

## **Entomopathogenic Fungi as Effective Insect Pest Management Tactic: A Review**

**Hafiza Tahira Gul<sup>1</sup>, Shafqat Saeed<sup>1, 2</sup> & Fawad Zafar Ahmad Khan<sup>2\*</sup>**

<sup>1</sup>Faculty of Agricultural Science and Technology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan

<sup>2</sup>College of Agriculture, Bahauddin Zakariya University Multan, Bahadur Campus, Layyah, Pakistan

\*Email: [fawaduca@gmail.com](mailto:fawaduca@gmail.com)

### **Abstract**

Entomopathogenic fungi, with its cosmopolitan existence and rich diversity, present a sustainable solution towards integrated pest management programs. These entomopathogens, due to their eco-friendliness and bio-persistence, are preferred to kill insects at various stages of its life cycle. The most important entomopathogens that have been commercially produced are *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea*. These are classified into different phylums including Oomycota, Ascomycota, Chytridiomycota and Zygomycota. These entomopathogens have contact mode of action in most cases. It contacts the cuticle, forms appresoria, penetrates into the insect, proliferates, produces toxins and ultimately kills the insect. The insect host shows reaction of reduced or increased feeding, behavioral fever, aberrant mating, and changed ovipositional preferences. The spores are disseminated by the insect cadaver, air, soil and water. The factors affecting entomopathogens are high relative humidity, temperature and sunlight. These are used for all bio-control methods including inundative and classical biological control. For future prospects, improvements are needed in the research methods, mass production, formulation and the application techniques. The proper selection of strains to kill specific host range without disturbing non-target insects is another point of study. It should be possible that the best use of microbial insecticides at wider range eradicate the chemicals use to a large level.

**Keywords:** Entomopathogenic Fungi, Biological Control, Insect Pest Management

## 1. Introduction of Entomopathogenic Fungi

Entomopathogenic fungi are considered to play vital role as biological control agent of insect populations. A very diverse array of fungal species is found from different classes that infect insects. These insect pathogenic species are found in a wide range of adaptations and infecting capacities including obligate and facultative pathogens. Spreading of fungal diseases is common in many insect species while some species may not be affected. In 1980s, the first insect pathogenic studies were carried out and their focus was to find the methods of disease management of the silkworm (Steinhaus, 1975). Bassi in 1835, first time formulated the germ theory by the use of white muscardine fungus on the silkworm that was then named in his honor as *Beauveria bassiana*. Gilbert and Gill (2010) described that this silkworm disease gave the idea of using insect infecting fungi for the control of insect pest management. Many commercial products are available globally (Shah and Goettel, 1999) that are formulated by utilization of less than ten species of fungi (Copping and Menn, 2000).

A group of fungi that kill an insect by attacking and infecting its insect host is called entomopathogenic fungi (Singkaravanit *et al.*, 2010). The main route of entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trachea (Holder *et al.*, 2005). The divisions of fungi are Ascomycota, Zygomycota and Deuteromycota (Samson *et al.*, 1988), and the divisions Oomycota and Chytridiomycota were also included in the previous classification of fungi. At the recent times, about 90 genera and almost above 700 species are considered as insect infecting fungi that represent about all the major classes of fungi (Moorhouse *et al.*, 1992; Hajek and St. Leger, 1994).

The differences between fungi and protozoa have been revised with the advancements in molecular biology that is being used as a tool of classification in modern sciences. But even now, the fungal taxonomy is still unstable. Fungi may consist of a monophyletic assembly that has some characters of animals such as structure of chitin and storage of glycogen. Scientists describe that the primitive property of smooth, single, posterior flagellum is possessed by the Chytridiomycetes (Cavalier-Smith, 1987). Some phylogenists classified it in four major classes the Ascomycetes, Oomycetes, Chytridiomycetes and Zygomycetes. Most of the insect disease causing fungus species is found in Ascomycetes and Zygomycetes while some other uncommon species are found in other classes. A clear distinction should be found between the two groups i.e., one group of disease causing resulting in insect deaths and the other group that attack on insects but do not cause mortality in the end (Gilbert and Gill, 2010). The other group includes Laboulbeniomyces species that may be obligate insect parasites but their lethal effect on insect host is very little, and Basidiomycota such as Uredinella and Septobasidium (Tanada and Kaya, 1993).

Entomopathogenic fungi are a major component of integrated pest management techniques as biological control agents against insect pests and other arthropods and are an integral part of mycoinsecticides in horticulture, forestry and agriculture (Inglis *et al.*, 2000; Carruthers *et al.*, 1997). The mass production of Hyphomycete fungi is not very expensive. Its storage is very easy and it is efficient on a wide range of humidity and temperature. Insect pathogenic fungi are being developed and produced in mass production globally to control a wide range of harmful insects of crops (Wraight and Carruthers, 1999). In integrated pest management programs of field and stored product pests as well as for the production of organic agricultural products, bio-friendly approaches are more safe (Roy *et al.*, 2006; Khan *et al.*, 2013a,b).

In this short review article, a brief detail of the entomopathogenic fungi is given according to its classification and its existing possible participation in the control of insect pests populations in the integrated pest management systems.

## 2. Classification of Entomopathogenic Fungi

### 2.1. Phylum Oomycota

The fungi that have cellulose in their coenocytic hyphae, without chitin and biflagellate zoospores are considered as oomycetes. Sexual reproduction can occur either on the same hyphae or on different hyphae between archegonia and antheridia (collectively called as gametangia). Numerous species are parasites and saprophytes of plants and animals while species of two genera are infecting to mosquito larvae. A mosquito larvae pathogen, *Lagenidium giganteum* is studied best (Glare, 2010; Scholte *et al.*, 2004). Some other *Lagenidium* species are pathogenic to crabs and other aquatic crustaceans (Hatai *et al.*, 2000).

The other species pathogenic to mosquito larvae, other Diptera and Chironomids are *Leptolegnia* spp. (Saprolegniales). Zattau and McInnis (1987) thought that these species infect the insect through secondary zoospore.

## 2.2. Phylum Chytridiomycota

The fungal groups that are without cellulose and contain chitin walls are included in this phylum. Single flagellum zoospores and gametes are settled and a thallus is grown which then converts into a coenocytic hyphae or a resting spore. When the comparisons are based on SSU rRNA phylogenetic, then this fungal group is considered as basal. Most of the usual insect infecting Chytridiomycetes is contained in Blastocladales genus Coelomomyces. In Coelomomyces, above 70 insect pathogenic species are described (Barr, 2001). Mostly the Hemipterans and Dipterans were recognized as the source of these fungi. Sporangia of these fungi like copepods, is thick walled and resistant and the zoospores are flagellated. Insect pathogenic *Myriophagus* (Chytridiales) are found on pupae of Diptera and Coelomycidium (Blastocladales) found on mosquitos and blackflies contain some other species (Samson *et al.*, 1988).

## 2.3. Phylum Zygomycota

Hyphae that are multicellular, non-septate, and zygospores by the joining of gametangia, are considered as of phylum Zygomycota conventionally. But, Zygomycota has not been found as monophyletic on the basis of molecular analyses (Tanabe *et al.*, 2000). The class Trichomycetes, within the phylum Zygomycota, consists of species that are mostly related with insects. The species *Smittium morbosum* (Trichomycetes) was reported by Cooper and Sweeney (1986), as mosquito pathogen. Trichomycetes has mostly weak or symbiotic associations unlike true infectious agents (Beard and Adler, 2002). Insect death is mostly related to some species of *Mucor* (Mucorales). The order Entomophthorales, contain above 200 insect infecting species within phylum Zygomycota. Some species are capable of producing secondary spores from primary spores and some produce long lasting resting spores.

## 2.4. Ascomycota and Deuteromycota

Ascomycota includes the fungi with haploid and septate mycelia and the ascospores (sexual spores), are produced in a sac called ascus on the ascomata, the fruiting body. Normally, in every ascus, the number of ascospores produced is eight. Zoospores are not produced in ascomycetes (Shah and Pell, 2003). The taxonomy of sexual (teleomorphic) and asexual (anamorphic) conidial stages always remained in confused classification when their production is not on single occurrence.

More than 300 insect infecting species are present in Cordyceps that is best known ascomycete. Chalkbrood disease in bees occurs by the genus *Ascosphaera*, which has dimorphism in sexuality. Klich, (2007) described that the spores of *Ascosphaera*, in an unusual manner of insect infecting species are ingested by the bee larvae that germinate in gut causing infection. The most important insect infecting species occur in *Aspergillus*, *Metarhizium*, *Hirsutiella*, *Beauveria*, *Aschersonia*, *Culicinomyces*, *Lecanicillium*, *Paecilomyces*, *Tolypocladium* and *Sorospora*. Mostly, these genera have a linkage with one or many genera that can be verified with biological studies or by the molecular studies presenting the genetic relationship between teleomorphs and anamorphs (Huang *et al.*, 2010; Bischoff *et al.*, 2009).

## 2.5. Basidiomycota

A very few Basidiomycetes have been noticed that act as insect pathogenic. It has been described by some researchers that *Uredinella* and *Septobasidiales* genera *Septobasidium* are considered infection causing agents in insects but most of them have a symbiotic relationship with insects like scales (Samson *et al.*, 1988).

## 3. Biology and Pathogenesis

In a general view, the life cycle of insect pathogenic fungi, an infective spore stage are required generally that germinates on the host cuticle. It forms a germ tube penetrating the host cuticle and occupies the (Hajek and St. Leger, 1994, Akbar *et al.*, 2012). The infecting fungal spores then increase in number, causing toxin production that ultimately kills the insect. The fungus comes out of the insect cadaver in suitable temperature and humidity conditions and disseminates in the environment. Several species form the resting spores that become capable of infecting at the time of favorable environmental conditions. The fungus needs a strategy for the dissemination to infect new hosts (Shahid *et al.*, 2012). In general, the vital factors for the survival and reproduction in fungus infection are host and suitable environmental conditions.

### 3.1. Encounter with Host

Host plays two types of roles for fungus that is either active (under favorable situations, fungi need to contact with host) or passive (as it is the main source of nutrition for fungus). It is difficult to describe the role of environmental

factors for land-dwelling fungi. In most cases, for all developmental stages of fungi, high humidity is required while for infection, temperature is limiting within certain ranges (Meyling and Pell, 2006). The optimum temperature for Hyphomycetes is generally between 20-30°C while for Entomophthorales, it is considered as 15-25°C. The optimum temperature for all stages of fungus development can vary among the individuals of same species. Some species require high humidity than others such as *Lecanicillium lecanii* (hyphomycete) requires about 16 h of 100% relative humidity to kill whiteflies at leaf level. While some species require low relative humidity, such as *Entomophthora muscae*, that causes infection in Diptera (Mullens and Rodriguez, 1985; Milner and Lutton, 1986). It is considered difficult to have an estimate of the actual humidity due to variability in microclimate (Fargues *et al.*, 2003; Glare, 2010). Soil moisture, organic matter in soil and pH are important in infection levels. According to Inglis *et al.*, (1998), a fungus is considered very effective in sterilized soils than in the unsterilized soils.

Wind and sunlight are the other abiotic conditions influencing pathogen and host encounter. Spores dispersal can be assisted with wind but in some cases, it decreases the humidity by removing free water. In most fungi, sunlight and ultraviolet (UV) rays can detriment to the infection persistence. But, a substantial variation in vulnerability to sunlight and UV is found among different species and strains (Fargues *et al.*, 1996). For aquatic species of entomopathogens, humidity is not a limiting factor but the temperature, chemical factors like organic pollutants and salinity are important factors (Gilbert and Gill, 2010).

### 3.2. Specificity and Host Range

Entomopathogenic fungus varies in specificity widely within genera, between genera and among species. *B. bassiana* (muscardine fungus) and *M. anisopliae* are best studied and have a wide host range including hundreds of insect species. Many other hosts have been reported in the recent years including species of Coleoptera, Lepidoptera, Diptera, Homoptera and Hymenoptera. The prevalent hosts of *M. anisopliae* are mostly coleopteran species that include more than 70 scarab species. The isolate of any fungus is more important than species for bio insecticides (Vestergaard *et al.*, 2003). The strains of insect infecting fungus are specific to hosts and very rarely infect both pest and beneficial species. The insect pathogenic fungus, *Zoophthora radicans* has been reported from over 80 species of insects of Coleoptera, Diptera, Homoptera and Lepidoptera.

With many exceptions, the specific strains are usually more infection causing to the insects mostly to the original host that is closely related. While other groups of insect infecting fungi are limited to host range. In rare cases, insect infecting fungus can kill some species in specific situations such as *M. anisopliae* is hardly reported as mosquito pathogen, but in controlled laboratory conditions, many isolates are pathogenic to the larvae of mosquito (Daoust and Roberts, 1982). In certain cases, the pathogenic fungus can kill only the vulnerable hosts. The laboratory and field vulnerability of different insects to different entomopathogenic fungi and even to different isolates is different. Similarly, the virulence of different life stages of insect is different towards the entomopathogenic fungi. Mostly, the fungi opposite to viral, bacterial and protozoan pathogens, penetrates the insect directly without digestion and due to this reason non-feeding stages of insect such as pupae are also susceptible to pathogenic fungi. *B. bassiana* and *M. anisopliae* both can infect all the life stages of insects.

Usually, fungi are called as allergens as they produce toxin and have the capacity to infect vertebrates. Therefore, fungi are an important source of allergic reactions in humans. But, the fungi that cause infections in insects are not allergic to human beings. Above all, vertebrates are not susceptible to the insect infecting fungi (Lacey *et al.*, 2001). The exception comes in certain cases such as *Aspergillus flavus* is famous pathogen of vertebrates. This is why; they are not used for commercial production.

### 3.3. Mode of Action and Host Reactions

The mode of action of insect pathogenic fungi varies and kills the insect by different ways such as causing starvation to toxin production. These insect pathogenic fungi produce many toxins and extracellular enzymes such as proteases and chitinases. Cuticle is the main hurdle to infection in insects as it is the main path of fungus penetration. Hence, it needs some physical or enzymatic means to pierce the hard cuticle. The infection process starts by contact of the spores to the host cuticle. Sometimes, conidium attaches to the cuticle or secrete mucus for adhesion during its germination and swelling (Hajek and St. Leger, 1994). Some infective sessile spores (Capilliconidia), have an adhesion drop at spore end to aid attachment for insect penetration (Glare *et al.*, 2010).

Some structures and general processes are involved in the penetration of host cuticle and the mechanisms of each fungus may also differ. As described by Hajek (1997) that *M. anisopliae* develop certain structures from conidia to aid attachment and germ tube penetration. The infection pegs (Appressoria), aid the germ tube penetration. After the penetration of germ tube through the cuticle and insect epidermis, the fungus multiplies into the body cavity of

insect. In some Entomophthorales, this multiplication may be by the protoplasts while in some hyphomycete (e.g., *M. anisopliae*), blastospores are involved in the initial proliferation (Bidochka and Hajek, 1998). With the presence of cuticle, some other humoral and cellular defense methods are used by the insect against invasion of fungus. Toxins are produced by some insect pathogenic fungi and more of them aid to increase pathogenesis and play an insecticidal role. While some other such fungi produce antimicrobial metabolites. Destruxins of *Metarhizium* species are among such fungal metabolites that increase the pathogenicity of fungus. Beauvericins are produced by *Beauveria* species. These metabolites also show invertebrate toxicity (Roberts, 1992). Some beauvericins are also isolated from *Isaria fumosoroseus* and some plant pathogenic *Fusarium* species (Vestergaard *et al.*, 2003). Other products produced by *B. bassiana* are isarolides, bassianolides and beauverolides (toxic to Blattaria).

In the initial infection stages, no considerable behavioral symptoms are observed. But, some days before death, symptoms start to appear such as reduced co-ordination, feeding (e.g., grasshopper and locust infected with *M. anisopliae*) and activity. Some other behavioral responses include behavioral fever, increased feeding (e.g., Colorado potato beetles infected with *B. bassiana*), positive or negative photo- or geotropism and altered mating (Noma and Strickler, 2000). “Behavioral fever”, is another response of fungal infection in which the insect changes its body temperature by basking in the sun or using warm surfaces for their positioning e.g., response of locusts and grasshoppers in the infection of *M. anisopliae* and *B. bassiana* (Blanford and Thomas, 2001).

### 3.4. Spore Production on Host

Main source of reproduction in insect pathogenic fungi are spores. For example, the fungus *Strongwellsea castrans* sporulates in living flies from one or two abdominal holes. But, in many cases, spores production occurs after the host death and making colonies in cadaver of insect. Major Entomophthorales species can discharge primary spores by using force. These spores then come out of the cadaver and spreads to seek out new hosts for pathogenesis (Luz and Fargues, 1998; Eilenberg and Meadow, 2003).

### 3.5. Transmission and Dispersal

Different strategies are used by the fungi to enhance the chances of encountering new host. For the groups producing abundant spores such as Hyphomycetes, rain, wind and invertebrates help in transmission of spores. Wind is a major source of spore distribution. Growing hyphae out of insect cadaver is also a major source of conidia dispersion. High humidity and moisture necessary for germination and sporulation are the depending sources for the transmission of terrestrial fungi. Fungus ability to infect multiple stages of life of an insect is helpful in disease spreading. For example, winged insects can spread spores and ultimately the disease in insect populations (Steinkraus *et al.*, 1999; Dromph, 2001).

### 3.6. Interactions between Pathogens and Other Natural Enemies

Many insects have several species of natural enemies such as parasitoids, predators and other pathogens. They have a competition among them for same food resource. There may be chances that a single insect may be infected by one or more natural enemies or pathogens. An interaction is found among them such as natural enemies can feed upon host that is already infected with pathogen. While on the other hand, Entomopathogenic fungus may infect natural enemies or other non-target insects. Another aspect is that these natural enemies and non-target insects become a source of fungal spore's dispersion (Feng *et al.*, 1990; Pell *et al.*, 2010).

## 4. Epizootiology and Its Role in Suppressing Pest Populations

Insect outbreaks are quickly reduced by the use of entomopathogenic fungi through epizootics. A vital factor in controlling insect pest population is natural epizootics. Epizootiology occurs in different environments.

### 4.1. Epigeal Environment

Epigeal environment is suitable for epizootics. Many sources of infection are in this environment such as from infected insects to non-infected insects, from the conidia on leaves to those insects feeding on leaves and through dispersion of spores in the air surrounding. Major occurrence is mostly found in aphid species (Hatting *et al.*, 2000). This occurrence could reach to 90% according to Steinkraus (1999).

### 4.2. Soil Environment

Natural epizootics mostly prevail due to the cryptic nature of soil living insects. It is seen that soil is major inoculum source of many Hyphomycetes (Steenberg *et al.*, 1995) and most of the epizootics have been noted in the stages of insect that live in soil. Many researches have reported the spores present in the soils are of the genera *Metarhizium*, *Beauveria*, *Tolypocladium* and *Isaria* (Keller *et al.*, 2003). The soil may act as a storage place for spores.



*Cordyceps* spp. (Ascomycetes) has been found on soil surface from the insect cadavers. During the spring, for epizootics initiation, presence of spores in the upper layers of soil surface is important in the epigeal environment.

#### 4.3. Aquatic Environment

This environment is quite different from soil environment. The Entomopathogenic species that infect aquatic insects have motile spores, zoospores. Disease can be affected by a wide range of biotic and abiotic factors such as pH, salinity, organic and inorganic pollutants and water movement. Another major influencing source is temperature affecting disease and epizootiology (Apperson *et al.*, 1992).

### 5. Development as Microbial Control Agents

When the living organisms are used by releasing to control pests, this is called as Inundative biological control (Eilenberg *et al.*, 2001). Some characteristics of Entomophthoralean fungi suggest that these can be used for Inundative biological control (Pell *et al.*, 2010). For this purpose these are produced in masses and commercial production is also done. Well studied Hyphomycetes have many commercial products globally that are based on six to seven species (Shah and Goettel, 1999; Bateman and Chapple, 2001). These are mostly produced due to wide host range, shelf life, ease of production, application and persistence (Wraight *et al.*, 2001; Alves *et al.*, 2003). *B. bassiana* is broadly used commercially to use against a broad range of insect pests such as banana weevils, pine caterpillars, European corn borer and greenhouse aphids etc, (Alves *et al.*, 2003). These formulations are in the form of emulsifiable oil or wettable powders. Commercial products of *M. anisopliae* are also available against a wide range of pests such as locusts and grasshopper (Lomer *et al.*, 2001). Formulations of *I. fumosoroseus* are also available against thrips, whiteflies, aphids and spider mites. Several other products are also available in many nations.

### 6. Conclusion and Future Prospects

Many insect pathogenic fungus based bio-insecticides have been produced and commercially manufactured so far. Though, a number of studies have been done for the improvements in production, pesticide formulation and practical application, even then many improvements are required to search and study, and implementation. Improvement in strains by the use of guides and selections will be a best strategy in the future. The use of microbial insecticides should be a contribution towards all fields of agriculture, sustainable agriculture, forestry and horticulture. It should be cared that the Entomopathogenic fungus should not destroy beneficial natural fauna in the environment. Strategies should be made at small and large levels for the mass production of conidia. Use of insect pathogenic fungus is unavoidable as it is an integral part of integrated pest management programs in many ecological zones.

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