Chapter 18
Panorama of *Metarhizium*: Host Interaction and Its Uses in Biocontrol and Plant Growth Promotion

Srinivas Patil, Gargi Sarraf, and Amit C. Kharkwal

Abstract Vectors have been wreaking a fatal havoc on mankind by causing diseases in agriculturally important plants and humans. Not only diseases caused by them are a hefty task to deal with, but their increasingly successful survival in human settlements is also a rising concern. The entomopathogenic fungi are considered amongst the first organisms for bio management of agriculturally important pests as they are eco-friendly, economically sustainable, and effective. With this, the collateral need for biocontrol in human disease vectors is also being felt. The first observation of fungi infecting insects was in as early as 900 AD, to the first data published in 1726 about entomopathogenic fungi. *Metarhizium* is a widespread fungus found all over the globe. More than 200 species of insects are infected by the fungus thereby making it one of the most sought biocontrol agents. This chapter gives an understanding of interaction between an arthropod host and entomopathogenic fungi genera *Metarhizium*, description of the host and fungal structure, what are some of the conventional and recent efforts done in order to improve the application strategies and what could be some of the possible uses of *Metarhizium* in enhancing plant health. Some of the plant pests and animal vectors which have been explored as host for *Metarhizium* are also mentioned.

Keywords Entomopathogenic fungi · *Metarhizium* · Insect–fungus interaction · PGP · Arthropod

18.1 Introduction

Metarhizium is distributed very much uniformly across the globe, from arctic to tropics and this feature gives it an edge over the other biocontrol agents. Being a fungus it does not need to be necessarily ingested, mostly all it requires is contact

S. Patil · G. Sarraf · A. C. Kharkwal (✉)
Amity Institute of Microbial Technology, Amity University Noida, Noida, Uttar Pradesh, India
e-mail: ackharkwal@amity.edu

© The Editor(s) (if applicable) and The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

N. Shrivastava et al. (eds.), *Symbiotic Soil Microorganisms*, Soil Biology 60,
https://doi.org/10.1007/978-3-030-51916-2_18

with the host cuticle (Kamareddine 2012) also it is very much prolific in terms of mass production (Rasgon 2011). *Metarhizium* genus comprises of species that have both narrow-spectrum entomopathogenecity and broad-spectrum entomopathogenecity, however, one of the most sought after species is *Metarhizhium anisopliae* (Aw and Hue 2017). *M. anisopliae* is a generalist that has been known to infect more than seven orders (Aw and Hue 2017). Majorly the earlier discovered species are *M. anisopliae, M. truncatum, M. cicadinum, M. brunneum, M. flavoviride, M. taii, M. cylindrosporum,* and *M. viridicolumnare* (Bischoff et al. 2009). However with advent of time several species and improved strains were also added to the list. The fungus is target specific, their generation time is short and it can survive in the environment for long when no host is available due to its ability of resting stage (Sandhu et al. 2012). Entomopathogenic fungi can be instilled in IPM (integrated pest management) as they show synergistic activity to control pestiferous insects in addition to the use of natural predators and other biocontrol agents like parasitoids and innovative approaches can be met in IPM using genomic techniques (Chandler et al. 2011; Erler and Ates 2015).

This fungus has many reasons to be used some of which are: firstly, effective broad-spectrum mortality, Secondly, fast and inexpensive mass production (Scholte et al. 2004). The complexity of using fungal spores as biocontrol agent is that the spores need optimal level of abiotic factors namely temperature, relative humidity, salinity, sunlight, and UV light exposure to break dormancy. Also, it needs contact with the host at all times to germinate which means it requires repeated applications if there are no hosts present for prolonged time and when we use it as a vector control agent it might be harmful to many nontarget insects (Scholte et al. 2004). The toxin destruxin affects structural integrity of cell membrane of host thereby damaging host tissue and it also causes fluid loss (Scholte et al. 2004)

18.2 Hosts

Certain strains of *Metarhizium* have successfully shown significant pathogenicity toward certain human disease vectors and plant pests. However, there have been some studies pertaining to some other strains which do not have a firm conclusion. So all in all there are several hosts for different *Metarhizium* strains. But in this chapter only selectively significant *Metarhizium*—host interactions have been mentioned (Table 18.1). The studies mentioning these interactions were aimed at the possible use of *Metarhizium* as a biocontrol agent (Table 18.2).

18.3 Structure and Mechanism

Although the insect anatomical structure is highly detailed, in this chapter we have elaborated certain portions of the arthropod anatomy, which is relevant in understanding the mode of infection. *Metarhizium* spore (conidia) generally germinates

Table 18.1 *Metarhizium* interaction with human and animal disease vectors

Species/strain	Parasitic on	Disease	References
Metarhizium pingshaense (Met_S26 and Met_S10)	*Anopheles coluzzii*	Malaria	Liao et al. (2017), Bilgo et al. (2018)
Metarhizium anisopliae ICIPE-30	*Anopheles gambiae*	Malaria	Mnyone et al. (2011)
M. anisopliae	*Aedes aegypti*	Yellow fever mosquito, chikungunya, dengue, etc.	Carolino et al. (2014)
M. anisopliae	*Culex quinquefasciatus*	Wuchereria, West Nile Virus, avian pox	Lacey et al. (1988)
M. anisopliae	*Aedes albopictus*	Dengue fever, Chikungunya, also capable of hosting Zika virus and certain nematodes	Scholte et al. (2007)
M. anisopliae	*Rhipicephalus sanguineus*	Canine ehrlichiosis	Kirkland et al. (2004)
	Ixodes scapularis	Lyme disease	
M. anisopliae (Ma959, MaE9 and Ma319)	*Anocentor nitens*	Tropical horse tick	Bittencourt et al. (2000)
M. anisopliae	*Ixodes scapularis*		Kurtti and Keyhani (2008)
	Amblyomma americanum	Rocky Mountain spotted fever (*Rickettsia rickettsiae*)	
M. anisopliae ICIPE 30	*Glossina fuscipes fuscipes* (controls parasite *Trypanosoma congolense*)	Trypanosomiasis	Wamiti et al. (2018)
Metarhizium brunneum ARSEF 4556 (with *Toxorhynchites brevipalpis*)	*Aedes aegypti*	Yellow fever mosquito, chikungunya, dengue, etc.	Alkhaibari et al. (2018)
M. anisopliae	*Rhodnius prolixus*	Chagas disease	Garcia et al. (2016)

when it comes into contact with the host's cuticle and then it outgrows the arthropod's body by draining its nutrition, resulting in the death of the mosquito (Fig. 18.1). The journey of the fungal spore from epicuticle (outermost interaction site) to hemocoel (terminal) witnesses the upregulation and production of many genes and proteins in both host and fungus. Prominent ones have been described in pathogenesis section.

18.3.1 Host Structure

A well-known fact about the insects is that they are devoid of an endoskeleton. It has only an exoskeleton. The integument is the outermost layer divided into epidermis

Table 18.2 *Metarhizium* interaction with various plant pests

Species/strain	Parasitic on (common name)	References
Metarhizium anisopliae	*Polyphylla fullo* (june beetle)	Erler and Ates (2015)
M. anisopliae (*ESALQ1604*)	*Mahanarva* (spittlebugs)	Iwanicki et al. (2019)
M. anisopliae (Metschnikoff) Sorokin variety anisopliae	*Tuta absoluta* (tomato borer); *Aethina tumida* (small hive beetle)	Muerrle et al. (2006), Contreras et al. (2014)
M. *brachyspermum* sp. nov. (Clavicipitaceae)	Elateridae (click beetles)	Yamamoto et al. (2019)
M. anisopliae	*Oryctes rhinoceros* (coconut rhinoceros beetle)	Indriyanti et al. (2017)
M. anisopliae	*Culicoides* spp.	Narladkar et al. (2015)
M. brunneum	*Bactrocera oleae* (olive fly)	Yousef et al. (2018)
M. anisopliae	*Bactrocera cucurbitae* (melon fly)	Sookar et al. (2014)
M. anisopliae (ICIPE 69 and ICIPE 18)	*Zeugodacus cucurbitae* (melon fly)	Onsongo et al. (2019)
M. acridum	*Locusta migratoria manilensis* (oriental migratory locust)	Zhang et al. (2015)
M. anisopliae	*Thaumatotibia leucotreta* (false codling moth)	Mkiga et al. (2020)

and cuticle. The cuticle is a chitinous structure in which the host arthropod body is enclosed. Protein makes 70% of the host cuticle (Charnley 2003). It can be interpreted that the cuticle is segregated into the epicuticle and procuticle. The procuticle is coated with a thin waxy and slimy layer known as the epicuticle. The procuticle is comprised of exocuticle and endocuticle. Mesocuticle is sclerotized and hardened region, which sometimes might be present in between them (Chapman 2012). It is mainly the pro cuticle, which is composed mainly of chitin and several other proteins. As discussed above the pro cuticle has endo- and exocuticle. So the endocuticle is a matrix of chitin intermixed with protein, providing a different property to the pure chitin. The modification of chitin with the protein confers its additional properties. While the exocuticle is mainly composed of sclerotin which essentially is a cross-linked form of certain proteins (Pryor 1940; Li and Ortiz 2014). Not only this sclerotin is present in exoskeleton, but it is distributed among the mouthparts used for biting and the dorsal and ventral sides. The composition of sclerotin varies among the insect hosts and also within the host sclerotin composition varies among different body parts, also certain regions are less sclerotinised certain regions are more sclerotinised (Russell et al. 2016). Beneath the exoskeleton lies the body which is divided into head, thorax, and cuticle (Chapman 2012).

18.3.1.1 Hemolymph

Hemolymph, which is considered as blood of arthropods, flows through sinuses referred to as a hemocoel. Hemocytes are the cells, which get circulated through hemolymph. Hemolymph mainly comprises of water, chlorine, sodium, potassium,

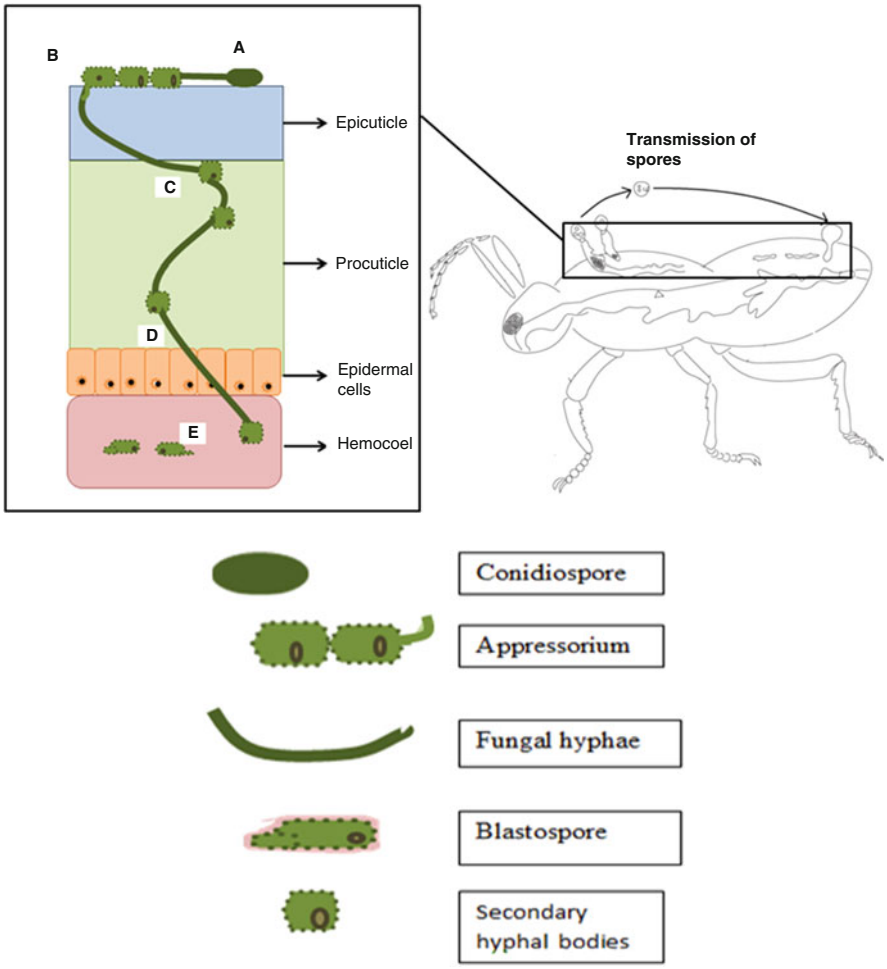

Fig. 18.1 The figure shows the path of fungal spore via the lateral cross-section of anatomical layers of the host and its life cycle. (**A**) conidiospore adhesion, (**B**) appressorium formation and peg penetration, (**C**) hyphal infection progressing in pro cuticle, (**D**) hyphae invading epidermal cells and (**E**) hemocoel colonization blastospores formation. Reference: based on Constanza Mannino et al. (2019)

calcium, magnesium, and biomolecules. Hemocyanin an oxygen-carrying protein is also directly present in hemolymph (Sowers et al. 2006). Significant concentration of free amino acids, presence of glucose, fructose, and sucrose are also found, although concentrations of these substances might vary from stage to stage (Wyatt 1961). As hemolymph is also the site for humoral defense responses for which many proteins are present like antimicrobial peptides, enzymes (later mentioned pathogenesis section), and also in some cases free amino acids like tyrosine aid in a process called humoral encapsulation (Chapman 2012), which contains the foreign body into a thick covering.

18.3.1.2 Hemocoel

Arthropods have an open circulatory system and their body cavity is called hemocoel. The space is for open blood (hemolymph) circulation. It is divided into three sinuses (pericardial, perineural and visceral sinus) and into compartments where respective organs directly bathe in hemolymph (Chapman 2012; Theopold et al. 2004). In the end of infection process, the fungal spores through the hemolymph reach hemocoel (Mondal et al. 2016) and subsequently all the organs.

18.3.1.3 Hemocytes

They can be called as the blood cells of the insect circulatory system and have an integral role to play in insect defense mechanism. As mentioned by Chapman (2012), they can be majorly classified as prohemocytes, plasmocytes, granulocytes, adipohemocytes, oenocytoids, and spherule cells.

Prohemocytes are basically the progenitor for many other types of hemocytes. Plasmocytes are present in large quantities mainly phagocytizing and encapsulating the foreign bodies, for example, *Beauveria* an entomopathogenic fungus is phagocytized but it can suppress the immune response. Granulocytes -as the name suggests contain large amounts of membrane-bound structures which are granules. These granules are released as a part of defense mechanism.

Adipohemocytes are lipid containing hemocytes. Oenocytoids might not present in all the orders, mainly present in lepidoptera (Chapman 2012). As such there is no special function for this, but a study by (Wang and St Leger 2007) shows that conversion of prophenoloxidase (which is stored into oenocytoids) into phenol oxidase by eicosanoids plays a role in the defense response.

Spherule cells contain small spherical bodies which are called as spherules and their function is unknown (Chapman 2012)

18.3.1.4 Fat Bodies

The biomolecules mostly present in the hemolymph are synthesized or stored at some time in the fat bodies. Fat bodies are a tissue-like organization of trophocytes or adipocytes which may be supplemented by the presence of urate cells, tracheal cells. It synthesizes many hemolymph proteins, stores glycogen which is later converted into trehalose a key sugar source in the hemolymph. It also serves as a storage site for most lipids in insects (Chapman 2012).

18.3.2 Fungal Spores

Metarhizium belongs to the Hypocreales order and the family Clavicipitaceae. The peculiar characteristic is that they are widely used insect pathogens. The spores of fungi are reproductive agents adapted for survival during unfavorable conditions and proliferate in a conducive environment. Conidiospores are asexual, exogenic spores formed mitotically. The hyphae are aseptate and the shape of each conidia is ovoid or cylindrical. It forms chains that appear cylindrical column-like or prismatic. It becomes conical at the apex and asci are arranged in dense hymenium (Money 2016; Sinha et al. 2016). The conidia grow on phialides which are whorls of branches ramified from conidiophores (Sinha et al. 2016). Conidiophores which bear conidiospores are translucent in appearance. The conidia can differ from species to species. The conidia can be straight-sided and small as of *M. anisopliae var anisopliae* or another var. *majus* can be large measuring up-to 18 μm. *M. flavoviride* have swollen light green conidia with club-shaped phialides. The rate of growth of conidia may differ too (Glare et al. 1996). The maximum temperature for most *M. anisopliae* isolates is 37 °C. However, there can be variability in thermotolerance (Fernandes et al. 2010).

18.3.2.1 Appressorium as a Structure

Appressorium is a specialized invasive structure which is basically an extension of germ tube to penetrate the host tissues which are intact. Appressorium is one of the salient features of both plant and arthropod pathogenic fungi. A study on in vitro appressorium production (Butt et al. 2016a) states that after germination as soon as the germ tube comes into contact with a hard surface infection structures are produced. As a plant defense response waxes may entrap and inhibit the germination of fungal conidia (Butt et al. 2016a, b). From a general prospect, appressorium can exist in unicellular, multicellular, or simply as a terminal swollen part of germ tube or completely differentiated structure in certain plant pathogens (Liu et al. 2012). Also as explained by Liu et al. (2012), in studies done on *Magnaporthe oryzae* during the development of appressorium—after germination, the conidiospore undergoes a set of events in the cell cycle and cell division. This is followed by appearance of an actomyosin ring which partitions the cell and the structure which will penetrate the host. Autophagy is then initiated in the spore cells which causes the cellular contents to flow inside the appressorium which makes it turgid and enhance its mechanical strength for penetration. Whereas in *Metarhizium*, MPL1 gene which produces perilipin homolog, Ca^{+2}, etc. maintains turgidity, actin cytoskeleton, chitin and dihydroxynaphthalene maintains structural support (Leger et al. 1991b; Gauthier and Keller 2013)

18.3.3 Pathogenesis

Metarhizium spore (conidiospore) generally adheres and germinates when it comes into contact with the host's cuticle and then it outgrows the arthropod's body by draining its nutrition, resulting in the death of the mosquito. Initially it starts with the development of appressorium, which penetrates into the host and then subsequently forms an infection peg, and then when it enters hemolymph, the formation of hyphae takes place, which releases toxins and then leads to death of the host (Scholte et al. 2004). The toxin destruxin affects the structural integrity of cell membrane of host thereby damaging host tissue and it also causes fluid loss (Scholte et al. 2004).

As referred from Aw and Hue (2017), there are six stages in the pathogenesis of *Metarhizium* (Aw and Hue 2017). These are given as below:

18.3.3.1 Adhesion

It is the initial event in which the asexual spores, which are conidia get attached to the cuticle of the host. The *Metarhizium* conidia are surrounded by an outer layer of rodlet cells. These rodlet cells consist of a protein "hydrophobin."

Hydrophobins are cysteine-rich proteins which are present in majority of fungi. These hydrophobins confer an amphipathic nature to rodlets aiding them in attachment to the hydrophobic epicuticle. Hydrophobins also play a major role in reducing the spore wettability thus forming a water-resistant layer (Sunde et al. 2008). When the spores are dispersed aerially they land on the epicuticle and the attachment of spore is due to hydrophobic interactions, electrostatic forces, and interaction of proteins (Aw and Hue 2017). Various external factors affect the attachment such as water, oxygen, nutrients, pH, hydrophobicity of host surface, and environmental conditions. The fungi can have specific requisites to infect restricted hosts (Sandhu et al. 2012).

Another recent study states that Mad 1 and Mad 2 are responsible for anchorage to insects as well as plants, respectively. Mad are *Metarhizium* adhesin like protein (Wang and St Leger 2007).

As explained by Greenfield et al. (2014) the adhesion follows a two-step process. Initially the superficial attachment occurs by electrostatic and hydrophobic forces or by attachment via adhesion proteins. The next step is to release enzymes to facilitate cuticle penetration. Also, the release of hydrolytic enzymes degrades fatty acids and release nutrients, which might aid in germination. The adhesins protein Mad 1 and 2 further strengthen the attachment (Greenfield et al. 2014).

18.3.3.2 Germination

The germination step is initiated by various non-specific exogenous nitrogen and carbon sources (Sandhu et al. 2012; Aw and Hue 2017). A study done by Santi et al. (2010) has reported different enzymes acting on the spore surface proteins. The spore surface proteins have been found to undergo degradation by different proteases.

These degrading enzymes comprise majorly of trehalase, seven different chitinases, two lipolytic enzymes have been detected. The trehalase enzyme ensures steady supply of glucose by breakdown of trehalose. Also phospholipase C which cleaves phospholipids was detected in *M. anisopliae* spores. The spore surface proteins not only have spore proteolytic activities but activities against reactive oxygen species (Santi et al. 2010), which might be produced on the host cuticle as defense response. Expression of Mest1 gene in *M. robertsii* helps aids in fast lipid hydrolysis and germination, on the contrary, *M. acridum* is helped by broadened host range expression of same gene. The expression of particular gene can be specific to particular host (Wang et al. 2011).

During the spore germination the spore absorbs water and nutrition from the host surface by osmosis and develops a germ tube which is an elongated structure (R. Barkai-Golan 2001)

18.3.3.3 Formation of Appressorium

Apart from the general organization and development of appressorium explained above, there are many specific molecular features in *Metarhizium*, which are explained below. Although some of them might be common to certain other entomopathogenic fungi. We can understand appressorium formation given below by bifurcating it into two major points. First is differentiation of germ tube end into appressorium and second maintaining of the turgor pressure for penetration into host cuticle (Fig. 18.2). Expression of ODC1 gene aids in appressorium formation. It encodes for ornithine decarboxylase. As it is known that ornithine decarboxylase enzyme is essential for cell growth as it stabilizes the DNA structure which prevents apoptosis (and supports the excess cell proliferation).The ornithine decarboxylase causes decarboxylation of ornithine which in turn aids in production polyamines which are directly involved in DNA structure stabilization (Pendeville et al. 2001). The expression of this gene is increased during appressorium formation and germination (Pulido et al. 2011). Pmk1 MAP promotes the appressorium maturation (Kershaw and Talbot 2009; Gauthier and Keller 2013). Chitin and dihydroxynaphthalene melanin deposits in appressorium act as structural support against the turgor pressure (Gauthier and Keller 2013). A study done by (Wang and St Leger 2007) shows that *Metarhizium* produces a protein MPL1 which is similar to a mammalian protein perilipin. The study reports that MPL1 confines the lipid molecules into droplets by binding with them similar to perilipin. Phosphorylation

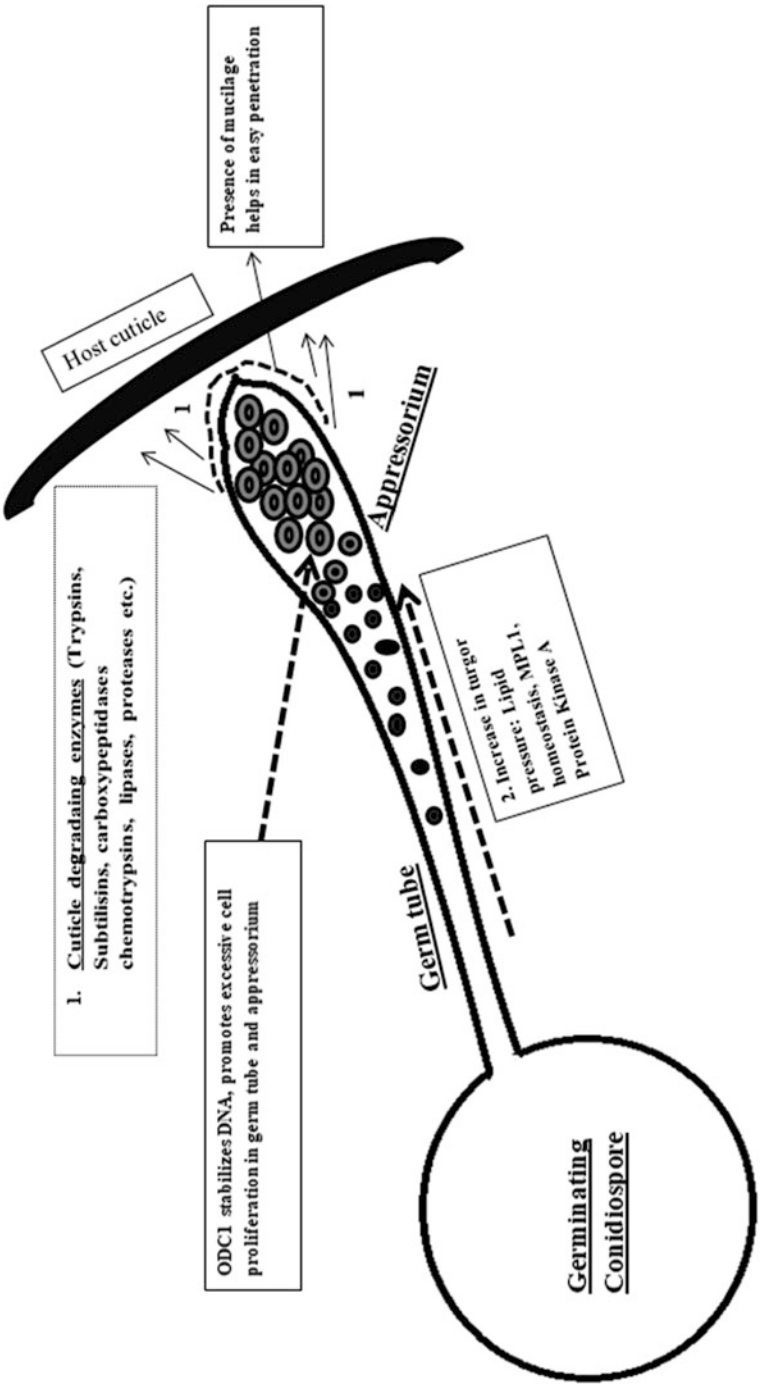

Fig. 18.2 The figure above explains appressorium formation

of this protein by cAMP dependent Protein Kinase A leads to release of the lipids and fatty acids contained in the lipid droplet. This helps in maintaining the turgor pressure, for example, in case of *M. grisea* a lipid breakdown product—glycerol acts as a solute. Its accumulation increases the water uptake (Thines et al. 2000; Wang and St Leger 2007). MAPK—mitogen activates protein kinase—plays a role in appressorium differentiation, Thines et al. (2000) reported that a mutant of *M. grisea* without MAPK did not had lipid mobilization. Aw and Hue (2017) stated about MAPK's intermediate role in adherence and lipid metabolism by regulating their respective genes. Leger et al. (1991a) proposed that Ca^{+2} ions get disrupted in the apical region of hyphae and get redirected to the region of cell enlargement in appressorium. The role of Ca^{+2} is also for actin cytoskeleton maintenance, which helps the appressorium to maintain its structure despite the turgor pressure (Leger et al. 1991b). Zhang et al. mention that chitin synthase MaChsIII, MaChsV, and MaChsVII are also involved in the disruption of host defense responses apart from Appressorium development (Zhang et al. 2019).

18.3.3.4 Penetration

Penetration into the host body involves cuticle breakdown. As cuticle composition varies from host to host as a result the amount and diversity of hydrolytic enzymes released by the fungus also varies. So, different hosts have specificity for different proteins and their concentration (Aw and Hue 2017). Studies by Leger et al. (1991a) have shown the presence of cuticle degrading enzymes in ungerminated conidia of *Metarhizium* namely esterase, chymoelastase protease, and *N*-acetylglucosaminidase. Also, the same study reported that the amount of these enzymes was more on an infected host when compared to in vitro conditions.

Trypsins, subtilisins, carboxypeptidases, and chemotrypsins and other proteases are secreted which degrades the protein part of procuticle of hosts. Pr1 and Pr2 are spore surface proteins responsible for proteolytic activities (Santi et al. 2010). Pr1 is a serine protease which degrades the cuticle by hydrolyzing proteins (Screen et al. 1997). Apart from proteases, chitinases are also involved.

Precisely chitin isoforms are secreted to limit its specificity to the host. Lipases found on conidial surface which interact with the lipid-rich epicuticle release fatty acids which leads to the enhanced hydrophobic interactions between conidia and host (Beys da Silva et al. 2010; Aw and Hue 2017). Also free fatty acid may act as a nutrition source.

18.3.3.5 Colonization

Host hemolymph is colonized next, after breaching the cuticle (Branine et al. 2019). The hemolymph is the site for the host's defense responses. The insect defense system is divided into humoral and cellular responses. The humoral response includes the antimicrobial peptides, reactive oxygen species, etc. The cellular

response comprises of encapsulation and phagocytosis by the hemocytes (Lavine and Strand 2002). To counteract the defense response when the encapsulated spores release from the hemocytes, destruxin is released (Aw and Hue 2017).

Destruxins are the insecticidal secondary metabolites responsible for the fungi's virulence (Dornetshuber-Fleiss et al. 2013). The role of destruxins in being virulent to the insect primarily includes (Golo et al. 2014)—suppressing the defense response, hindering the fluid secretion by malpighian tubules thereby interfering with the osmoregulation (James et al. 1993), they are also shown to block vacuolar H^+ ATPases (V-ATPases). V-ATPases via ATP hydrolysis pump the protons into lysosomes, Golgi, late endosomes, and other membrane-bound compartments to maintain the required acidic conditions (Toei et al. 2010) and it also possesses anti-feedant properties (Amiri et al. 1999; Golo et al. 2014).

Presence of catalase, peroxidase (for breakdown of certain reactive oxygen species), genes which code for proteins, which help in breakdown of antimicrobial peptides, MaAC, and certain other genes protect the fungal cells from chemical and physical stresses (Aw and Hue 2017) These stress might arise as a consequence of host immune response. Trehalose makes up the major part of the insect hemolymph. Instant energy to the insects and survival in abiotic stresses are its role. As it is a disaccharide it proves to be the main carbon source for fungal spore growth when it grows in insect hemolymph (Shukla et al. 2015).

Trehalase is also present to hydrolyze the trehalose in the hemolymph into glucose, secreted as extracellular enzyme by the fungi (Xia et al. 2002), Presence of MOS1 gene increases the fungal survival at high osmotic pressure (Wang et al. 2008). All these factors aid in the survival of the fungi in the harsh environment of hemolymph. Inside the hemolymph, some fungal cells proliferate into protoplasts which prevents their recognition by host defense system because the recognition proteins may be present at the cell wall (Mondal et al. 2016).

18.3.3.6 Sporulation

After the fungi have proliferated into host hemolymph and Hemocoel, it feeds on host's nutrients, which leads to the death of the host insect. After this, upon receiving suitable conditions the fungal spore germinates and the hyphae extrude out of the corpse, although spores of *M. anisopliae* have shown to germinate internally in dried corpses (Mondal et al. 2016). Spore formation is an important procedure for the dissemination of fungal diseases. Metalloproteases Mrmep1 and Mrmep2 have been shown to be responsible for the sporulation of *M. robertsii*, (Zhou et al. 2018). The conidia of *M. anisopliae* were present before it fully colonized the host. Mycelium growth is usually observed near the antennae base, on cervix, and its mouthparts (Sun et al. 2002).

18.4 *Metarhizium* Application Methods in Vector Control: A Superfluity

As discussed in this text, it is quintessential need for the fungal spore to come into direct contact with the host in order to infect it so the application strategies must be wisely chosen. Use of *Metarhizium* in agricultural biocontrol procedures mostly comprises of powder-like substances or wettable suspensions as a carrier material but in case of vector biocontrol this entomopathogenic fungi can be inoculated into the breeding grounds, stagnant waters and in some studies have also reported an increase in mortality rate of *Anopheles gambiae* when use of mud panels, polyester netting, and cotton cloth as a holding material was done for the fungus (Aw and Hue 2017). In another study, it was observed that *Metarhizium* was able to interfere with the DDT and permethrin resistance genetically, due to which chances of susceptibility to these insecticides were increased (Farenhorst et al. 2009). Although insecticide-treated net and residual sprays are most popularly used options but apart from them plethora of setups and carrier materials have been experimented with but here we shall discuss some important conventional and recent molecular approaches.

18.4.1 Experimental Huts

A study was carried out in the malaria-endemic region of Tanzania (Mnyone et al. 2012). Local housing huts were designed to contain fungal suspension (which comprised of *B. bassiana* and *M. anisopliae*) infected polyester nets, curtains, bed net strips, panels, and baffles. The results showed a decrease in survival rate of mosquitos and a significant percentage (68%–76%) of mosquitos had fungal growth which shows increased contact surface (Mnyone et al. 2012). It also showed the effect of this strategy on biting behavior and malaria transmission from the mosquito.

18.4.2 Using Paper Substrates as a Resting Material for Fungal Spores

In another study (Farenhorst and Knols 2010) smooth paper pieces were coated with fungal formulations (*Metarhizium anisopliae*) made in low viscous material both manually and mechanically and were used instead of sprays. A stainless steel bar was used to apply this suspension. This method was very effective in standardizing the amount of fungal biomass used and its exposure time to the mosquito.

18.4.3 *Water Storage Pots as a Carrier Material for* Metarhizium

In this project (Farenhorst et al. 2008) African water storage pots were used as wet clay pots were an attractive breeding site for mosquitos. In this study, *Anopheles gambiae* and *Anopheles funestus,* which are an evident malaria vectors, were used. Oil formulated conidia were sprayed uniformly inside the surface of clay pots. The dead mosquito cadavers after the application were studied for fungal growth. The results inferred that clay pots were an attractive sight for mosquito breeding, also these studies (Farenhorst et al. 2008) suggested that in future some other mosquito attractants could also be combined in this clay pot approach.

18.4.4 *Combination of* Metarhizium *with Insecticide-Treated Nets*

With the ease of availability and inexpensive nature, insecticide-treated nets (ITN) have become the mainstay in vector control. But, while combining ITNs with a fungal suspension major focus is to enhance the functional ability of ITNs. A study was done (Hancock 2009) in order to check the performance of fungal suspension intervened ITNs, in this various factors were considered like fungal infection, gonotrophic feeding process, ITNs, etc. then a model was proposed for which mathematical analysis was done. As a result (Hancock 2009) an extensive information was produce which had many suggesting reasons to use fungal biocontrol integrated with ITNs such as high virulence, more probability of fungal infection in host, prolonged period of fungal exposure, etc.

18.4.5 Metarhizium *in Odor Bait Stations (OBS)*

This was a study done by Lwetoijera et al. (2010) where an OBS was used against a malaria vector *Anopheles arabiensis*. OBS are box-like structures made on a wooden frame which is covered with a canvas. The entire device except the floor is covered with a black cloth and inside this device there is a mosquito lure which mainly consists of carboxylic acids, carbon dioxide, and ammonia (Okumu et al. 2010; Lwetoijera et al. 2010). It contains a funnel-like opening and it also has *Metarhizium* conidia treated baffles. The results showed an increase in infection rates and also it is much anticipated because using OBS is much more safe than using fungal suspension in human dwellings as done in experimental huts (Mnyone et al. 2012) and other such methods.

18.4.6 Oil as a Carrier Material

18.4.6.1 Mineral Oil

A study done by (Bukhari et al. 2011) focused on comparing the efficiency of an aqueous substance, a synthetic oil formulation (shellSol T) and dry carrier material (wheat flour, white pepper, and fine bicarbonate particles) as a carrier material of fungal spores in aquatic habitats. The results revealed that Shell Sol T a synthetic oil is an effective spore carrier material in comparison to aqueous and the dry carrier material in both laboratory and field trials. In fact the field trials witnessed a decrease by 39–50 percent of the *Anopheles gambiae* population. In comparison to non-formulated fungal spores where efficacy was very low, Shell Sol T formulated spores showed a promising effect and increased persistence in water. This study gives another perspective—usage of synthetic oils as a carrier material. Also, the oil used here had minimal toxicity to the aquatic habitat as the quantity used here was very less compared to that of the safe limit.

18.4.6.2 Vegetable Oil

This experiment focused to check the viability of *M. flavoviride* spores after storage in different formulations and variable temperature. As a diluent, dedeorized kerosene oil preserved the spores better than Shellsol K, apparently during short duration of storage; however, mean conidial growth was found more in Shellsol K after 32 weeks. Vegetable oils were effective but its efficacy was improved with addition of antioxidant in case of groundnut oil. It was also observed that lower temperature led to increased germination of spores. The addition of silica gels, which aided by drying out spores, showed significant results as well. Still there was requirement for further studies to get the better understanding of storage techniques under variable conditions. (Moore et al. 1995).

18.4.7 Mosquito Landing Boxes (MLBs) for Metarhizium

MLBs are devices that have natural or synthetic human odor as a mosquito attractant. It is a system based on odor baiting technology. These devices are basically a wooden box with solar panels on the top which powers the odor dispenser. Particles of the odor solution lands on the walls of the device which may have fungal spore coating or some insecticide. In a study Lwetoijera et al. (2010) used this device and the walls of the MLB were coated with spores of the entomopathogenic fungi *M. anisoplieae* and vector targeted here was *Anopheles arabiensis.* This trial was conducted in a semi-field system. Separate cups containing larvae were placed near both MLBs and control system and it was supposed that if a mosquito captured from the semi-field system is let into the cups containing larvae and the larvae is not able

to survive, and then the mosquito has been contaminated with the fungal spores and vice versa. Many factors like larval mortality, pupation, amount of hyphae growth on cadavers, etc. were assessed to check the level of contamination of the mosquitos with the fungi. The results revealed that 43% of mosquitos were contaminated with the fungal spores compared to 0% in the control. In this study alternatively, a chemical pyriproxyfen was also used in the system. This study inferred that MLBs are an effective tool for delivering fungal biopesticides as a decrease in the survival rate and direct killing of host-seeking mosquitos was witnessed.

18.4.8 Metarhizium *in Combination with Phytochemicals*

Use of neem oil as an insecticide is a very popular and old method but despite that today we see very less use of such phytochemical in vector control. A study done by Simone A. Gomes and coworkers (Gomes et al. 2015) focused on the use of neem oil as an adjuvant for the entomopathogenic fungi *Metarhizium*. In their study, they used two systems, one to check the effect of neem oil used solely on *Aedes aegypti* and another one to check the effect of neem oil in combination with *Metarhizium*. Neem oil of variable concentrations was used. Statistical analysis of the survival curve was done. The results revealed that at the concentration of 1×10^8 conidia per ml, a very low survival rate of 12% was observed. Also, later it was suggested that the addition of neem enhanced virulence. This study suggested the use of adjuvants such as phytochemicals along with the fungal biopesticides. In future, many other potential phytochemicals can be used as an adjuvant to variety of fungal biopesticides.

18.4.9 Metarhizium *for Chemical Resistant Vector Hosts*

Insecticide resistance is a prominent and emerging problem in the area of vector control. But the use of entomopathogenic fungi for insecticide-resistant vectors is one of the ways to fight insecticide resistance. An interesting study by Blanford et al. (2009), which was done on the vector *Anopheles gambiae* which was insecticide resistant—has interacted with *Metarhizium anisopliae* and another entomopathogenic fungi and results revealed increased susceptibility of the insecticide-resistant strain of host. The resistant strain used here was *Anopheles gambiae s.s.* VKPER, which is a pyrethroid-resistant strain. Also an insecticide susceptible strain *Anopheles gambiae* SKK strain was treated with the same procedure. Various mechanisms were used to deliver the fungal isolates to the mosquito-like formulations in synthetic oils (Kamareddine 2012), dry conidia (Ondiaka et al. 2015), etc.

18.4.10 Delivery System in Agriculture Fields

Apart from the above-given methods, there are some specialized methods that can be instilled for the delivery of fungal spores to the host. The formulations are designed to increase the viability of spores and to expose them lucratively to the host.

18.4.10.1 Kaolin Based

The spores of fungi are mixed with 80% kaolin. Prior to use, it is mixed with water and wetting agent solution. Then solution is sprayed directly onto the plant (Goble et al. 2016).

18.4.10.2 Patty Blend Formulation

Vegetable oil and sugar are mixed with pre-weighed conidiospores. Conidial viability is increased by the addition of Silwet and Saboraud maltose agar. Lactophenol cotton blue is added to stop its germination. The insects are treated with the strip containing serially diluted formulation (Kanga et al. 2010).

18.4.11 Molecular Approaches

The DNA technologies in the new age facilitate the addition of new gene into fungi and perform gene manipulation to increase efficacy of the fungi. Due to various stress condition their efficiency gets decreased but the DNA technology can be useful to improve the ability of virus to sustain in unfavorable conditions and more virulence. By expressing the endogenous proteins in the *Metarhizium* the pathogenesis success rates increases by targeting the cuticle, physiology, and hormones (Lovett and St Leger 2018).

In an experiment performed by Leger et al. (1991b), a genetic modification was done in *Metarhizium* in which more number of copies of *Pr1 gene,* which basically codes for protease that degrades cuticle of host, was inserted. When newly engineered *Metarhizium* was made to infect *Manduca sexta* there was overproduction of gene and activation of phenoloxidase system. The results include reduction in death timing, food consumption, and biological containment of the fungi. One of the reasons for biological containment was due to the accelerated melanization of the host cadaver which in turn provides insufficient substrate source for fungal spores growth (St Leger et al. 1996). Phenoloxidase is activated by prophenoloxidase cascade and provides immunity to the insects and polymerization of the indole group of phenoloxidase leads to the formation of melanin which leads to melanization precisely upon with infection with fungal spores (González-Santoyo and Córdoba-Aguilar 2012; Carolino et al. 2014; Butt et al. 2016b; Zhang et al. 2017).

Peng et al. (2015) performed an experiment in which *ATM1 gene* was overexpressed which codes for trehalase. Trehalase degrades trehalose which provides fungus with the carbon source in hemolymph of insect host. When results were compared to the wild strain of *Metarhizium* the genetically engineered fungi showed more degradation of trehalose and growth enhancement of fungi in host hemolymph.

In an another experiment, scorpion toxin *BjαIT* was used for genetic manipulation of *Metarhizium* the results showed enhanced virulence by the fungi in host infection. The growth of fungal spores on cadaver did not report any drop which might not affect the transmission. Though the yield was reduced but germination and formation of appressorium were the same as the wild-type strain and the lethal dose and lethal time were less to (Peng and Xia 2015).

Transgenic *M. pingshaense* was used to control the insecticide-resistant *Anopheline* mosquitoes. The new genetically modified fungi were hybrid it had voltage-gated calcium blocker with *kappa hexatoxin Hv1a and calcium-activated potassium genes*. The results in labs showed that efficacy was increased and it was able to control the resistant malaria vector. This can be used for on field application in the future (Lovett et al. 2019).

In heat stress conditions hyphal cells may start producing Reactive oxygen species (ROS). Pyruvate acts as ROS scavengers but the rate of formation of pyruvate is slower than the formation of ROS. A transgenic *Metarhizium* was designed so as there is overexpression of genes and therefore increased concentration of pyruvate kinase will be produced. During conidia formation, the pyruvate kinase gets accumulated and this helps conidia to survive during heat stress (Wu et al. 2019).

Genetic modification to create transgenic *Metarhizium* has been successful in many of the cases as mentioned above and showed an increase in virulence as performed by Peng et al. (2015), Lovett et al. (2019) and some (Wu et al. 2019) showed considerable efficacy in lab as well which can further be implemented in field and tested for the outcome. Extensive research in knowing the enzymes, genes, and host immune system in addition to fungi evasion and invasion techniques can help to genetically manipulate the fungus and increase its efficacy.

18.5 Plant Growth Promotion

Mycorrhizae are obligate biotrophs and endophytes aids in improving plant growth and nutrients acquisition from soil to the plants (Karandashov and Bucher 2005; Behie et al. 2017). Unlike mycorrhiza, *Metarhizium* as an endophyte shows no obligatory nature as it can survive in soil freely, as entomopathogens or as saprophytes (Behie et al. 2017). Beyond the activity of being pathogenic to the insects, there is one more benefit which is, plant growth promotion; although this is an area that has not been extensively studied (Canassa et al. 2019). This improves the yield In addition to the pest management of the plants. For sustainable agriculture plant growth promotion coupled with pest inhibiting capabilities can help to discontinue the use of heavy pesticides and fertilizers (Senthil Kumar et al. 2018). *Metarhizium* can induce root hair development, nitrogen translocation, improved absorption of iron, and auxin production (Behie and Bidochka 2014; Moonjely et al. 2019). Colonization of plant tissue makes it an endophytic fungi, which enhance plant biomass as well as it can increase nutrient mobilization and its transfer (Krell et al.

2018). The soil fertility may affect fungal activity and nutrient metabolization. Many studies have been performed but some of them lack a firm conclusion thus it requires further research to be done.

18.5.1 Exchange of Nutrients and Endophytic Nature

Nitrogen is crucial for plant growth and the loss of nitrogen due to insect herbivory leads to deprived nitrogen content available for plants (Behie and Bidochka 2014). Several fungi in symbiotic association with plants transfer nitrogen (Wang et al. 2017a, b). *Metarhizium* has a wide host range and is pervasive worldwide (Hajek and St. Leger 1994). *Laccaria bicolor* transfers the nitrogen derived from insects back to the plant white pine. *Metarhizium* has shown similar results when experimented with the insect *Galleria mellonell* (waxmoth) N^{15} labeled. This was performed on Switchgrass and haricot beans. The *Metarhizium* spp. were able to increase the plant productivity (Behie et al. 2012). In another experiment, five species of *Metarhizium* were tested all expressed positive results. *M. robertsii* was tested on field in natural conditions, showed significant results (Behie and Bidochka 2014). A recent experiment conducted shows that MepC and Mep2 are two ammonium permease genes which have been involved in nitrogen derived from insects and also in colonization process (Moonjely et al. 2019). Research for finding genes responsible for symbiosis of Insect pathogenic fungi can help to understand the functioning elaborately.

A recent study conducted by Behie et al. (2017) gives evidence that *Metarhizium* derives carbon from the plants as much as other endophytes. The carbon translocation can sustain fungi when insect host is absent. Reportedly, when the host was present there was significant increase in carbon transfer to the fungi (Behie et al. 2017). This nature of reciprocation of nutrients helps both plants and fungi.

Plant photosynthates containing carbon were found in the roots of the plants and in the rhizosphere which are utilized by the fungi as substrate. The ^{13}C (CO_2 given to plants had ^{13}C isotope) used was found to be incorporated in the fungi which had been provided by the plants as a symbiotic relationship. In fungi, it was traced to NAG and other Carbon-based components (Behie et al. 2017). The decomposing cadaver when added with *Metarhizium* spores lead to increase in ammonium and nitrate in the soil and spores were able to colonize plants as well that resulted in better plant growth (Kryukov et al. 2019).

Metarhizium has evolved as an endophyte (Moonjely et al. 2016). In an experiment performed by Barelli et al. (2018), the analysis was made between how extensively the *Metarhizium* colonizes the plant roots. For precise detection Plate culture method (c.f.u count) and quantitative PCR, both were done. The results showed that there was fungal colony in rhizosphere, rhizoplane, and within the roots too. Another thing that was noticed that the population of fungi did vary with the number of days post-inoculation (Barelli et al. 2018) but with this experiment it is

evident that *Metarhizium* can colonize roots efficiently. Thus there is scope of further investigation, whether this fungi can colonize phyllosphere or not.

18.5.2 Improved Iron Absorption on Calcareous Substrates

In an experiment performed by Raya-Diaz et al. (2017) on sorghum plants, the relationship between plants, entomopathogenic fungi, and soil can be established well. It concluded that the entomopathogenic fungi tested in case of calcareous soil, *M. brunneum* turned out to be the most efficient fungus which lowered the pH of the alkaline soil by releasing the organic acids. The plants suffering from iron chlorosis are iron-deficient plants. This is common concern for the plants growing in calcareous soil either acidic or basic as it leads to the less iron availability in the form plants requires for its uptake (Brown 1956). As tested in vitro, iron oxides changed to dissolved iron forms. The Fe chlorosis symptoms were seen to be assuaged by *M. brunneum* on the sorghum plants which were grown on the artificial substrate that was calcareous. The best method was soil inoculation method (Raya-Diaz et al. 2017). In another experiment performed by Sánchez-Rodríguez et al. (2016) the wheat and sorghum plant showed increase in growth and chlorophyll content by improving the iron bioavailability in soil.

18.5.3 Auxin Formation for Plant Growth

Auxin is a plant growth hormone which influences plant physiology and developmental process to the stimuli sunlight and gravitropism (Bhattacharya 2019; Pandey et al. 2019). In an experiment performed by Liao et al. (2017) vegetative growth of corn plants has shown improvement and the yield was increased. Avirulent *Metarhizium* strain (*Δmcl1*) contributed to the growth of plants as well proving that the plant growth promotion activity is not influenced by entomopathogenic activity. Auxin was produced by fungi, there was increase in formation of leaf collar, foliage biomass, and the length of the stalk where the spores were able to colonize. The culture filtrate even contained auxin which showed positive result (Liao et al. 2014). The growth of plants is due to combined effect of chemicals and auxin (IAA) dependent pathways importantly which is produced by *Metarhizium*; promoting the lateral root and root hair development (Liao et al. 2017).

18.5.4 Proliferation of Plant Cells and Disease Suppression

The *Metarhizium* species can be potential plant endophytes and can live inside plant tissues. In an experiment performed it was noticed that their role can be in

increasing the size of stalk, root length, and weight of the root (Mantzoukas et al. 2015; Greenfield et al. 2016). It can cause proliferation of lateral root hair for enhanced plant growth (Sasan and Bidochka 2012). The growth rates differ with respect to the strain used, duration, and the inoculation amount (Jaber and Enkerli 2017). In bean plants, there were significant results to show that it improved the reproductive and vegetative growth also (Canassa et al. 2019).

Jaber (2018) performed an experiment whether *M. brunneum* can be effective for suppression of disease-causing pathogens. The results showed significant decrease in the occurrence of disease, its development, and intensity. The fungus was able to promote shoot and root growth and the weights.

18.6 Conclusion: In the Light of Recent Advances

With the increased understanding of the molecular aspects of *Metarhizium* such as the genes and proteins involved in pathogenesis and their upregulation, secondary metabolites and small molecules, molecular aspects of host immunity, genome-wide studies, etc. have made the scope of research on *Metarhizium* infinitely vast. Donzelli and Krasnoff (2016) state that the recently available genome sequences give many biosynthetic pathways and ability to produce secondary metabolites which surpasses the current knowledge of chemistry. Basically, this study focuses on details of genes involved in the production of secondary metabolites. Also, there have been studies (Brancini et al. 2019) where light has shown to affect the gene expression in *Metarhizium*, after periodic exposure to light upregualtion and down regulation of certain proteins takes place. One such protein is photolyase which is upregulated and is responsible for UV tolerance. The changes concluded in inference that light is involved in stress and signaling. So light might be a factor in controlling the efficacy of *Metarhizium*. Studies by Mukherjee and Vilcinskas (2018) and Hussain (2018) discuss about changes in the gene expression. On recognition of the fungal spore by the host immune system, the host increases the expression of certain antifungal peptides in response to overcome the hostile environment in host, the fungi also increases the expression of certain proteins that destroy these peptides which are epigenetically controlled. This way of the counteracting molecular responses in host and the fungi give further insights into the coevolution process.

Except from entomopathogenecity *Metarhizium* has certain effects on growth promotion in plants. A recent review by Hu and Bidochka (2019) has mentioned that species from *Metarhizium* genera are root endophytes and have a symbiotic relation as they provide insect-derived nitrogen and get photosynthates in return. They have further reviewed the factors governing the rhizospheric interactions. Also, there has been a recent study on the host cadaver decomposition affecting plant growth promotion (Kryukov et al. 2019). The decomposed cadavers contain more ammonia and nitrogen compared to cadavers overgrown by fungus. It was concluded that

fungi were unable to sporulate on decomposed cadavers and provided nitrogen faster from the cadavers overgrown by fungus.

So all these facts and studies boil down to some important inferences:

1. Evolution of *Metarhizium defense response*—As discussed in the pathogenesis section there are several lytic enzymes which, facilitate the entry of *Metarhizium* into the host. As stated by Mukherjee and Vilcinskas (2018), host can recognize and counteract these proteins by releasing antimicrobial compounds, proteinase inhibitors, and antifungal compounds. Same study has shown that the expression of chymotrypsin and metalloproteinases by *Metarhizium* can counteract the host defense compounds. An *in vitro* increase in metalloprotease activity was observed in response to the antimicrobial peptides (AMP) like metschnikowia, lysozyme, and proteinase inhibitor. Another significant component of the insect defense system is hemocytes. The presence of destruxin has been mentioned in the text previously. This protein causes actin remodeling, pyknotic nuclei, and blebbing in plasmocytes (Götz et al. 1997). Another toxin cytochalasin is also involved but it is less toxic than destruxin. Both these toxins selectively regulate the expression of IMPI and lysozyme, which are antimicrobial peptides. Also induction of genes involved in epigenetic responses of histone acetylation and deacetylation in *M. robertsii* against an AMP shows, how specific modification in *M. robertsii* at transcriptional level is made to counteract host defense system. This might suggest lesser chances of host gaining resistance against the fungi. (Mukherjee and Vilcinskas 2018)
2. *An ideal biocontrol strategy must focus on enhancing efficacy of both the biological agent and its carrier*—There have been several attempts in creating a recombinant strain of *Metarhizium,* which is more effective by selecting genes like toxin genes as a candidate some of which were discussed in this article. Also carriers with improved efficacy are in a need to be developed considering certain environmental factors which have shown to have a stressful impact on the growth of *Metarhizium.* Study done by Wang et al. (2017a, b) on *Galleria mellonella* and *M. robertsii* mentions an important role of DNA methyltransferases, which are responsible for epigenetic or gene expression control. Here they have shown to have a role in stress tolerance and virulence of the fungi. This shows how transgenic fungi can be effective.
3. *Metarhizium as a complete plant health package*—With the well-established entomopathogenic effects and some endophytic plant growth promotion activities, *Metarhizium* can be used as a holistic supplement. A suggestable effort could be improving *Metarhizium* transgenically with plant growth promotion activity besides its entomopathogenecity. Although all these have been proven experimentally, still there are requirements of field assays. Possessing the knowledge about timely usage of biocontrol agents is very important to avoid emergency pest mitigation; especially when using a fungus, because it is temperature and humidity dependent. All of these characteristics can be brought to better use by focusing on synergistic approach like combining it with bio-fertilizers and using it with other biopesticides. Not only nitrogen translocation but also other mineral utilization by plants can be improved with scientific studies. So as with time,

entomopathogenicty of *Metarhizium* is being explored in newer hosts we are becoming more molecularly aware about it. The confluence of this scientific awareness and industry requirements is where *Metarhizium* promises a vast scope.

References

Alkhaibari AM, Maffeis T, Bull JC, Butt TM (2018) Combined use of the entomopathogenic fungus, Metarhizium brunneum, and the mosquito predator, Toxorhynchites brevipalpis, for control of mosquito larvae: is this a risky biocontrol strategy? J Invertebr Pathol 153:38–50. https://doi.org/10.1016/j.jip.2018.02.003

Amiri B, Ibrahim L, Butt TM (1999) Antifeedant properties of Destruxins and their potential use with the Entomogenous fungus Metarhizium anisopliae for improved control of crucifer pests. Biocontrol Sci Tech 9:487–498. https://doi.org/10.1080/09583159929451

Aw KMS, Hue SM (2017) Mode of Infection of Metarhizium spp. fungus and their potential as biological control agents. J Fungi 3(2):30

Barelli L, Moreira CC, Bidochka MJ (2018) Initial stages of endophytic colonization by metarhizium involves Rhizoplane colonization. Microbiol (United Kingdom). https://doi.org/10.1099/mic.0.000729

Behie SW, Bidochka MJ (2014) Ubiquity of insect-derived nitrogen transfer to plants by endophytic insect-pathogenic fungi: an additional branch of the soil nitrogen cycle. Appl Environ Microbiol 80:1553–1560. https://doi.org/10.1128/AEM.03338-13

Barkai-Golan R (2001) chapter 2 - postharvest disease initiation. In: Barkai-golan (ed) RBT-PD of F and V. Elsevier, Amsterdam, pp 3–24

Behie SW, Zelisko PM, Bidochka MJ (2012) Endophytic insect-parasitic Fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577. https://doi.org/10.1126/science.1222289

Behie SW, Moreira CC, Sementchoukova I et al (2017) Carbon translocation from a plant to an insect-pathogenic endophytic fungus. Nat Commun 8:14245. https://doi.org/10.1038/ncomms14245

Beys da Silva WO, Santi L, Schrank A, Vainstein MH (2010) Metarhizium anisopliae lipolytic activity plays a pivotal role in Rhipicephalus (Boophilus) microplus infection. Fungal Biol 114 (1):10–15

Bhattacharya A (2019) Effect of high-temperature stress on the metabolism of plant growth regulators, chapter 6. Academic Press, New York, pp 485–591

Bilgo E, Lovett B, St. Leger RJ et al (2018) Native entomopathogenic Metarhizium spp. from Burkina Faso and their virulence against the malaria vector Anopheles coluzzii and non-target insects. Parasit Vectors 11:209. https://doi.org/10.1186/s13071-018-2796-6

Bischoff JF, Rehner SA, Humber RA (2009) A multilocus phylogeny of the Metarhizium anisopliae lineage. Mycologia 101:512–530. https://doi.org/10.3852/07-202

Bittencourt VREP, Mascarenhas AG, de Menezes GCR, Monteiro SG (2000) In vitro action of Metarhizium anisopliae (Metschnikoff, 1879) Sorokin, 1883 and Beauveria bassiana (Balsamo) Vuillemin, 1912 on eggs of the tick Anocentor nitens (Neummann, 1897) (Acari: Ixodidae). Rev Bras Med Veterinária 22:248–251

Blanford S, Read AF, Thomas MB (2009) Thermal behaviour of Anopheles stephensi in response to infection with malaria and fungal entomopathogens. Malar J 8:72. https://doi.org/10.1186/1475-2875-8-72

Brancini GTP, Ferreira MES, Rangel DEN, Braga GÚL (2019) Combining Transcriptomics and proteomics reveals potential post-transcriptional control of gene expression after light exposure in Metarhizium acridum. G3 (Bethesda) 9:2951–2961. https://doi.org/10.1534/g3.119.400430

Branine M, Bazzicalupo A, Branco S, Hogan DA (2019) Biology and applications of endophytic insect-pathogenic fungi. PLoS Pathog 15(7):e1007831

Brown JC (1956) Iron chlorosis. Annu Rev Plant Physiol 7:171–190. https://doi.org/10.1146/annurev.pp.07.060156.001131

Bukhari T, Takken W, Koenraadt CJM (2011) Development of Metarhizium anisopliae and Beauveria bassiana formulations for control of malaria mosquito larvae. Parasit Vectors 4:23. https://doi.org/10.1186/1756-3305-4-23

Butt TM, Coates CJ, Dubovskiy IM, Ratcliffe NA (2016a) Chapter nine - Entomopathogenic Fungi: new insights into host–pathogen interactions. In: Lovett B, St. Leger RJ (eds) Genetics and molecular biology of Entomopathogenic fungi. Academic Press, New York, pp 307–364

Butt TM, Coates CJ, Dubovskiy IM, Ratcliffe NA (2016b) Entomopathogenic Fungi: new insights into host-pathogen interactions. Adv Genet 94:307–364. https://doi.org/10.1016/bs.adgen.2016.01.006

Canassa F, Tall S, Moral RA et al (2019) Effects of bean seed treatment by the entomopathogenic fungi Metarhizium robertsii and Beauveria bassiana on plant growth, spider mite populations and behavior of predatory mites. Biol Control 132:199–208. https://doi.org/10.1016/J.BIOCONTROL.2019.02.003

Carolino AT, Paula AR, Silva CP et al (2014) Monitoring persistence of the entomopathogenic fungus Metarhizium anisopliae under simulated field conditions with the aim of controlling adult Aedes aegypti (Diptera: Culicidae). Parasit Vectors 7:198. https://doi.org/10.1186/1756-3305-7-198

Chandler D, Bailey AS, Tatchell GM et al (2011) The development, regulation and use of biopesticides for integrated pest management. Philos Trans R Soc Lond Ser B Biol Sci 366:1987–1998. https://doi.org/10.1098/rstb.2010.0390

Chapman RF (2012) The insects: structure and function, 5th edn. Cambridge University Press, Cambridge

Charnley AK (2003) Fungal pathogens of insects: cuticle degrading enzymes and toxins. Academic Press, New York, pp 241–321

Constanza Mannino M, Huarte-Bonnet C, Davyt-Colo B, Pedrini N (2019) Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. J Fungi 5(2):33

Contreras J, Mendoza JE, Martinez-Aguirre MR et al (2014) Efficacy of enthomopathogenic fungus Metarhizium anisopliae against Tuta absoluta (Lepidoptera: Gelechiidae). J Econ Entomol 107:121–124. https://doi.org/10.1603/ec13404

Donzelli BGG, Krasnoff SB (2016) Molecular genetics of secondary chemistry in Metarhizium fungi. Adv Genet 94:365–436. https://doi.org/10.1016/bs.adgen.2016.01.005

Dornetshuber-Fleiss R, Heffeter P, Mohr T et al (2013) Destruxins: fungal-derived cyclohexadepsipeptides with multifaceted anticancer and antiangiogenic activities. Biochem Pharmacol 86:361–377. https://doi.org/10.1016/j.bcp.2013.05.022

Erler F, Ates AO (2015) Potential of two entomopathogenic fungi, Beauveria bassiana and Metarhizium anisopliae (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. J Insect Sci 15:44. https://doi.org/10.1093/jisesa/iev029

Farenhorst M, Knols BGJ (2010) A novel method for standardized application of fungal spore coatings for mosquito exposure bioassays. Malar J 9:27. https://doi.org/10.1186/1475-2875-9-27

Farenhorst M, Farina D, Scholte E-J et al (2008) African water storage pots for the delivery of the entomopathogenic fungus Metarhizium anisopliae to the malaria vectors Anopheles gambiae s.s. and Anopheles funestus. Am J Trop Med Hyg 78:910–916

Farenhorst M, Mouatcho JC, Kikankie CK et al (2009) Fungal infection counters insecticide resistance in African malaria mosquitoes. Proc Natl Acad Sci USA 106:17443–17447. https://doi.org/10.1073/pnas.0908530106

Fernandes EKK, Keyser CA, Chong JP et al (2010) Characterization of Metarhizium species and varieties based on molecular analysis, heat tolerance and cold activity. J Appl Microbiol 108:115–128. https://doi.org/10.1111/j.1365-2672.2009.04422.x

Garcia ARM, de Rocha AP, Moreira CC et al (2016) Screening of fungi for biological control of a Triatomine vector of Chagas disease: temperature and trypanosome infection as factors. PLoS Negl Trop Dis 10:e0005128–e0005128. https://doi.org/10.1371/journal.pntd.0005128

Gauthier GM, Keller NP (2013) Crossover fungal pathogens: the biology and pathogenesis of fungi capable of crossing kingdoms to infect plants and humans. Fungal Genet Biol 61:146–157. https://doi.org/10.1016/J.FGB.2013.08.016

Glare TR, Milner RJ, Beaton CD (1996) Variation in Metarhizium, a genus of fungal pathogens attacking Orthoptera: is Phialide morphology a useful taxonomic criterion? J Orthoptera Res 5:19–27. https://doi.org/10.2307/3503572

Goble TA, Gardescu S, Jackson MA, Hajek AE (2016) Evaluating different carriers of Metarhizium brunneum F52 microsclerotia for control of adult Asian longhorned beetles (Coleoptera: Cerambycidae). Biocontrol Sci Tech 26:1212–1229. https://doi.org/10.1080/09583157.2016.1192103

Golo PS, Gardner DR, Grilley MM et al (2014) Production of destruxins from Metarhizium spp. fungi in artificial medium and in endophytically colonized cowpea plants. PLoS One 9: e104946–e104946. https://doi.org/10.1371/journal.pone.0104946

Gomes SA, Paula AR, Ribeiro A et al (2015) Neem oil increases the efficiency of the entomopathogenic fungus Metarhizium anisopliae for the control of Aedes aegypti (Diptera: Culicidae) larvae. Parasit Vectors 8:669. https://doi.org/10.1186/s13071-015-1280-9

González-Santoyo I, Córdoba-Aguilar A (2012) Phenoloxidase: a key component of the insect immune system. Entomol Exp Appl 142:1–16. https://doi.org/10.1111/j.1570-7458.2011.01187.x

Götz P, Matha V, Vilcinskas A (1997) Effects of the entomopathogenic fungus Metarhizium anisopliae and its secondary metabolites on morphology and cytoskeleton of plasmatocytes isolated from the greater wax moth, galleria mellonella. J Insect Physiol 43(12):1149–1159. https://doi.org/10.1016/s0022-1910(97)00066-8

Greenfield BPJ, Lord AM, Dudley E, Butt TM (2014) Conidia of the insect pathogenic fungus, Metarhizium anisopliae, fail to adhere to mosquito larval cuticle. R Soc Open Sci 1:140193. https://doi.org/10.1098/rsos.140193

Greenfield M, Gómez-Jiménez MI, Ortiz V et al (2016) Beauveria bassiana and Metarhizium anisopliae endophytically colonize cassava roots following soil drench inoculation. Biol Control theory Appl Pest Manag 95:40–48. https://doi.org/10.1016/j.biocontrol.2016.01.002

Hajek AE, St. Leger RJ (1994) Interactions between fungal pathogens and insect hosts. Annu Rev Entomol 39:293–322. https://doi.org/10.1146/annurev.en.39.010194.001453

Hancock PA (2009) Combining fungal biopesticides and insecticide-treated bednets to enhance malaria control. PLoS Comput Biol 5:e1000525–e1000525. https://doi.org/10.1371/journal.pcbi.1000525

Hu S, Bidochka MJ (2019) Root colonization by endophytic insect-pathogenic fungi. J Appl Microbiol. https://doi.org/10.1111/jam.14503

Hussain A (2018) Reprogramming the virulence: insect defense molecules navigating the epigenetic landscape of Metarhizium robertsii. Virulence 9:447–449. https://doi.org/10.1080/21505594.2017.1421828

Indriyanti DR, Widiyaningrum P, Haryuni H et al (2017) Effectiveness of Metarhizium anisopliae and entomopathogenic nematodes to control Oryctes rhinoceros larvae in the rainy season. Pakistan J Biol Sci 20:320–327. https://doi.org/10.3923/pjbs.2017.320.327

Iwanicki NSA, Pereira AA, Botelho ABRZ et al (2019) Monitoring of the field application of Metarhizium anisopliae in Brazil revealed high molecular diversity of Metarhizium spp in insects, soil and sugarcane roots. Sci Rep 9:4443. https://doi.org/10.1038/s41598-019-38594-8

Jaber LR (2018) Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by Fusarium culmorum in wheat. Planta 248:1525–1535. https://doi.org/10.1007/s00425-018-2991-x

Jaber LR, Enkerli J (2017) Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Sci Technol 27:28–41. https://doi.org/10.1080/09583157.2016.1243227

James PJ, Kershaw MJ, Reynolds SE, Charnley AK (1993) Inhibition of desert locust (Schistocerca gregaria) Malpighian tubule fluid secretion by destruxins, cyclic peptide toxins from the insect pathogenic fungus Metarhizium anisopliae. J Insect Physiol 39:797–804. https://doi.org/10.1016/0022-1910(93)90056-W

Kamareddine L (2012) The biological control of the malaria vector. Toxins (Basel) 4:748–767. https://doi.org/10.3390/toxins4090748

Kanga LHB, Adamczyk J, Patt J et al (2010) Development of a user-friendly delivery method for the fungus Metarhizium anisopliae to control the ectoparasitic mite Varroa destructor in honey bee, *Apis mellifera*, colonies. Exp Appl Acarol 52:327–342. https://doi.org/10.1007/s10493-010-9369-5

Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. Trends Plant Sci 10:22–29. https://doi.org/10.1016/j.tplants.2004.12.003

Kershaw MJ, Talbot NJ (2009) Genome-wide functional analysis reveals that infection-associated fungal autophagy is necessary for rice blast disease. Proc Natl Acad Sci 106(37):15967–15972

Kirkland BH, Westwood GS, Keyhani NO (2004) Pathogenicity of Entomopathogenic Fungi Beauveria bassiana and Metarhizium anisopliae to Ixodidae tick species Dermacentor variabilis, Rhipicephalus sanguineus, and Ixodes scapularis. J Med Entomol 41:705–711. https://doi.org/10.1603/0022-2585-41.4.705

Krell V, Unger S, Jakobs-Schoenwandt D, Patel AV (2018) Endophytic Metarhizium brunneum mitigates nutrient deficits in potato and improves plant productivity and vitality. Fungal Ecol 34:43–49. https://doi.org/10.1016/J.FUNECO.2018.04.002

Kryukov VY, Kabilov MR, Smirnova N et al (2019) Bacterial decomposition of insects post-metarhizium infection, possible influence on plant growth. Fungal Biol 123(12):927–935. https://doi.org/10.1016/J.FUNBIO.2019.09.012

Kurtti TJ, Keyhani NO (2008) Intracellular infection of tick cell lines by the entomopathogenic fungus Metarhizium anisopliae. Microbiology 154:1700–1709. https://doi.org/10.1099/mic.0.2008/016667-0

Lacey CM, Lacey LA, Roberts DR (1988) Route of invasion and histopathology of Metarhizium anisopliae in Culex quinquefasciatus. J Invertebr Pathol 52:108–118. https://doi.org/10.1016/0022-2011(88)90109-7

Lavine MD, Strand MR (2002) Insect hemocytes and their role in immunity. Insect Biochem Mol Biol 32:1295–1309. https://doi.org/10.1016/s0965-1748(02)00092-9

Leger RJS, Goettel M, Roberts DW, Staples RC (1991a) Prepenetration events during infection of host cuticle by Metarhizium anisopliae. J Invertebr Pathol 58:168–179. https://doi.org/10.1016/0022-2011(91)90061-T

Leger RJS, Roberts DW, Staples RC (1991b) A model to explain differentiation of appressoria by germlings of Metarhizium anisopliae. J Invertebr Pathol 57:299–310. https://doi.org/10.1016/0022-2011(91)90134-C

Li L, Ortiz C (2014) Pervasive nanoscale deformation twinning as a catalyst for efficient energy dissipation in a bioceramic armour. Nat Mater 13:501–507. https://doi.org/10.1038/nmat3920

Liao X, O'Brien T, Fang W, St Leger R (2014) The plant beneficial effects of Metarhizium species correlate with their association with roots. Appl Microbiol Biotechnol 98. https://doi.org/10.1007/s00253-014-5788-2

Liao X, Lovett B, Fang W, St Leger RJ (2017) Metarhizium robertsii produces indole-3-acetic acid, which promotes root growth in Arabidopsis and enhances virulence to insects. Microbiology 163:980–991. https://doi.org/10.1099/mic.0.000494

Liu X-H, Gao H-M, Xu F et al (2012) Autophagy vitalizes the pathogenicity of pathogenic fungi. Autophagy 8:1415–1425. https://doi.org/10.4161/auto.21274

Lovett B, St Leger RJ (2018) Genetically engineering better fungal biopesticides. Pest Manag Sci 74:781–789. https://doi.org/10.1002/ps.4734

Lovett B, Bilgo E, Millogo SA et al (2019) Transgenic Metarhizium rapidly kills mosquitoes in a malaria-endemic region of Burkina Faso. Science 364:894–897. https://doi.org/10.1126/science.aaw8737

Lwetoijera DW, Sumaye RD, Madumla EP et al (2010) An extra-domiciliary method of delivering entomopathogenic fungus, Metharizium anisopliae IP 46 for controlling adult populations of the malaria vector, Anopheles arabiensis. Parasit Vectors 3:18. https://doi.org/10.1186/1756-3305-3-18

Mantzoukas S, Chondrogiannis C, Grammatikopoulos G (2015) Effects of three endophytic entomopathogens on sweet sorghum and on the larvae of the stalk borer Sesamia nonagrioides. Entomol Exp Appl 154:78–87. https://doi.org/10.1111/eea.12262

Mkiga AM, Mohamed SA, du Plessis H et al (2020) Metarhizium anisopliae and Beauveria bassiana: pathogenicity, horizontal transmission, and their effects on reproductive potential of Thaumatotibia leucotreta (Lepidoptera: Tortricidae). J Econ Entomol 113(2):660–668. https://doi.org/10.1093/jee/toz342

Mnyone LL, Kirby MJ, Mpingwa MW et al (2011) Infection of Anopheles gambiae mosquitoes with entomopathogenic fungi: effect of host age and blood-feeding status. Parasitol Res 108:317–322. https://doi.org/10.1007/s00436-010-2064-y

Mnyone LL, Lyimo IN, Lwetoijera DW et al (2012) Exploiting the behaviour of wild malaria vectors to achieve high infection with fungal biocontrol agents. Malar J 11:87. https://doi.org/10.1186/1475-2875-11-87

Mondal S, Baksi S, Koris A, Vatai G (2016) Journey of enzymes in entomopathogenic fungi. Pacific Sci Rev A Nat Sci Eng 18:85–99. https://doi.org/10.1016/j.psra.2016.10.001

Money NP (2016) Chapter 3 - Spore production, discharge, and dispersal. In: Watkinson SC, Boddy L, Money NP (eds) The fungi, 3rd edn. Academic Press, Boston, pp 67–97

Moonjely S, Barelli L, Bidochka MJ (2016) Insect pathogenic fungi as endophytes. Adv Genet. https://doi.org/10.1016/bs.adgen.2015.12.004

Moonjely S, Zhang X, Fang W, Bidochka MJ (2019) Metarhizium robertsii ammonium permeases (MepC and Mep2) contribute to rhizoplane colonization and modulates the transfer of insect derived nitrogen to plants. PLoS One 14:e0223718. https://doi.org/10.1371/journal.pone.0223718

Moore D, Bateman RP, Carey M, Prior C (1995) Long-term storage of Metarhizium flavoviride conidia in oil formulations for the control of locusts and grasshoppers. Biocontrol Sci Technol 5:193–200. https://doi.org/10.1080/09583159550039918

Muerrle TM, Neumann P, Dames JF et al (2006) Susceptibility of adult Aethina tumida (Coleoptera: Nitidulidae) to entomopathogenic fungi. J Econ Entomol 99:1–6. https://doi.org/10.1093/jee/99.1.1

Mukherjee K, Vilcinskas A (2018) The entomopathogenic fungus Metarhizium robertsii communicates with the insect host galleria mellonella during infection. Virulence 9(1):402–413. https://doi.org/10.1080/21505594.2017.1405190

Narladkar BW, Shivpuje PR, Harke PC (2015) Fungal biological control agents for integrated management of Culicoides spp. (Diptera: Ceratopogonidae) of livestock. Vet World 8:156–163. https://doi.org/10.14202/vetworld.2015.156-163

Okumu F, Biswaro L, Mbeleyela E et al (2010) Using nylon strips to dispense mosquito attractants for sampling the malaria vector Anopheles gambiae s.s. J Med Entomol 47:274–282. https://doi.org/10.1603/me09114

Ondiaka SN, Masinde EW, Koenraadt CJM et al (2015) Effects of fungal infection on feeding and survival of Anopheles gambiae (Diptera: Culicidae) on plant sugars. Parasit Vectors 8:35. https://doi.org/10.1186/s13071-015-0654-3

Onsongo SK, Gichimu BM, Akutse KS et al (2019) Performance of three isolates of Metarhizium Anisopliae and their virulence against Zeugodacus Cucurbitae under different temperature

regimes, with global extrapolation of their efficiency. Insects 10:270. https://doi.org/10.3390/insects10090270

Pandey V, Bhatt ID, Nandi SK (2019) Chapter 20 - Role and regulation of auxin signaling in abiotic stress tolerance. In: MIR K, Reddy PS, Ferrante A, Khan N (eds) Plant signaling molecules. Woodhead Publishing, Sawston, pp 319–331

Pendeville H, Carpino N, Marine J-C, Takahashi Y, Muller M, Martial JA, Cleveland JL (2001) The ornithine decarboxylase gene is essential for cell survival during early murine development. Mol Cell Biol 21(19):6549–6558

Peng G, Xia Y (2015) Integration of an insecticidal scorpion toxin (BjαIT) gene into Metarhizium acridum enhances fungal virulence towards Locusta migratoria manilensis. Pest Manag Sci 71:58–64. https://doi.org/10.1002/ps.3762

Peng G, Jin K, Liu Y, Xia Y (2015) Enhancing the utilization of host trehalose by fungal trehalase improves the virulence of fungal insecticide. Appl Microbiol Biotechnol 99:8611–8618. https://doi.org/10.1007/s00253-015-6767-y

Pryor MGM (1940) On the hardening of the cuticle of insects. Proc R Soc Lond Ser B Biol Sci 128:393–407

Pulido JM, Guerrero IP, Martínez IJM, Valadez BC, Guzman JCT, Solis ES, Corona JFG, Schrank A, Bremont FJ, Hernandez AG (2011) Isolation, characterization and expression analysis of the ornithine decarboxylase gene (ODC1) of the entomopathogenic fungus, Metarhizium anisopliae. Microbiol Res 166(6):494–507

Rasgon JL (2011) Using infections to fight infections: paratransgenic fungi can block malaria transmission in mosquitoes. Future Microbiol 6:851–853. https://doi.org/10.2217/fmb.11.71

Raya-Diaz S, Sanchez-Rodriguez AR, Segura-Fernandez JM et al (2017) Entomopathogenic fungi-based mechanisms for improved Fe nutrition in sorghum plants grown on calcareous substrates. PLoS One 12:e0185903. https://doi.org/10.1371/journal.pone.0185903

Russell PJ, Hertz PE, McMillan B (2016) Biology: the dynamic science. Cengage Learning, Belmont, CA

Sánchez-Rodríguez AR, Barrón V, Del Campillo MC, Quesada-Moraga E (2016) The entomopathogenic fungus Metarhizium brunneum: a tool for alleviating Fe chlorosis. Plant Soil 406:295–310. https://doi.org/10.1007/s11104-016-2887-0

Sandhu SS, Sharma AK, Beniwal V et al (2012) Myco-biocontrol of insect pests: factors involved, mechanism, and regulation. J Pathog 2012:126819. https://doi.org/10.1155/2012/126819

Santi L, Beys da Silva WO, Berger M et al (2010) Conidial surface proteins of Metarhizium anisopliae: source of activities related with toxic effects, host penetration and pathogenesis. Toxicon 55:874–880. https://doi.org/10.1016/j.toxicon.2009.12.012

Sasan RK, Bidochka MJ (2012) The insect-pathogenic fungus Metarhizium robertsii (Clavicipitaceae) is also an endophyte that stimulates plant root development. Am J Bot 99:101–107. https://doi.org/10.3732/ajb.1100136

Scholte E-J, Knols BGJ, Samson RA, Takken W (2004) Entomopathogenic fungi for mosquito control: a review. J Insect Sci 4:19. https://doi.org/10.1093/jis/4.1.19

Scholte E-J, Takken W, Knols BGJ (2007) Infection of adult Aedes aegypti and Ae. albopictus mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. Acta Trop 102:151–158. https://doi.org/10.1016/j.actatropica.2007.04.011

Screen S, Bailey A, Charnley K, Cooper R, Clarkson J (1997) Carbon regulation of the cuticle-degrading enzyme PR1 from Metarhizium anisopliae may involve a trans-acting DNA-binding protein CRR1, a functional equivalent of the Aspergillus nidulans CREA protein. Curr Genet 31 (6):511–518

Senthil Kumar CM, Jacob TK, Devasahayam S et al (2018) Multifarious plant growth promotion by an entomopathogenic fungus Lecanicillium psalliotae. Microbiol Res 207:153–160. https://doi.org/10.1016/J.MICRES.2017.11.017

Shukla E, Thorat LJ, Nath BB, Gaikwad SM (2015) Insect trehalase: Physiological significance and potential applications. Glycobiology 25(4):357–367

Sinha KK, Choudhary AK, Kumari P (2016) Chapter 15 - Entomopathogenic fungi. In: Omkar BT (ed) Ecofriendly pest management for food security. Academic Press, Cambridge, MA, pp 475–505

Sookar P, Bhagwant S, Allymamod MN (2014) Effect of *Metarhizium anisopliae* on the fertility and fecundity of two species of fruit flies and horizontal transmission of Mycotic infection. J Insect Sci 14:1–12

Sowers AD, Young SP, Grosell M, Browdy CL, Tomasso JR (2006) Hemolymph osmolality and cation concentrations in Litopenaeus vannamei during exposure to artificial sea salt or a mixed-ion solution: relationship to potassium flux. Comp Biochem Physiol A Mol Integr Physiol 145 (2):176–180

St Leger R, Joshi L, Bidochka MJ, Roberts DW (1996) Construction of an improved mycoinsecticide overexpressing a toxic protease. Proc Natl Acad Sci USA 93:6349–6354. https://doi.org/10.1073/pnas.93.13.6349

Sun J, Fuxa JR, Henderson G (2002) Sporulation of Metarhizium anisopliae and Beauveria bassiana on Coptotermes formosanus and in vitro. J Invertebr Pathol 81:78–85. https://doi.org/10.1016/s0022-2011(02)00152-0

Sunde M, Kwan AHY, Templeton MD et al (2008) Structural analysis of hydrophobins. Micron 39:773–784. https://doi.org/10.1016/j.micron.2007.08.003

Theopold U, Schmidt O, Söderhäll K, Dushay MS (2004) Coagulation in arthropods: defence, wound closure and healing. Trends Immunol 25:289–294. https://doi.org/10.1016/j.it.2004.03.004

Thines E, Weber RW, Talbot NJ (2000) MAP kinase and protein kinase A: dependent mobilization of triacylglycerol and glycogen during appressorium turgor generation by Magnaporthe grisea. Plant Cell 12:1703–1718. https://doi.org/10.1105/tpc.12.9.1703

Toei M, Saum R, Forgac M (2010) Regulation and isoform function of the V-ATPases. Biochemistry 49:4715–4723. https://doi.org/10.1021/bi100397s

Wamiti LG, Khamis FM, Abd-Alla AMM et al (2018) Metarhizium anisopliae infection reduces Trypanosoma congolense reproduction in Glossina fuscipes fuscipes and its ability to acquire or transmit the parasite. BMC Microbiol 18:142. https://doi.org/10.1186/s12866-018-1277-6

Wang C, St Leger RJ (2007) The Metarhizium anisopliae Perilipin homolog MPL1 regulates lipid metabolism, appressorial turgor pressure, and virulence. J Biol Chem 282:21110–21115. https://doi.org/10.1074/jbc.M609592200

Wang C, Duan Z, St. Leger RJ (2008) MOS1 Osmosensor of Metarhizium anisopliae Is Required for Adaptation to Insect Host Hemolymph. Eukaryot Cell 7(2):302–309

Wang S, Fang W, Wang C, St Leger RJ (2011) Insertion of an esterase gene into a specific locust pathogen (Metarhizium acridum) enables it to infect caterpillars. PLoS Pathog 7:e1002097–e1002097. https://doi.org/10.1371/journal.ppat.1002097

Wang Y, Wang T, Qiao L, Zhu J, Fan J, Zhang T, Wang Z-X, Li W, Chen A, Huang B (2017a) DNA methyltransferases contribute to the fungal development, stress tolerance and virulence of the entomopathogenic fungus Metarhizium robertsii. Appl Microbiol Biotechnol 101 (10):4215–4226. https://doi.org/10.1007/s00253-017-8197-5

Wang W, Shi J, Xie Q et al (2017b) Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Mol Plant 10:1147–1158. https://doi.org/10.1016/J.MOLP.2017.07.012

Wu C, Zhang X, Fang W (2019) Increasing pyruvate concentration enhances conidial thermotolerance in the entomopathogenic fungus Metarhizium robertsii. Front Microbiol 10:519. https://doi.org/10.3389/fmicb.2019.00519

Wyatt GR (1961) The Biochemistry of Insect Hemolymph. Annu Rev Entomol 6(1):75–102

Xia Y, Clarkson JM, Charnley AK (2002) Trehalose-hydrolysing enzymes of Metarhizium anisopliae and their role in pathogenesis of the tobacco hornworm, Manduca sexta. J Invertebr Pathol 80(3):139–147

Yamamoto K, Ohmae M, Orihara T (2019) Metarhizium brachyspermum sp. nov. (Clavicipitaceae), a new species parasitic on Elateridae from Japan. Mycoscience 61(1): 37–42. https://doi.org/10.1016/J.MYC.2019.09.001

Yousef M, Alba-Ramírez C, Garrido Jurado I et al (2018) Metarhizium brunneum (Ascomycota; Hypocreales) treatments targeting olive fly in the soil for sustainable crop production. Front Plant Sci 9:1. https://doi.org/10.3389/fpls.2018.00001

Zhang W, Chen J, Keyhani NO et al (2015) Comparative transcriptomic analysis of immune responses of the migratory locust, Locusta migratoria, to challenge by the fungal insect pathogen, Metarhizium acridum. BMC Genomics 16:867. https://doi.org/10.1186/s12864-015-2089-9

Zhang J, Huang W, Yuan C et al (2017) Prophenoloxidase-mediated ex vivo immunity to delay fungal infection after insect Ecdysis. Front Immunol 8:1445. https://doi.org/10.3389/fimmu.2017.01445

Zhang J, Jiang H, Du Y et al (2019) Members of chitin synthase family in Metarhizium acridum differentially affect fungal growth, stress tolerances, cell wall integrity and virulence. PLoS Pathog 15:e1007964–e1007964. https://doi.org/10.1371/journal.ppat.1007964

Zhou R, Zhou X, Fan A, Wang Z, Huang B (2018) Differential functions of two metalloproteases, Mrmep1 and Mrmep2, in growth, sporulation, cell wall integrity, and virulence in the filamentous fungus Metarhizium robertsii. Front Microbiol 9:1528