

Entomopathogenic Fungi and their Potential Role in the Sustainable Biological Control of Storage Pests

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Abstract: Chemical control methods are mostly preferred in the control of pests that cause qualitative and quantitative losses in stored products instead of physical or biological control applications. The increasing consumer response to pesticide use and the insect resistance to many pesticides have reversed this situation and interest in biological control has increased. Entomopathogenic fungi (EPF) are biological control agents that are safer than synthetic pesticides. EPF play major roles in the natural regulation of many insect and mite species. Sustainable Biological Control with EPF could make a substantial contribution to the control of storage pests. When storage pests' interactions are complex with EPF, we can notice both positive and negative impacts. EPF disrupts the host cuticle and proliferates as hyphae in the hemolymph, secreting toxins responsible for the death of host insects. Subsequent saprophytic growth leads to the production of fungal spores that can reinfect other hosts. For a successful infection, the fungus must be effective on the host's defense system. In order to determine the optimum conditions of myco-insecticides in biological control programs, specific research is required to understand the interaction between EPF, host insects, crops, and their environment. This review includes an overview of EPF, its host defense mechanism, pathogenicity, infection occurrence, the potential for use, and prospects. Furthermore, this review extensively investigates the contribution of EPF to biological control in sustainable agricultural practices.

Keywords: Storage pests, Fungal pathogenicity, host defense mechanism, sustainable agriculture, myco-insecticide.

Entomopatojenik Mantarlar ve Depo Zararlılarının Sürdürülebilir Biyolojik Kontrolündeki Potansiyel Rollerini

Öz: Depolanan ürünlerde kalitatif ve kantitatif kayıplara neden olan zararlıların kontrolünde fiziksel veya biyolojik mücadele uygulamaları yerine çoğunlukla kimyasal mücadele yöntemleri tercih edilmektedir. Pestisit kullanımına karşı artan tüketici tepkisi ve birçok pestisiteye karşı böceklerin direnci bu durumu tersine çevirmiş ve biyolojik mücadeleye olan ilgi artmıştır. Entomopatojenik mantarlar (EPF), sentetik pestisitlerden daha güvenli biyolojik kontrol ajanlarıdır. EPF, birçok böcek ve akar türünün doğal regülasyonunda önemli roller oynar. Entomopatojenik mantarlarla Sürdürülebilir Biyolojik Kontrol, depolama zararlılarının kontrolüne önemli bir katkı sağlayabilir. Depolama zararlıları etkileşimleri entomopatojenik mantarlarla karmaşık olduğunda, hem olumlu hem de olumsuz etkiler görülebilir. EPF konak kütikülünü bozar ve hemolenfte hif olarak çoğalarak konak böceklerin ölümünden sorumlu toksinleri salgılar. Saprotik büyüme ile, diğer konakları yeniden enfekte edebilen mantar sporlarının üretimine yol açar. Başarılı bir enfeksiyon için mantarın konağın savunma sistemi üzerinde etkili olması gerekir. Biyolojik kontrol programlarında miko-böcek öldürücülerin optimum koşullarını belirlemek için EPF, konak böcekler, ekinler ve bunların çevreleri arasındaki etkileşimi anlamak için özel araştırmalar gereklidir. Bu inceleme, EPF'ye, konakçı savunma mekanizmasına, patojenitesine, enfeksiyon oluşumuna, kullanım potansiyeline ve beklentilerine genel bir bakış içerir. Bu derlemede ayrıca EPF kullanımının sürdürülebilir tarım uygulamalarında biyolojik mücadeleye katkısı ayrıntılı olarak incelenmiştir.

Anahtar kelimeler: Depo zararlıları, Fungal patojenite, konak savunma mekanizması, sürdürülebilir tarım, miko-insektisit.

1. Introduction

1.1 Biological Control with Storage Pests

Grains are indispensable in terms of their high nutritional value and easy cultivation and they are the most important food source for all people. Production of high protein and granular plants (such as barley, wheat, and corn) used to make flour, known as cereals, is increasing rapidly around the world (Güneş & Turmuş, 2020). The losses that occur at every stage of the production, storage, and use of cereals are serious. Among these, most losses occur during storage (Kumar & Kalita, 2017). The damage to stored food products caused by insects accounts for about 5–10 % in the temperate regions of the world and 20–30 % in the tropical countries (Rajashekar et al., 2010; Manivannan, 2015). Insects cause qualitative and quantitative losses in stored products and this is an

important worldwide problem (Mason & McDonough, 2012). While chemical methods are mostly preferred for pest control before harvest, less physical or biological control practices are also used (Zettler & Arthur, 2000). The food industry's enthusiasm for chemical control has decreased due to the growing consumer concern about pesticide use and the development of insect resistance to commonly used pesticides (Altıkat et al., 2009). The utilization of physical storage methods such as heat treatment and freezing is restricted due to their high energy requirements (Adler, 2010; Fields et al., 2012). In biological control applications, natural enemies such as predators, parasitoids, and pathogens are involved in order to keep the number of pests below the economic threshold. *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae), *Lariophagus distinguendus* Forster (Hymenoptera: Pteromalidae) and *Habrobracon hebetor*

Say (Hymenoptera: Braconidae) are known as natural enemies of storage crop pests. These control agents are used effectively in the protection of post-harvest storage crops (Whiting, 2005). Historically, the use of insect pathogens against stored product pests coincides with the isolation and identification of the bacterial pathogen *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) from the Mediterranean flour moth *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) (Rumbos & Athanassiou, 2017). Following these studies, applications have accelerated with the use of biological control agents against storage pests (Moore et al., 2000). Research on the use of macrobiological control agents (such as predators and parasitoids) in biological pest control in storage facilities is limited due to the extended time required for pest suppression. Moreover, these studies necessitate precision in timing and release of significant number of beneficial insects (Flinn & Scholler 2012; Rumbos & Athanassiou, 2017). In general, microbiological control agents are considered to be more compatible with insect species in a few stored products and with grain storage systems. Insect pathogens, unlike beneficial insects, have a wide host range even though most of these are host-specific (Kavallieratos et al., 2006, 2014).

1.2. Entomopathogenic Fungi

The entomogenous word has been derived from two Greek words, "entomon" meaning insects and "genes" meaning arising in. Therefore, the etymological meaning of entomogenous microorganisms is "microorganisms which arise in insects" (Sandhu, 2012). Entomopathogenic fungi (EPF) consist of the genus of fungi that establish various relationships with insects and other arthropods (parasitic, communalistic, pathogenic and saprophytic) (Demirbağ, 2008). There are more than 1000 species of EPF belonging to at least 100 genera in the fungal kingdom (Chen et al., 2021). Many of these are within the Ascomycota and Zygomycota divisions. In Ascomycota, many species are included in the orders Hypocreales, Zygomycota, and Entomophthoralean (Roy et al., 2006). In the grouping revealed by looking at the systematic status of EPF, the entomopathogenic or entomoparasitic fungi Blastocladiomycota (*Coelomomyces* spp., *Coelomycidium simuli*), Entomophthoromycotina, Kickxellomycotina (Harpellales and Asellariales), Eurotiomycetes (*Ascospaera* and other genera), Laboulbeniomycetes (ectoparasitic Ascomycetes), Dothideomycetes (Myriangium), Sordariomycetes (mostly in Hypocreales), and Pucciniomycetes (Humber, 2008). EPF can grow on standard media such as potato dextrose agar (PDA) or malt extract agar (MEA) and do not require additional nutrients. While the best growth temperature is 20-35° C, *Beauveria bassiana* grows at a wide temperature range (from 8 to 35 °C) with a maximum thermal threshold for growth at 37°C (Fernandes et al., 2008). Fungi easily produce asexual spores on the host or in culture in humid conditions, which are the parts that provide infection in nature (Demirbağ, 2008).

Soil is an important source of EPF and EPF in the soil are of great importance in terms of biological control (Jackson et al., 2000). In soil, the excessive proliferation and spread of EPF are limited. Soils with elevated levels of organic matter and more clay than sand have a more

significant distribution of EPF whereas alkaline and sandy soils contain less of it (Elkhateeb, 2021). The population forms as a result of converting the materials within the deceased organism into spores capable of causing infection when released. The dispersal and spread of infective structures are very important in the development of a disease caused by a pathogen. The infective structures of EPF belonging to the Hypocreales order are passively dispersed to the environment via dead insects. This distribution occurs with factors such as wind and rain (Meyling & Eilenberg, 2007). The spores of fungi of the Entomophthoralean order are actively released under hydrostatic pressure and spread with the wind. In some cases, EPF can spread by passing from living infected insect to another insect. For example, Flies infected with *Entomophthora thripidum* and *Strongwellsea catran* and some aphid species can migrate long distances by carrying fungi. In addition, spores allow fungi to expand their distribution and colonize new environments: spores are an asexual form of reproduction of fungi, not only resting structures (resting spore). When the number of hosts decreases and adverse environmental conditions begin, 16 fungal species belonging to many Entomophthoralean orders were observed to produce resting structures (resting spore) composed of mitosis (conidia) or meiosis (zygospore) that can remain in the soil for a long time. This is one of the factors contributing to the spread of the fungus (Shah & Pell, 2003).

1.2.1. Pathogenicity and infection mechanism of entomopathogenic fungi

For virulence in fungal pathogens of humans, plants, and insects, their dimorphic form must be converted to the yeast form (Gauthier, 2015). In human pathogenic fungi, temperature (from 22–25 °C in the soil to 37 °C in the host) is the most important factor for dimorphic transition while other factors such as CO₂, cysteine, and estradiol are also effective for both transformation and growth (Klein & Tebbets, 2007). In plant pathogens, nitrogen sources, some branched-chain amino acids, and enzyme activity of lipoxygenases and cyclooxygenases constitute important structures that contribute to yeast-mycelial dimorphism (Berrocal et al., 2012; Naruzawa & Bernier, 2014). In insect pathogenic fungi, the mechanisms of the structures that form the transition from germ tubes to hyphae at the beginning of infection and from hyphae to mycelium in the later stages of infection have not been adequately clarified (Boucias et al., 2016). However, there is evidence that the high osmotic pressure found in the hemocoel may trigger the first switch (Butt et al., 2016). It is known that there are various biological and non-biological factors that affect the growth and development of insects. Among them, one of the most important biological factors is EPF. Pathogens need a high metabolic rate in order to ensure their development and reproduction in the host organism. They cause oxidative stress in the host and the emergence of large amounts of toxic substances and by-products produced by the parasites. The life cycles of EPF usually occur simultaneously with the development time of their hosts (Shah & Pell, 2003). EPF can infect their hosts not only from the gut but also from the respiratory holes of insects and the surface of the integument. This feature leads to the fact that EPF can directly infect insects

regardless of their feeding activities; thus, it does not need to be eaten by the host and the host range is not limited to chewing insects (Castrillo et al., 2005; Shah & Pell, 2003). The following flow chart (Fig. 1) shows briefly the steps of infection by EPF.

Entomopathogenic fungal infection begins with the attachment of the fungal conidia to the insect cuticle. This occurs through hydrophobic and electrostatic interactions between the conidia and the insect cuticle. The fungal spore can attach to any part of the host cuticle. The components of the cuticle differ from insect to insect and are depending on the developmental stage of the insect (Ye et al., 2021). In the outermost of the *B. bassiana* conidia, there are hydrophobic rodlets covered with protein hydrophobins. Two hydrophobins, Hyd1 and Hyd2, in *B. bassiana* are responsible for hydrophobic rodlets and conidia hydrophobicity (Cho et al., 2007). In EPF living in aquatic environments, the attachment process is followed by the formation of pouches by zoospores (Castrillo et al., 2005). The second stage of infection is the germination of the spore of the fungus attached to the cuticle. Spore germination is affected by temperature, pH, humidity, oxygen, and nutrient availability. Although it varies according to the fungal species, the optimum temperature required for germination is between 20-30°C (Skinner et al., 2014). It has been observed that fungi with a wide host distribution do not need specific carbon and nitrogen sources in the host cuticle for germination while fungi with a narrow host range need specific compounds in the insect cuticle to germinate (Ortiz-Urquiza et al., 2013). The third stage of infection is penetration. It puts mechanical pressure on the cuticle by forming a structure called the fungus appressorium. This structure and the cuticle-degrading enzymes synthesized by the fungus

enable the fungus to penetrate the cuticle of the host more easily (Ortiz-Urquiza & Keyhani, 2013; Soliman, 2020). The epicuticle of the host insect consists of proteins, lipids, sterols, and fatty acids. Cuticle-degrading enzymes - lipase, protease, and chitinase- play an important role in the entry of the fungus into the host. The lipase enzyme synthesized by the fungus breaks down lipids and lipoproteins in the epicuticle (Pedrini et al., 2007). Lipases also increase the hydrophobic interaction between the fungus and the host cuticle surface (Santi et al., 2010). The proteolytic enzymes synthesized by the fungus break down the proteins in the insect cuticle, resulting in the emergence of chitin fibrils. The chitinase enzyme breaks down the chitin in the insect cuticle, allowing the fungus to progress in the insect cuticle. Fungus passing through the hemolymph proliferates in this same structure and forms yeast-like cells (blastospores). The proliferation of the fungus leads to disruption of the tissue integrity of the host insect. Meanwhile, the fungus synthesizes secondary metabolites that weaken the insect's immune system. In addition, the fungus synthesizes acid trehalase enzyme and decomposes it to use trehalose, an important disaccharide found in the insect's hemolymph, as an energy source. Thus, the feeding of the insect is interrupted (Litwin et al., 2020). In a recent study, it was demonstrated that inactivation of the *ATM1* gene that encodes an acid trehalase enzyme, which is responsible for breaking down the trehalose in the fungus *Metarhizium acridum*, resulted in a significant decrease in the fungus's ability to infect and kill insects (Jin et al., 2015). Finally, the fungus sporulates on the dead insect and newly formed spores can infect another host. Under favorable conditions, this situation continues. Figure 2 depicts the invasion of the host insect (*Galleria mellonella*) by *B. bassiana*.

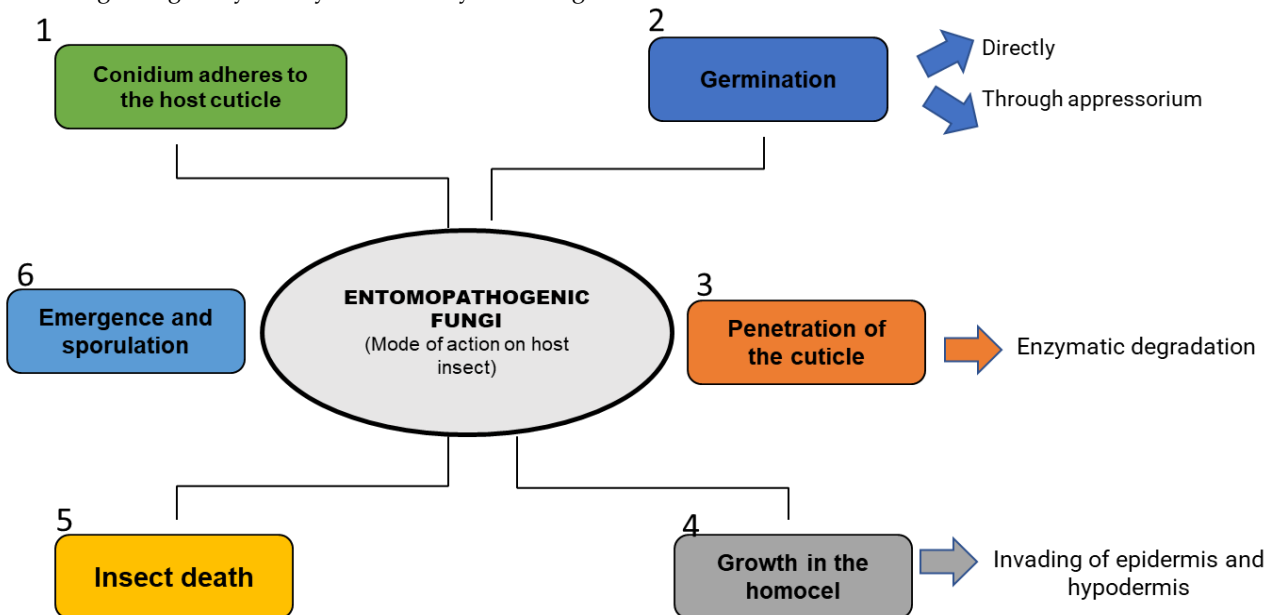


Figure 1. Diagrammatic representation of infection steps by EPF. (Modified from Sinha et al., 2016; Sharma et al., 2023).

1.2.2. Production of Toxins

The production of multiple toxic secondary metabolites, known as immunosuppressive compounds (Altimira et al., 2022) or which can facilitate fungal invasion (Zhang et al., 2020), is also seen as an important step in the infection

process. Secondary metabolites include non-ribosomal peptides and polyketides with many different chemical structures. The precise role of secondary metabolites is unknown but these structures are thought to be related to the virulence of fungal strains (Zhang et al., 2020). EPF kill their hosts in different ways by producing different

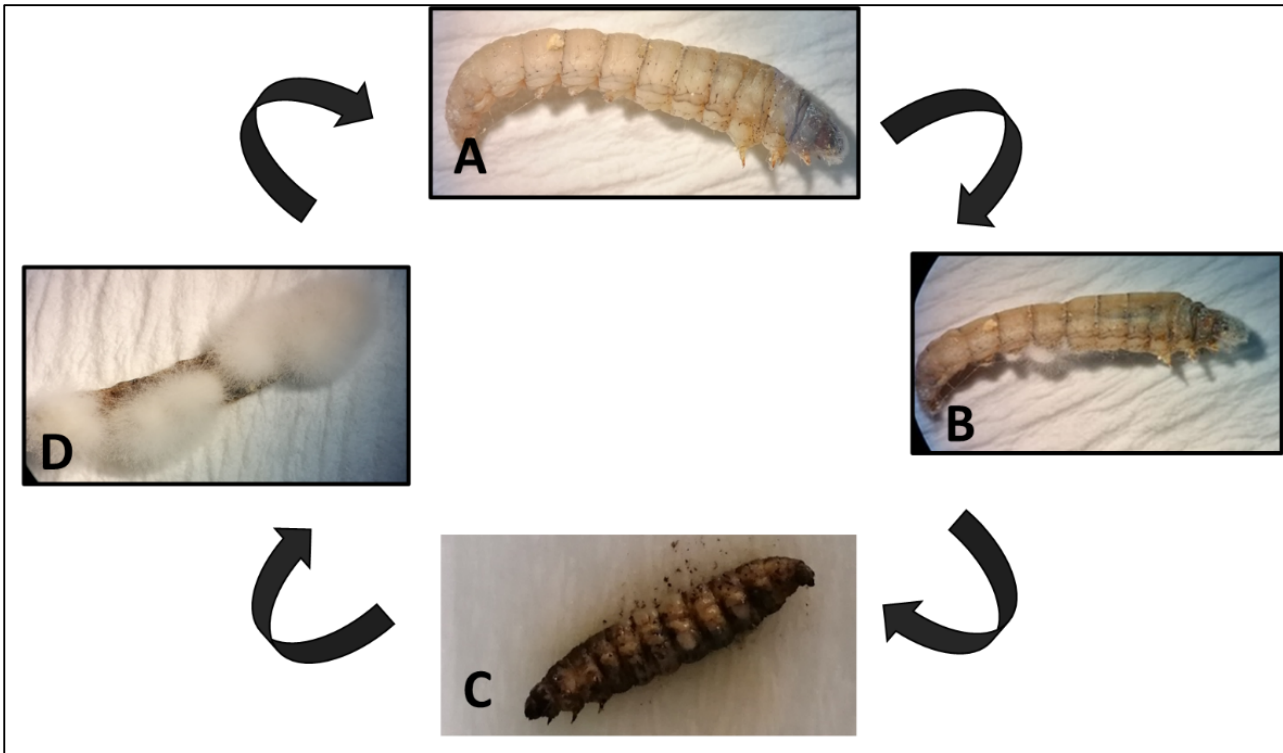


Figure 2. Life Cycle of Entomopathogenic Fungus in the Presence of a Suitable Substrate and Environment. A: Adhesion of spores on the insect, B: Formation of Germination, Hyphae, Conidia and Appressorium, C: After the fungus enters the body cavity (hemocoel), depletion of nutrients and destruction of tissues until the death of the host, D: Proliferation of Spores to the Body Surface for Continuity of Infection and Spread in the External Environment.

metabolites (Zimmermann, 2007b). A plethora of work with circumstantial evidence is available from Deuteromycete pathogens for the involvement of fungal toxins in host death. The action of cytotoxins is suggested by cellular disruption prior to hyphae penetration. Behavioral symptoms such as partial or general paralysis, sluggishness, and decreased irritability in mycosed insects are consistent with the action of neuromuscular toxins (Sandhu et al., 2012). Chemically different toxic metabolites have been identified in biological control agents such as *Beauveria*, *Fusarium*, *Gliocladium*, *Metarhizium*, *Paecilomyces*, and *Verticillium*. Some of these metabolites are known to be important pathogenicity factors (Strasser et al., 2000). Many researchers have focused on the metabolites produced by *Beauveria* species and *M. anisopliae*, the two most known important microbial control agents. Table 1 lists some of the metabolites produced by EPF. (Zimmermann, 2007a; Zimmermann, 2007b; Strasser et al., 2000). *Beauveria* spp. produces many toxic compounds such as beauvericin, bassianin, bassianolide, oosporin, and destruxin B in vivo and in vitro (Zimmermann 2007a). In addition, *Metarhizium* spp. produces a variety of metabolites, including destruxins (28 types), cytochalasin C, and hydroxyfungerin A and B. So far, it has not been determined whether EPF produce any toxin during disease or whether the toxin is required for virulence. Quesada-Moraga and Vey (2003) stated that *B. bassiana* does not need toxin production to be pathogenic against locusts. In some cases, although toxin production is suspected, it is not definitively indicated. Some fungi belonging to *Coelomycidium*, *Coelomyces* genus and Entomophthoralean order may have some very weak toxins. However, most likely, these fungi kill their hosts

by invading vital tissues (Goettel et al., 2005). In addition, fungal infection may cause changes in host movements such as fever, elevation, increase or decrease in activity, decreased response to semi-chemicals, and changes in reproductive behavior (Roy et al., 2006). *B. bassiana* and *Paecilomyces fumosoroseus* cause death by defeating the immune system of the insect with their synthesized secondary metabolites such as beauvericin, bassianolides, ennalin, and oosperin during infection (Kidanu, 2020). Beauvericin has anti-tumor, anti-fungal, anti-bacterial, and insecticidal effects. The insecticidal property of beauvericin was first tested on *Artemia salina* (brine shrimp) and it was found to be effective.

1.2.3. Host Defense Mechanism against Entomopathogenic Fungus

Insects exhibit a complex immune response consisting of both cellular and humoral responses to defend themselves against pathogens (Cooper & Eleftherianos, 2017; de Oliveira Barbosa Bitencourt et al., 2020). Hemocytes, which are involved in the phagocytosis, encapsulation, and nodulation of pathogens, play a role in the cellular response (Strand, 2008).

The humoral response includes the recognition of pathogen-associated molecular patterns (PAMPs) on the surfaces of pathogenic microorganisms, resulting in the induction of lectins, the prophenoloxidase cascade, and the biosynthesis of antimicrobial peptides (AMPs), which are various molecular groups (Hultmark, 2003; Pal & Wu, 2019).

For successful infection, the fungus must overcome adverse physical and chemical factors and the insect's immune system. The insect's humoral immune system

Table 1. Metabolites of Entomopathogenic Fungi

FUNGUS	METABOLITE	EFFECTS	REFERENCES
<i>Beauveria bassiana</i>	Beauvericin, Bassianin, Bassianolide, Beauverolides, Tenellin, Oosporein, Oxalic Acid, Bassiacridin, Oxalic Acid, Bassianolides, Pyridovericin	Anti-Bacterial, Anti-Fungal, Anti-Viral, Insecticidal, Anti-Tumor, Ionophoric, Cytotoxic	Zimmermann 2007a; Roy et al., 2006; Kidanu, 2020
<i>B. brongniartii</i>	Oosporein, Bassianolides	Anti-Bacterial, Anti-Fungal, Anti-Oomycotic, Anti-Tumor, Insecticidal Activity	Zimmermann 2007a; Goettel et al., 2005; Butt et al., 2001
<i>B. caledonica</i>	Oosporein	Anti-Bacterial, Anti-Fungal, Anti-Oomycotic, Anti-Tumor, Insecticidal Activity	Zimmermann 2007a; Roy et al., 2006; Kidanu, 2020
<i>B. felina</i>	Cyclodepsipeptides (iso-isariin B, and isaridin E)	Insecticidal Activity	Langenfeld et al., 2011
<i>Metarhizium anisopliae</i>	Destruxins (28 types), swainsonie, cytochalasin C	Insecticidal Activity	Zimmermann 2007a; Goettel et al., 2005
<i>Metarhizium sp.</i>	Hydroxyfungerin A and B	Insecticidal Activity	Zimmermann 2007a
<i>Paecilomyces fumosoroseus</i>	Beauvericin, beauverolides, Oxalic acid	Anti-Bacterial, Anti-Fungal, Anti-Viral, Insecticidal, Anti-Tumor, Ionophoric	Zimmermann 2007a
<i>P. tenuipes</i>	Beauvericin	Anti-Bacterial, Anti-Fungal, Anti-Viral, Insecticidal, Anti-Tumor, Ionophoric	Zimmermann 2007a; Kidanu, 2020
<i>Verticillium lecanii</i>	Dipicolonic acid, hydroxycarboxylic acid, cyclosporine	Insecticidal Activity	Zimmermann 2007a
<i>Hirsutella thompsonii</i>	Hirsutellin A, hirsutellin B, fomalactone	Insecticidal Activity	Zimmermann 2007a; Maimala et al., 2002
<i>Fusarium spp</i>	Beauvericin, Bassiatin, Cyclosporine A, Fusaric acid	Anti-Bacterial, Anti-Fungal, Anti-tumor, Insecticidal	Zimmermann 2007a; An, 2004

responds to the infection by increasing the synthesis of antifungal compounds and reactive oxygen species (ROS) and by activating innate immune factors such as melanization and phagocytosis (Zibae & Malagoli, 2014). Melanin is involved in the insect's cellular defense system (pathogen encapsulation) and in the synthesis of antimicrobial peptides (Langfelder et al., 2003). The fungus can protect itself by increasing the synthesis of enzymes such as superoxide dismutase by activating genes that reduce the effects of ROS in response to the insect's immune system (Xie et al., 2010). Oosporein, a metabolite synthesized by *B. bassiana*, suppresses the immune system of the host insect by inhibiting the cleavage of prophenoloxidase (PO) into polyphenol oxidase (PPO) and the expression of the antifungal peptide Gallerimycin (from the *Gal* gene) (Feng et al., 2015). The MCL1 protein synthesized by *B. bassiana* allows it to escape from the hemocyte cells located in the hemolymph of the insect (Wang et al., 2021). Like vertebrates, insects also have enzymatic and non-enzymatic defense systems. The main elements of the enzymatic system are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) enzymes (Krishnan et al., 2006). Therefore, the successful adaptation of insects to environmental conditions is achieved by effective detoxification mechanisms and the removal of these substances from their bodies (Wu et al., 2004). The role of these detoxification enzymes is not limited to protecting insects from the negative effects of insecticides, various plant metabolites or entomopathogenic microorganisms. Infections with EPF, which are considered xenobiotics today, can also cause inhibition of detoxification enzymes or changes in their activity in insects (Serebrov et al., 2006).

1.3. The Potential of Entomopathogenic Fungus for Biological Control

The EPF have some advantages and disadvantages in

microbial biological control. The advantages of using fungi as insecticides are that they have high host selectivity in some cases in terms of pest control: EPF can be used in the control of harmful insects without affecting non-harmful parasites and beneficial insect populations. They do not have any negative effects on mammals and; thus, the damage encountered as a result of insecticide applications such as environmental pollution. EPF can be used to reduce problems such as insecticide resistance, provide a long-term control, be developed with biotechnological research, and stay in the environment for a long time after application (Lengai et al., 2020; Fenibo et al., 2021). However, there are some drawbacks of using fungi as insecticides. They require a longer time to kill insects than chemical insecticides (sometimes 10-15 days). EPFs are more selective than chemical insecticides which means that they may not be effective against all pests. They can be more expensive to produce and require cold storage conditions compared to chemical insecticides. Their efficacy and persistence on pest populations can vary depending on the host insect which requires long-term studies and research to optimize insect-specific application techniques. Furthermore, EPFs may pose potential risks to immunocompromised humans as some fungi secrete various toxins to kill the target insect and the effects of these toxins on other organisms are not fully known (Lengai et al., 2020; Fenibo et al., 2021).

The first step in the development of commercial microbial control agents is the isolation, characterization, and determination of their pathogenicity against the target insect. There are many studies conducted in our country for this purpose. Old Chinese texts on medicinal herbs such as "*Bencao gangmu*" (from the Middle Ages) have records of *Cordyceps sinensis*-infected insects (Hepialidae family of Lepidoptera order), *Cordyceps sobolifera* infected cicadas, and *B. bassiana* infected silkworm (Shin et al., 2020). Sevim et al. (2010b)

demonstrated the activity of various EPF against *M. melolontha* L. (Coleoptera: Scarabaeidae) and *Evlachovaea* sp. They determined that KTU-36 isolate caused 86.6% mortality under laboratory conditions. Gökçe & Er (2005) obtained *Paecilomyces* sp. isolates from different sources and found out that most of the isolates cause mortality (>70%) to the greenhouse whitefly (*Trialeurodes vaporariorum* (Westwood) (Aleyrodidae: Homoptera)). İnanlı et al. (2012) tested commercial preparations of *B. bassiana* and *M. anisopliae* on tomato moth (*Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and found 66% of mortality rate from *B. bassiana* and 100% from *M. anisopliae*. Çam et al. (2002) tested the fungus *B. bassiana* against the potato insect (*Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae) and achieved a mortality rate of over 80% on the larvae at the end of the 6th day. Apart from these studies, various isolates of EPF were tried against various harmful insects that spread in the forests of our country and promising results were obtained. Many species of EPF were tested against the pine processionary beetle (*Thaumetopoea pityocampa* (Schiff.) (Lepidoptera: Thaumetopoeidae) which is harmful in pine forests (Sevim et al., 2010b; Er et al., 2007), giant bark beetle (*Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae) which is harmful in spruce forests (Sevim et al., 2010a; Tanyeli et al., 2010), sycamore lace beetle (*Corythucha ciliata* (Say) (Hemiptera: Tingidae) which is harmful to plane trees (Sevim et al., 2013) and poplar small buckthorn (*Saperda populnea* (L.) (Coleoptera: Cerambycidae) which damages poplar trees (Eken et al., 2006) and promising results were obtained for further studies. Screening tests are typically the first step in the development of commercial EPF. These tests are conducted in the laboratory to select the EPF with the highest virulence against the target insect pest. However, further studies are needed to develop a commercial product, including field trials to assess the efficacy of the EPF under realistic conditions.

2. Conclusion

Pesticides used in agricultural production can contaminate soil, surface water, and groundwater, and can have a toxic effect on organisms living in these ecosystems. The degradation products of some pesticides may be more toxic and their toxic effects may increase due to their increasing concentrations through the food chain (Hassaan & El Nemr, 2020). Biological control, in comparison with other control methods, has several advantages such as targeting only the pest species, not causing resistance problems, protecting the natural balance, and having no negative effects on humans or the environment. Other advances of biological control include sustainable agricultural production, obtaining high-quality products without pesticide residues, and protecting biological diversity. In pest control with chemical pesticides, many problems such as resistance development of insects, the emergence of secondary pests, negative effects on beneficial non-target species, negative effects on human and animal health, pollution of groundwater and reduction of biodiversity have emerged. The continuity of sustainable agricultural practices in the 21st century will largely depend on the development of environment-friendly alternative control techniques in which the use of chemical pesticides in pest control is reduced (Kesin et al., 2019). Organic agriculture

and biological control have gained great importance in these practices aimed at the effective use of sustainable agriculture, protection of the environment and biological diversity, reduction of chemical residues and deterioration of the ecological balance. To ensure sustainability in agriculture, agriculture based on the use of entomopathogenic bioinsecticides, which is an alternative to agriculture based on the use of chemical pesticides, should be supported and given more importance. In this context, the discovery of new microbial control agents in the fight against harmful insects in organic farming practices and the dissemination of the use of existing entomopathogenic bioinsecticides are of great importance. Like other beneficial microorganisms, EPF also have positive effects on the soil structure and plant growth. Until recently, the relationship between EPF and plants was poorly understood. However, the discovery that these fungi can also promote plant growth, health, and yield in addition to their entomopathogenic role is an important development that could lead to their widespread use in agriculture. They can be an excellent pest management tool, especially in agricultural production systems where the use of pesticides is undesirable. They can also be used in plant management since they support plant growth with other positive effects (Dara, 2019).

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