrition source containing skimmed milk, yeast extract, and a low-cost fungal protein has increased biomass in a liquid shaking culture, according to the results of a recent experiment (Muskat et al., 2022, a). This is the first successful attempt to explore biomass production in a liquid media and it is a crucial step toward the fungus's potential for mass production.

CONCLUSIONS

Even though order Entomophthorales has some ecological advantages over order Hypocreales, there are no commercially available plant entomophthoralean mycoinsecticides on the market. They were not developed because the alternative was more economically viable. Despite mass production challenges, significant progress has been made in determining the best formulation for entomophthoralean species. The recent submerged fermentation laboratory experiment success paves the way for large-scale fermentation and formulation processes. Given the recent legislation relaxation regarding the use of microorganisms in pest control and the European Parliament's recommendations to reduce pesticide dependency, it is critical to investigate and utilize all available natural resources. The physiology of these fungi and the multitrophic interactions in the environment are not yet fully understood, and future studies will need to focus on this. Research on the physiology of entomphthoralean fungi is essential for developing strategies for mass production, storage, and application.

In the context of regulatory relaxation and the newest results on the mass production process, this paper outlined the major characteristics of entomophthoralean fungi and their current development potential as plant protection products.

REFERENCES

Bałazy S. (1993). *Flora of Poland. Fungi (Mycota), vol. 24, Entomophthorales*. Polish Academy of Science, Warsaw.

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model application of entomopathogenic fungi as alternatives to chemical pesticides: prospects, challenges, and insights for next-generation sustainable agriculture. *Frontiers in Plant Science, 12*. 741–804.

Batta, Y., Rahman, M., Powis, K., Baker, G., & Schmidt, O. (2011). Formulation and application of the entomopathogenic fungus: *Zoophthora radicans* (Brefeld) Batko (Zygomycetes: Entomophthorales). *Journal of Applied Microbiology*, 110. 831–839

Baverstock, J., Baverstock, K.E., Clark, S.J., & Pell, J.K. (2008). Transmission of *Pandora neoaphidis* in the presence of cooccurring arthropods. *Journal of Invertebrate Pathology*, 98. 356–359.

Blackwell, M. (2011). The fungi: 1, 2, 3..5.1 million species? *American Journal of Botany, 98*(3). 426–438.

Boomsma, J. J., Jensen, A. B., Meyling, N. V., & Eilenberg, J. (2014). Evolutionary interaction networks of insect pathogenic fungi. *Annual Review of Entomology, 59*. 467–485.

Boucias, D. G., & Pendland, J. C. (1998). *Principles of Insect Pathology.* Kluwer Academic Publishers, Boston/Dordrecht/London.

Britannica (2019) The Editors of Encyclopaedia. "entomology". *Encyclopedia Britannica*. Retrieved March 1, 2022 from https://www.britannica.com/science/entomology.

Butt, T.M., Coates, C.J., Dubovskiy, I.M., & Ratcliffe, N.A. (2016). Entomopathogenic Fungi: New Insights into Host-Pathogen Interactions. *Advances in Genetics, 94*. 307–64.

Committee on the Environment, Public Health and Food Safety of European Parliament (2019). Report on the implementation of Directive 2009/128/EC on the sustainable use of pesticides. Retrieved March 1st, 2022 from https://www.europarl.europa.eu/doceo/document/A-8-2019-0045_EN.html .

DeBach, P. (1964). *Biological Control of Insect Pests and Weeds.* London: Chapman & Hall.

Dinu, M. M., Bloemhard, C. M. J., van Holstein-Saj, R. & Messelink, G. J. (2017). Exploring opportunities to induce epizootics in greenhouse aphid populations. *Acta Horticulturae, 1164.* 371–376.

Eilenberg, J., Hajek, A., & Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl, 46.* 387–400.

Eilenberg, J., Thomsen, L., & Jensen A. (2013). A third way for entomophthoralean fungi to survive the winter: slow disease transmission between individuals of the hibernating host. *Insects,* 4. 392–403.

European Commission (2022). Questions and Answers: Farm to Fork: new rules for micro-organisms used in plant protection products. *Press material from the Commission Spokesperson's Service*. Retrieved March 1st, 2022 from https://ec.europa.eu/ commission/presscorner/detail/en/qanda_22_852 .

European Parliament and of the Council (2019). EU Directive 2009/128/EC establishing a framework for Community action to achieve the sustainable use of pesticides.

Evans, H.C. (1989). Mycopathogens of insects of epigeal and aerial habitats. In Wilding, N., Collins, N.M., Hammond, P.M. and Webber, J.F. (Ed.), *Insect-Fungus Interactions* (pp. 205–238). Academic Press, London.

Evans, H.F. (1997). Microbial insecticides: novelty or necessity? *BCPC Symposium Proceedings No. 68*. Coventry, BCPC, Farnham.

Ferron, P., Fargues, J., & Riba, G. (1991). Fungi as microbial insecticides. In Arora, D.K., Ajello, L. and Mukerji, K.G. (Ed.), *Handbook of Applied Mycology* (pp. 665–706). Marcel Dekker, New York.

Gonzalez, F., Tkaczuk, C., Dinu, M. M., Fiedler, Ż., Vidal, S., Zchori-Fein, E., & Messelink, G. J. (2016). New opportunities for the integration of microorganisms into biological pest control systems in greenhouse crops. *Journal of Pest Science*, 89. 295–311

Görg, L.M., Eilenberg, J., Jensen, A.B, Jensen, A. H., & Gross, J. (2021). Pathogenicity against hemipteran vector insects of a novel insect pathogenic fungus from Entomophthorales (*Pandora* sp. nov. inedit.) with potential for biological control. *Journal of Invertebrate Pathology*, *183:*107621.

Gryganskyi, A.P., Humber, R.A., Smith, M.E., Hodge, K., & Huang, B. (2013). Phylogenetic lineages in

Entomophthoromycota. *Molecular Phylogenetics and Evolution, 65*. 682–694.
Gryganskyi, A. P., Mullens, B. A., Gajdeczka, M. T., Rehner, S. A., Vilgalys, R., & Hajek, A. E. (2017). Hijacked: Co-option of host behavior by entomophthoralean fungi. *PLoS pathogens*, 13(5), e1006274.
Hajek, A. E., Humber, R. A., Elkinton, J. S., May, B., Walsh, S. R., & Silver, J. C. (1990). Allozyme and restriction fragment length polymorphism analyses confirm *Entomophaga maimaiga* responsible for 1989 epizootics in North American gypsy moth populations. *Proceedings of the National Academy of Sciences, 87*(18). 6979–6982.
Hajek, A. E., & St. Leger, R. J. (1994). Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology, 39*. 293–322.
Hajek, A. (2004). Classical biological control. In *Natural Enemies: An Introduction to Biological Control* (pp. 39–61). Cambridge: Cambridge University Press.
Hajek, A.E., & Goettel, M.S. (2008). Guidelines for evaluating effects of entomopathogens on non-target organisms. In L.A. Lacey and H.K. Kaya (Ed.), *Field manual of techniques in invertebrate pathology, 2nd edition* (pp. 815–833). Springer, Dordrecht.
Hajek, A.E., Steinkraus, D.C.,& Castrillo, L.A. (2018). Sleeping Beauties: Horizontal Transmission via Resting Spores of Species in the Entomophthoromycotina. *Insects*, 9(3):102.
Hatano, E., Baverstock, J., Kunert, G., Pell, J.K., & Weisser, W.W. (2012). Entomopathogenic fungi stimulate transgenerational wing induction in pea aphids, *Acyrthosiphon pisum* (Hemiptera: Aphididae). *Ecological Entomology, 37*. 75–82.
Hikal, W. M, Baeshen, R. S., & Said-Al Ahl, H.A.H. (2017). Botanical insecticide as simple extractives for pest control. *Cogent Biology, 3:1.*
Hu, Y., Chen, C.,Ye, S., & Hu, H. (2018). Development of a novel isolation unit for entomophthoralean fungi. *Journal of Invertebrate Pathology*, 155. 1–4.
Hua, L., & Feng, M. G. (2003). New use of broomcorn millets for production of granular cultures of aphid-pathogenic fungus *Pandora neoaphidis* for high sporulation potential and infectivity to *Myzus persicae*. *FEMS Microbiology Letters, 227*. 311−317.
Humber, R. A. (2012a). Identification of entomopathogenic fungi. In Lacey L.A. (Ed), *Manual of Techniques in Invertebrate Pathology*, *2nd ed.* (151–187). Academic Press; San Diego, CA, USA.
Humber, R. A. (2012b). Entomophthoromycota: a new phylum and reclassification for entomophthoroid fungi. *Mycotaxon, 120*(1). 477–492.
Inglis, G. D., Goettel, M.S., Butt, T. M., & Strasser, H. (2001). Use of hyphomycetous fungi for managing insect pests. In T.M. Butt, C. Jackson and N. Magan (Ed.), *Fungi as biocontrol agents: progress, problems and potential* (pp. 23–69). CAB International, Wallingford.
Islam, W., Adnan, M., Shabbir, A., Naveed, H., Abubakar, Y. S., Qasim, M., Tayyab, M., Noman, A., Nisar, M. S., Khan, K.A., & Ali, H. (2021). Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests. *Microbial Pathogenesis, 159*. 105–122.
Jaronski, S.T. (2014). Chapter 11 - Mass Production of Entomopathogenic Fungi: State of the Art. In Morales-Ramos, J.A., Rojas, G. M., and Shapiro-Ilan, D. I (Ed.), *Mass Production of Beneficial Organisms* (pp. 357-413). Academic Press.
Keller, S. (1987). Observations on the overwintering of *Entomophthora planchoniana*. *Journal of Invertebrate Pathology*, 50. 333–335
Keller, S., & Zimmerman, G. (1989). Mycopathogens of soil insects. In Wilding, N., Collins, N.M., Hammond, P.M. and Webber, J.F. (Ed.), *Insect–Fungus Interactions* (pp. 240–247). Academic Press, London.
Keller, S. (1997). The genus Neozygites (Zygomycetes, Entomophthorales) with special refere-nce to species found in tropical regions. *Sydowia, 49*. 118-146.
Keller, S. (1998). Use of fungi for pest control in sustainable agriculture. *Phytoprotection, 79*(4). 56–60.
Khachatourians, G. G., & Qazi, S. S. (2008). Entomopathogenic fungi: biochemistry and molecular biology. In Brakhage, A. A. and Zipfel, P. F. (Ed.), *Human and Animal Relationships* (33–61). Berlin, Heidelberg: Springer.
Latgé, J. P. (1981). Comparaison des exigences nutritionelles des Entomophthorales. *Annals of Microbiology (Inst Pasteur) 132B*. 299–306.
Li, Y., Steenwyk, J. L., Chang, Y., Wang, Y., James, T. Y., Stajich, J.E., Spatafora, J. W., Groenewald, M., Dunn, C. W., Hittinger, C. T., Shen,X. X., & Rokas, A. (2021). A genome-scale phylogeny of the kingdom Fungi. *Current Biology*, 31-*8*. 1653–1665.
Licht, H. D. F., Hajek, A. E., Eilenberg, J., & Jensen, A. B. (2016). Utilizing genomics to study entomopathogenicity in the fungal phylum Entomophthoromycota: a review of current genetic resources. *Advances in genetics*, 94. 41–65.
Litwin, A., Nowak, M. & Różalska, S. (2020). Entomopathogenic fungi: unconventional applications. *Reviews in Environmental Science and Bio/Technology* 19, 23–42
McCoy, C. W., Samsom, R. A., &. Boucias, D. G. (1988). Entomogenous fungi. In Ignoffo, C.M. (Ed.), *Handbook of natural pesticides, Volume 5: Microbial insecticides, Part A, Entomogenous protozoa and fungi* (pp. 151–236). CRC Press, Boca Raton.
Möckel, L., Meusemann, K., Misof, B., Schwartze, V. U., De Fine Licht, H. H., Voigt, K., Stielow, B., de Hoog, S., Beutel, R. G., & Buellesbach, J. (2022). Phylogenetic Revision and Patterns of Host Specificity in the Fungal Subphylum Entomophthoromycotina. *Microorganisms 10*, *256.*
Muskat, L.C., Görg, L.M., Humbert, P., Eilenberg, J., & Patel, A.V. (2022, a). Encapsulation of the psyllid-pathogenic fungus *Pandora* sp. nov. inedit. and experimental infection of target insects. *Pest Management Science. 78(3)*. 991–999.
Muskat, L.C., Przyklenk, M., Humbert, P., Eilenberg, J., & Patel, A.V. (2022, b). Fermentation of the psyllid-pathogenic fungus *Pandora* sp. nov. inedit. (Entomophthorales: Entomophthoraceae), *Biocontrol Science and Technology*, 0. 1–22.

Ødegaard, F. (2000) How many species of arthropods? Erwin's estimate revised. *Biological Journal of the Linnean Society 71.* 583–597.

Pell, J. K., Eilenberg, J., Hajek, A. E., & Steinkraus, D. C. (2001). Biology, ecology and pest management potential of entomophthorales. In Magan, N., Butt, T. M. and Jackson, C. (Ed.), *Fungi as biocontrol agents* (pp. 71-153). CABI International, Wallingford, Oxon.

Rajula, J., Karthi, S., Mumba, S., Pittarate, S., Thungrabeab, M., & Krutmuang, P. (2021). Chapter 4 - Current status and future prospects of entomopathogenic fungi: A potential source of biopesticides, Editor(s): Surajit De Mandal, Ajit Kumar Passari, *Recent Advancement in Microbial Biotechnology,* Academic Press. 71–98.

Ravensberg, W.J. (2010). The development of microbial pest control products for control of arthropods: a critical evaluation and a roadmap to success. *Ph.D. Thesis* Wageningen University, Wageningen, NL.

Reinbacher, L., Bacher, S., Praprotnik, E., & Grabenweger, G. (2021). Standard non-target tests for risk assessment of plant protection products are unsuitable for entomopathogenic fungi - a proposal for a new protocol. *Journal of Soils and Sediments,* 21, 2357–2368.

Roberts, D. W., & Hajek, A. E. (1992). Entomopathogenic Fungi as Bioinsecticides. In Leatham, G.F. (Ed.), *Frontiers in Industrial Mycology*. Springer, Boston, MA.

Roberts, D.W., Fuxa, J.R., Gaugler, R., Goettel, M., Jaques, R., & J. Maddox (1990). Use of insect pathogens. In Pimentel, D. and Hanson, A.A. (Ed.), *Handbook of pest management in agriculture, Vol.II.* (pp. 243–278). CRC Press, Boca Raton.

Roy, H. E., Steinkraus, D. C., Eilenberg, J., Hajek, A. E., & Pell, J. K. (2006). Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. Annual Review of Entomology, 51.331–357.

Shah, P. A., Clark, S. J., & Pell, J. (2004). Assessment of aphid host range and isolate variability in *Pandora neoaphidis* (Zygomycetes: Entomophthorales). *Biological Control,* 29. 90–99.

Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., Bonito, G., Corradi, N., Grigoriev, I., Gryganskyi, A., James, T. Y., O'Donnell, K., Roberson, R. W., Taylor, T. N., Uehling, J., Vilgalys, R., White, M. M., & Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, *108(5).* 1028–1046.

Steinhaus E. A. (1949). *Principles of Insect Pathology*. New York, McGraw-Hill Book Co., Inc.

Steinhaus E. (1956). Microbial control - the emergence of an idea. A brief history of insect pathology through the nineteenth century. *Hilgardia 26(2).*107–160.

Steinhaus, E. A. (1975). *Disease in a Minor Chord.* Ohio State University Press, Columbus.

Stern, V. M, Smith, R. F., van den Bosch, R., & Hagen, K. S. (1959). The integrated control concept. *Hilgardia*, *29.* 81–101.

Sundh I., & Eilenberg, J. (2021). Why has the authorization of microbial biological control agents been slower in the EU than in comparable jurisdictions? *Pest Management Science.77(5).* 2170–2178.

Tanada, Y., & Kaya, H. K. (1993). *Insect Pathology.* San Diego, Academic Press.

Tembo, Y., Mkindi, A. G., Mkenda, P. A., Mpumi, N., Mwanauta, R., Stevenson, P. C., Ndakidemi, P. A., & Belmain, S. R. (2018). Pesticidal Plant Extracts Improve Yield and Reduce Insect Pests on Legume Crops Without Harming Beneficial Arthropods. *Frontiers in Plant Science, 9*. 1425.

Van den Bosch, R. (1971). Biological control of insects. *Annual Review of Ecology and Systematics,* 2:1. 45–66.

Wells, P.M., Baverstock, J., Majerus, M.E.N., Jiggins, F.M., Roy, H.E., & Pell, J.K. (2011). The effect of the coccinellid *Harmonia axyridis* (Coleoptera: Coccinellidae) on transmission of the fungal pathogen *Pandora neoaphidis* (Entomophthorales: Entomophthoraceae). *European Journal of Entomology, 108*. 87–90.

Wilding, N., Mardell, S.K., & Brobyn, P.J. (1986). Introducing *Erynia neoaphidis* into a field population of *Aphis fabae*: form of the inoculum and effect of irrigation. *Annals of Applied Biology, 108 (2)*. 373–385.

Wraight, S.P., & Carruthers, R.I. (1999). Production, delivery, and use of mycoinsecticides for control of insects' pests on field crops. In: Hall, F.R. and Menn, J.J. (Ed), *Biopesticides: use and delivery* (pp. 233–269). Humana Press, Totowa.

Zhou, X., & Feng, M. G. (2009). Sporulation, storage and infectivity of obligate aphid pathogen *Pandora nouryi* grown on novel granules of broomcorn millet and polymer gel. *Journal of Applied Microbiology, 107*: 1847−1856.

Zhou, X., & Feng, M. G. (2010). Improved sporulation of alginate pellets entrapping *Pandora nouryi* and millet powder and their potential to induce an aphid epizootic in field cages after release. *Biological Control 54.* 153−158.

Zhou, X., Su, X., & Liu, H. (2015). A floatable formulation and laboratory bioassay of *Pandora delphacis* (Entomophthoromycota: Entomophthorales) for the control of rice pest *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). *Pest Management Science, 72(1).* 150–154.

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and Technology,17:9*. 879-920.