Fungal entomopathogens: a systematic review

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Abstract

Background: Apprehensions about the safety and the environment regarding the insecticidal application against insect infestations have directed our attention toward advancement of biological mediators so that they are assimilated into the concept of integrated pest management stratagems to develop a more practical approach for the management of insect pests. Management of insect pests by making use of biological approaches (such as fungal entomopathogens (EPF) or others which are antagonistic to insect population) provides a substitute approach which reduces the continuous use of chemical amalgams against insect pests.

Main body: The present review provides a framework of the present status of information on EPF as it identifies with their current use as biological control of pest infestations. To utilize a variety of biological control methodologies against insect hosts, it is essential to improve our comprehension of the ecology of EPF and also their role in nature. This article may assist us with understanding the virulence and the virulence factors related with EPF and present the latest developments and accomplishments in the significant field. We focus on recent instances of studies that show the overall patterns in interactions among insect pests and EPF prompting the advancement of epizootics. Also, we sum up the topical discoveries on current status of mycoinsecticides and propose future research needs.

Conclusions: As the current mechanism of fungal pathogenesis on insects is moderately slow and needs improvement, there is likewise the requirement for additional comprehension of the interactions among entomopathogens and insect pests so as to grow soundly planned procedures by identifying potential targets and via the improvement of fungal strains for improving the adequacy of these organisms in field applications.

Keywords: Entomopathogenic fungi, Biological control agents, Infection process, Enzymes, Pathogenesis, Host defense, Epizootiology, Mycoinsecticides

Background

Insect pests are regarded as major deterrents which have accounted for an estimated 10.80% crop losses on global scale in the era of post green revolution (Dhaliwal et al. 2015). Also, an estimated loss of global annual crop production corresponding to 18-26% valued at $470 billion has been observed recently (Mantzoukas and Eliopoulos 2020). To reduce these losses, insecticides are employed and, as a result, have become an essential method for suppressing the insect pest infestations due to their abstemiously minimal application effort, high usefulness and expediency (Sharma 2019), but concentrated application of chemicals has brought about the advancement of resistance to either one or even more classes of insecticides in as much as 500 species of pests (Kumar and Kalita 2017).

As a result, the microbial agents (entomopathogens) are viewed as regulatory operators of pest infestations and represent the different species of fungi, viruses, protozoa, and bacteria. Initial studies in reference to entomopathogens were carried out by Agostino Bassi who established that the infectious agent, instigating the
occurrence of muscardine disease in case of silkworms was *Beauveria bassiana* Bals. (Hypocreales: Cordycipitaceae). Entomopathogens which befall naturally are significant controlling factors against insect populace (Roy and Cottrell 2008). Tanzini et al. (2001) used the term entomopathogenic for the micro-organisms which regulate the population of insect pests to the levels wherein no economic damage to crop plants is observed. Delgado and Murcia (2011) defined the term in relation to the microbial populace which is proficient in attacking insect pests by incorporating them into their own life cycle and by utilizing them as hosts and also classified these micro-organisms as either facultative or obligate parasites attacking insect pests, having high potential for survival.

Use of microbial population as control agents is not only effective against insect pests but this approach is also environmentally safe and sound for humans (reduced pesticide residues) as well as the non-target organisms. The provocation for the impression of using microbial insect pathogens against insect pests resulted from close examination of the disease of silkworm (Audoin 1837). Afterward, the recommendation to assign microbial insect pathogens against insects came from LeConte (1874) and Pasteur (1874). The first efficacious mass-produced microbial control application on large scale was carried out by Krassiltschik (1888) against *Bothynoderes punctiventris* (Germar) (Coleoptera: Curculionidae) (sugar beet weevil) by making use of the antagonistic nature of the conidiospores of *Metarhizium anisopliae* (Metcninkoff) (Hypocreales: Clavicipitaceae) in Russia. The credit for examining infectious nature of *M. anisopliae* must be attributed to Elie Metchnikoff, who initially identified the microbial agent as *Entomophthora anisopliae* against wheat cockchafer, *Anisoplia austriaca* (Herbst) (Coleoptera: Scarabaeidae).

Out of all the other microbial control agent, EPF are the most imperative due to accumulation of various factors, such as easy distribution, easy manufacturing techniques, availability of large number of already identified strains, and over-expression of exogenous toxins and endogenous proteins (St Leger and Wang 2010). EPF legitimately influence plants more than the most compound pesticides. For instance, endophytic EPF have been archived in many plants, for example, soybeans, wheat, tomatoes, and bananas (Jaber 2015). As per the action mechanism of EPF is concerned, they release spores thereby infecting the body of the insect host. The fungal spores initially propagate on the exterior of host body and later penetrate the host. As a result, death of the insect is inevitable within 4-7 days (conditional on the quantity of spores). Cadaver of the insect serves as the origin of new spores, which further disseminate, and thus, the life cycle of entomopathogenic fungi is continued on new hosts.

EPF also play an important role as either colonizer in rhizosphere (Pava-Ripoll et al. 2011) or plant growth promoters (Jaber and Enkerli 2017). EPF account for the principal number of taxa as in the diverse group of fungi, there are more than 100 genera of EPF comprising of 750-1000 (St Leger and Wang 2010). The multi-layered jobs played by EPF could likewise be utilized in a roundabout way as biofertilizers (Jaber and Enkerli 2017), and microbial control specialists in contradiction of both pests and plant infections (Mantzoukas and Grammatikopoulos 2019).

**Main text**

**Biological control of insects**

Biological control of insect pests with EPF is one of the most desirable and effectual practice involving uses of natural microorganisms, which hinder their activity and can be used as an alternative to the chemical insecticides. There have been some genera of EPF, which are antagonistic toward field, greenhouse, forest, storage, and household pests. These can be incorporated as EPF, for instance *Beauveria, Metarhizium, Isaria, Lecanicillium,* and *Hirsutella.* The different species belonging to these genera are target specific and cause infection in many insects. EPF have several biological attributes, such as target-specificity, high reproductively, short generation time, and long survival, which play significant roles in biocontrol of insect pest.

EPF species are mostly isolated from the soil, which protects them from the damaging solar radiation (Meyling and Eilenberg 2007). It has been noted that certain species of *Beauveria* and *Metarhizium* can infect and kill insects in soil and also EPF interact with roots of plant for their growth and survival which predominately relies on insects for carbon and not on soil (Inglis et al. 2001).

Endophytic EPF evolve inside the above ground plant tissues and do not produce any perceptible symptoms of infection. Their usage provides numerous advantages consisting of high yield, cost-effectiveness, preservation of beneficial organisms, safe to humans, no harmful effect on environment, and varied biodiversity (Mantzoukas and Eliopoulos 2020). The most commonly applied and naturally occurring EPF endophyte species are *Beauveria bassiana,* *Isaria fumosorosea* Wize (Hypocreales: Clavicipitaceae), and *Metarhizium anisopliae* (Akutse et al. 2013). The endophytic EPF gives protection against numerous pests to host plant and aids to ameliorate plant response through production of compounds and inducing systemic resistance in the host plant. The endophytic EPF and host plant upon colonization secrete several types of chemicals such as secondary metabolites and enzymes. The secondary
plant metabolites (alkaloids, flavonoids, phenolics, etc.) are produced by the host plant as defense against fungal pathogens, and endophytic EPF produce secondary plant metabolites (benzopyranones, phenolic acids, quinones, and steroids) and enzymes (β-1,3-glucanases, chitinases, amylases, laccases, and cellulases) which helps in the interaction between endophyte and plant host (Zaynab et al. 2018).

Historical evidences show the application and efficacy of EPF have been many as the EPF M. anisopliae var acridum has been applied as spray suspension in Africa to control the locusts (Langewald and Kooyman 2007). Spray preparation of hydrophobic conidia was articulated in oil or as wettable agent, and hydrophilic blastospores were formulated as wettable powders. The solar radiation affects the persistence of fungal propagules and pores were formulated as wettable agent, and hydrophilic blastospores were formulated as wettable powders. The solar radiation affects the persistence of fungal propagules and their effectiveness can be improved by solar blockers (Inglis et al. 2001). Conidia of M. anisopliae were used on seeds of corn before planting to reduce the damage of wireworms and this increased fresh weight and density of corn (Kabaluk and Ericsson 2007).

**Pest control by EPF (approaches)**

Biological control is regarded as a pleasing technique for regulating insects, due to its insignificant environmental impact and inhibiting the development of resistance in vectors. EPF can be employed under three broad biological control approaches, *i.e.*, classical biological control, augmentation, and conservation, by making use of living organisms to suppress the insect population and making it less abundant or less damaging (Hajek 2004).

i. **Classical biological control.** Classical biological control has been a notable approach that includes the use of a biological control agent to manage the insect pest population. The introduction of an exotic biological control agent for the permanent establishment and long-term sustainable and economical pest control in the new location. The introduction of the Vidalia beetle, Rodolia cardinalis (Mulsant) (Coleoptera: Coccinellidae), from Australia to California in the late 1880s to control the scale insect, Icerya purchasi Maskell (Hemiptera: Margarodidae), is the successful example of classical biological control and this approach cannot be better understood without this historical dimension (Abdelghany 2018). The classical biological control needs strong, regional co-ordination of the efforts for the successful management of insect-pests. There are few examples of classical biological control with EPF such as Entomophthora mainaiga Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae), and Zoophthora radicans (Bre-feld) (Entomophthorales: Entomophthoraceae) (Shah and Pell 2003). The gypsy moth, Lymantria dispar Linnaeus (Lepidoptera: Erebidae), accidentally introduced in the USA during the 1860s, and E. mai-maiga introduced from Japan in the 1900s by placing infected cadavers onto tree trunks and able to control larvae of Gypsy moth which feed on the leaves of many trees (Hajek et al. 2004). The fungal movement of E. mai-maiga can take place through air-borne conidia (Shah and Pell 2003). Z. radicans were imported from Israel to release in Australia during 1970 to control spotted alfalfa aphid, Therioaphis trifolii f. maculata (Buckton) (Hemiptera: Aphididae), a serious pest of legume plants.

ii. **Augmentation.** The biological control agents of insect-pests are present in their indigenous pest populations in various circumstances. The natural enemies are either few or active late to restrict the crop damage. There are two strategies of augmentation: inoculation biological control and inundation biological control (Shah and Pell 2003).

a. **Inoculation biological control.** The intentional release of a minute amount of fungal biological control agents so that it will multiply and control the pest for an extended period and sustain the pest population below the economic threshold level. The natural enemies are inoculated in small to moderate amounts in the early season of the crop, increase the number of biological control agents, and spread over a period of time before the insect-pest population reaches the maximum potential. The natural enemies are not able to control the pest population permanently at a high population density. After a regular interval of time, the new inoculations must be made for reestablishment because the control is not achieved from released natural enemies (Abdelghan 2018). Soil can be inoculated with mycorrhiza to intensify growth and to advance up a natural process. Beauveria brongniartii inoculated on Barley seeds to produce mycelium and aerial conidia for the control of European cockchafer, Melolontha melolontha Linnaeus (Coleoptera: Scarabaeidae) in Central Europe (Keller et al. 1999). The consolidated use of Zoophthora radicans Batko (Entomophthorales: Entomophthoraceae) and semiochemicals for the control of Diamondback moth, Plutella xylostella Linnaeus (Lepidoptera: Plutellidae) which is a significant pest of Brassica (Furlong and Pell 2001).

b. **Inundation biological control.** Inundation biological control includes fungal biological control agent to control the population density of a nasty
pest. Inundative augmentation involves the release of a massive amount of natural enemy for accelerated short-term control of the insect-pest population (Liu and Li 2004). The control of the pest population is accomplished by the released natural enemies themselves. The pest population is controlled immediately, and the population of both pest and natural enemies diminishes with time. The fungus is employed as a chemical pesticide and different terms are used, i.e., mycospeticide, mycoinsecticide, and biopesticide have been associated with inundation biological control (Abdelghany 2018). 

Conservation biological control. Conservation biological control concerns with the modification of the environment or farming practices to defend and intensify specific entomopathogens to lessen the effect of pests. This approach accommodates to distinguish effective indigenous biological control agents and the selection of practices to conserve and raise their population. The practices which prefer fungal biological control agent may include irrigation and reduction in pesticide usage. The EPF increase in population size and their effective results in a low pest population. Natural enemies include all types of biological regulation: macro-and microorganisms controlling invertebrates, weeds, and plant diseases, including the antagonistic microorganisms responsible for suppressive soils. The conservation biological control approach is correlated to the chief principle of organic farming, which has the protection of the present natural enemies (Shah and Pell 2003 and Abdelghany 2018).

Classification of entomopathogenic fungi

The classification of fungal organisms has generally relied on their respective ultrastructure (e.g., structure of septum and that of the cell wall) and their morphology (e.g., mechanism of conidiogenesis) as essential standards. The more exact arrangement of EPF into characterized scientific taxa has been accomplished by the examination of their digestive systems (e.g., production of toxins and enzymes and utilization of nutrients) and utilizing genomic analysis (e.g., mtDNA restriction length polymorphism, karyotyping, and rRNA sequences (Khachatourians 1991).

Fungi (true) are presently positioned in four legitimate phyla (Basidiomycota, Ascomycota, Zygomycota, and Chytridioymycota). An artificial phylum, Deuteromycota is also documented comprising of filamentous fungi which exist in anamorphic forms (asexual). It is alleged that the affiliates of this phylum are in fact either basidiomycetes or ascomycetes which have lost their ability to multiply sexually or their sexual structures are not yet described (Alexopoulos et al. 1996). In addition, teleomorphs for various genera of deuteromycete (entomopathogen) have along these lines been recognized, and a portion of these teleomorphs can likewise be in fact entomopathogenic (e.g., Cordyceps and Torrubiella spp. are identified teleomorphs of Paecilomyces spp. (Samson et al. 1988)).

The oomycetes, once positioned in Mastigomycotina, have now been set in the kingdom of Stramenopila, phylum Oomycota (Barr, 1992). Individuals from this kingdom offer different highlights of monophyletic origin, including a comparative flagellum hair structure and incorporate labyrinthulids, diatoms, and algae (Patterson 1989). The phylogenetic connection among the four phyla of fungi has been proposed, in view of on ultrastructural and morphological highlights, and to a great extent affirmed by resulting analyses of DNA sequence (Alexopoulos et al. 1996) as stated earlier.

Chytridioymycetes might be well-thought-out as the most “crude” organisms since the predecessor of this group diverged primarily and retained flagella and centrioles (Barr, 1992). The zygomycetes diverged before the occurrence of a dikaryotic stage and consistently septate mycelium, which are normal for the basidiomycetes and ascomycetes and the flagella was lost eventually. After the progenitors of basidiomycetes and ascomycetes veered, the basidiomycetes’ predecessor developed basidiospores (meiospores ordinarily delivered four for each basidium), in addition to clamp connections and dolipore septa (barrel shaped) and the ascomycetes’ progenitors developed ascospores (i.e., meiospores for the most part delivered eight for every ascus). The scientific classification of the Ascomycota is all the more plainly characterized and contains two significant orders: Laboulbeniales (Class: Laboulbeniomycetes) and Hypocreales (Class: Sordariomycetes; subclass: Hypocreomycetidae) (Hibbett et al. 2007).

Hibbett et al. (2007) suggested an arrangement of characterization for all fungal groups dependent on molecular phylogenetic investigations. As stated in the background section, there are 750-1000 EPF, these can
be found primarily placed in two fundamental groups: phylum Zygomycota, subphylum Entomophthoromycotina, order Entomophthorales, and phylum Ascomycota (subkingdom Dikarya). Nonetheless, the Zygomycota is not an acknowledged phylum inside the re-examined classification. Hence, the Entomophthorales have not been allotted a phylum in the current arrangement. Most of EPF recognized to date have been positioned in the following classes: Zygomyctes (phylum Zygomycota), Hyphomycetes (phylum Deuteromycota), and Pyrenomycetes and Laboulbeniales (phylum Ascomycota) (Samson et al. 1988).

Infection process
EPF face numerous host challenges in each generation to produce adequate fresh infectious spores to sustain viable populations. The prosperous transmission usually needs the discharge of massive spore numbers and glutinous spore surfaces that enhance adhesion (Vega et al. 2012). The spores germinate and directly invade through the hard exoskeleton of the host insect (Vega et al. 2012). The fungi do not necessitate to be ingested. The EPF commonly invade through the mouthparts or spiracles because of non-sclerotized integument, and smooth penetration (Clarkson and Charnley 1996). The penetration of the epicuticle is directly by germ tubes or infection pegs originated from the underneath of appressoria (Zacharuk 1981). The fungal cells propagate inside the hemocoel, tissues, and muscles of the host’s body so that it dies (Vega et al. 2012). The EPF can infect non-feeding stages such as eggs and pupae. The fungal germings are steadily crossing distinct environments and counter to the variations invoking biochemical processes and cellular differentiation to form particular morphological structures. The fungal germings conform to colonize insect tissue and neutralize potential host responses. Infection structures reasonably emerge as a mechanism to surmount host barriers (St Leger and Wang, 2010). The process of infection can be restrained by low humidity conditions because fungus requires high humid conditions for germination and maturity of fungal structures.

The perception of a susceptive host can involve chemical and topographical signals. The cuticle of tobacco hornworm, Manduca sexta Linnaeus (Lepidoptera: Sphingidae), has been used to study the impact of surface topography on appressorium formation. The appressoria developed after extensive germination across the micro folds of the exoskeleton of 1-day fifth-instar larvae. The fungus does not get induction signals from the exoskeleton due to micro folds. Contrastingly, the appressoria germinate close to conidium on 5-day fifth-instar larvae (St Leger and Wang, 2010). The entomopathogen uses the host cadaver for spore production and dispersal under preponderating environmental circumstances (Roy et al. 2006).

Metarhizium spp. and Beauveria spp. belonging to the order Hypocreales are opportunistic hemibiotrophic in nature while fungi belonging to the order Entomophthorales are biotrophic. Metarhizium spp. and Beauveria spp. are entomaphagous that infect living insects and saprophagous that invade dead insect corpse. Entomophthorales exterminate their host by the colonization of tissues (Freimoser et al. 2003). Time between application of entomopathogens and revelation to the activity of various parasitoids is vital for the continued existence of the respective parasitoids (Oreste et al. 2015). The application of EPF in context of the time of application has been found to affect the rate of parasitization by a parasitoid. For instance, the treatments of entomopathogens M. anisopliae and B. bassiana and time of fungal treatment influenced the rate of parasitization by Encarsia formosa Gahan (Hymenoptera: Aphelinidae) against Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae) (Oreste et al. 2015).

Fungal invasion
The process of pathogenesis is initiated by the adhesion of conidia to the insect cuticle. The attachment of fungal propagules to the host exoskeleton is the first and foremost step of the infection process (Sevim et al. 2015). Fungi are heterotrophic organisms that consume the non-chemical compounds provided by different organisms as their chief source of energy. The non-specific adhesion mechanisms involved in binding are controlled by the hydrophobic attributes of the conidial cell wall (Boucias et al. 1988). This process includes interaction among the conidial proteins and the hydrophobic exoskeleton surface of the susceptible insects (Fang et al. 2005). This process happens in three consecutive stages:
1. Adsorption of the spores to the insect exoskeleton
2. Adhesion of pre-germinated spores to the host cuticle
3. Germination and development till appressoria formation (Tellez-Jurado et al. 2009)

The cuticle is a significant impediment to fungal invasion. The insect epicuticle is differentiated into multilayers; the outer epicuticle is mechanically fragile while the inner epicuticle designates toughness, although the enzymes produced by EPF conquer this obstruction (Charnley and St Leger, 1991). The outer epicuticle may be penetrated by an inadequate force and prevent the passage of cuticle-degrading fungal enzymes. Once the epicuticle is passed, penetrant structures may expand laterally propagating penetrant plates. The lateral expansions promote penetration by inducing fractures in the insect cuticle (Brey et al. 1986) and expedite dispersal of the pathogen cuticle-degrading enzymes (Goettel et al.
In addition, insect epicuticular lipids play important role in binding fungus to the host cuticle (Ferron 1978). The procuticle is impermeable to pathogen secretion; the degree of resistance depends on cuticle thickness and hardening. Insects having heavily sclerotized body segments and cuticular melanisation induced by physical damage or β-1,3 glucans on the fungal cell wall (Charnley 1989), is common (Butt et al. 1988). Melanisation support to prevent fast-growing pathogens (St Leger 1991), but evaluating these responses in disease resistance is challenging because of inadequate understanding of the amounts of melanin expected to influence infection and how melanin reactions might prevent fungal germination. The inhibitory compounds on the exoskeleton such as phenols, quinones, and lipids lead to the failure of fungal invasion (Kerwin 1984).

The Beauveria, Metarhizium, and Isaria belonging to order Hypocreales have hydrophobic conidia due to hydrophobins (cysteine-rich proteins) in the cell wall while Verticillium lecanii has hydrophilic conidia (Inglis et al. 2001). The conidia of the order Entomophthorales are large and originate from sporangia. The conidia attach themselves to the cuticle and support the adhesion process (Papierok and Hajek 1997). In addition, Neozygites and Zoophthora spp. exhibit elongated capilloconidia and adhere to the exoskeleton through an adhesive droplet (Glare et al. 1985).

The process of adhesion among the spore and the insect cuticle is mediated by the presence of molecules synthesized by the fungus, called adhesins. In M. anisopliae, MAD1 adhesin-like protein is present on the conidial surface to attach to the host surface, but for adhesion to plant surfaces MAD2 is present (Pava-Ripoll et al. 2011). MAD1 adhesin affects germination and blastospore formation, significantly reducing fungus virulence (Wang and St Leger, 2007). The conidium develops and produces penetration structures under promising conditions of temperature, relative humidity, nutritional, and physical requirements in the cuticle. The penetration structures such as appressoria or germ tubes penetrate the host insect through infection peg (Shah and Pell 2003).

Conidiobolus obscurus (Hall & Dunn), Pandora neoaphidis (Remaud & Hennebert), Entomophthora planchoniana Corru, and Batkoa apiculata (Thaxt.) (Entomophthorales: Entomophthoraceae) belong to order Entomophthorales. These can penetrate the cuticle directly from the germ tube without the formation of appressoria (Hajek and Delalibera 2010). B. bassiana requires carbon and fatty acids for the germination of conidia. In addition, insect epicuticular lipids may help in the germination of conidia by providing energy sources and have antifungal properties (Ferron 1978). The hydrolytic enzymes such as proteases, lipases, and chitinases synthesis help to degrade the cuticle and release nutrients for fungus germination (Franco et al. 2011).

M. anisopliae and B. bassiana spores are hydrophobic in nature so they bind to insect cuticle (Holder and Keyhani 2005). The conidia of M. anisopliae have cuticle-degrading catalysts and the potential to modify the surface of the integument for the attachment. During pre-germination development as the conidium dilates, excretion of adhesive mucus takes place which improves the initial hydrophobic interactions among the conidium and cuticular surface (Boucias and Pendland 1991). M. anisopliae strains are more precise toward the scarabid beetles. The cuticle of scarabids has antifungal compounds such as short-chain fatty acids. The EPF must have the capability to endure antifungal compounds for the successful invasion (Boucias and Pendland 1991). The germ tubes of M. anisopliae develop appressoria at the surface of the cuticle, infection peg in epicuticle, penetrant hyphae in procuticle, and yeast-like hyphal bodies in the hemocoel. The infection structures emerged as a mechanism to overwhelm host barriers.

M. anisopliae var acidum is specific for the locusts. The conidia may be produced in internal air spaces as the corpse shrivels out under unpropitious circumstances (Wang and St Leger, 2005). Once the EPF invades the cuticle comprised of a polysaccharide network and enters into the hemocoel, this induced biophysical or biochemical interruption in the insect which leads to death of the host insect (Charnley 2003). The fungus will burst out through the exoskeleton of the host insect, producing aerial spores following high relative humidity conditions. The unrestricted germination of the fungus may happen on the corpse of the host insect. The life cycle of the EPF gets completed if sporulated on the cadaver of the host (Samson et al. 1988 and Charnley 2003). Under adverse environmental conditions, resting spores produced within the dead insect may facilitate the fungus to persist for long periods (Samson et al. 1988).

**Host response**

After a successful invasion, the EPF proliferate inside the host insect and septicemia occurs. The fungus combats the host-induced restrictions and poisonous compounds additionally; the infection depends on the genetic potential of the pathogen. Structural features such as sclerotization hinder penetration while enzyme inhibitors and tyrosinases generate antimicrobial melanins comprise the frontline defense toward weak pathogens (Gillespie et al. 2000). The protease inhibitors are also present in the hemolymph of infected insects to limit lethal infections. Moreover, the distinct behaviors of the
host insect can defend. Mycosis provokes physiological symptoms of abnormalities in the insect-like lack of coordination, altered behavior, and paralysis. Death results from a succession of effects that involve the physical deterioration of tissues, toxicity, and dehydration of cells by loss of fluids, and consumption of nutrients (Bustillo 2001). The grasshopper Camnula pellucida (Scudder) (Orthoptera: Acrididae) retrieved from the infection of Entomophaga gryllii (Fresenius) (Entomophthoraceae: Entomophthoraceae) by raising internal body temperature (Carruthers et al. 1992). The prominent virulence constituent in M. anisopliae is PRI protease, which reduces the time of death by 25% in Manduca sexta (St Leger et al. 1996). Spores can germinate swiftly in the digestive tract of the insect where the relative humidity is high but digestive fluids degrade germinating hyphae (Charnley 2003).

**Virulence enzymes associated with EPF**

The process of pathogenesis is mediated by mechanical force and the enzymatic process. The EPF require virulence enzymes like proteases, peptidases, chitinases, and lipases for entry and successful growth (Khachatourians and Qazi 2008). The proteases and peptidases help in the degradation of the insect cuticle because insect cuticle is composed of chitin and protein. These also aid in the degradation of saprophytic fungi and activate prophenoloxidase in the hemolymph. The fungi B. bassiana, B. brongniartii, Lagenidium giganteum (Schenk) (Oomycota: Lagenidiales), Nomuraea rileyi (Farl.) Samson (Hypocreales: Clavicipitaceae), M. anisopliae, and V. lecanii have been identified with protein degrading enzymes (Sheng et al. 2006). B. bassiana has subtilisin-like serine endoprotease (Pr1 and Pr1B) and M. anisopliae has chymotrypsin (CHY1) (Screen et al. 2001). St Leger et al. (1996) constructed an engineered mycoinsecticides based on M. anisopliae by over-expressing the Pr1 toxic protease from M. anisopliae genome. This was engineered to enhance the killing speed of M. anisopliae. The Pr1 over-expression activates the phenoloxidase system in the hemolymph of Manduca sexta which causes a 25% reduction in the time of death. The EPF M. anisopliae, B. bassiana, and V. lecanii have grown in culture containing cuticle of locust produce various hydrolytic enzymes which are active against insect cuticle.

The chitinases (endo and exo-chitinases) play important roles in the cleavage of N-acetylglucosamine. The enzyme helps to break the insect cuticle polymer into monomers. The chitinolytic enzymes were present in M. anisopliae, M. flavoviride, and B. bassiana culture supplemented with insect cuticles (St Leger et al. 1996). The enzymatically produced protoplast and cell of M. anisopliae have chitinolytic enzymes. These enzyme activities were cell-bound and located in the membrane fraction (Valadares-Inglis and Peberdy 1997). Bchitl1 encoding gene was present in the B. bassiana genome and its amino acid sequence is similar to endochitinase of Streptomyces avermitilis Kim and Goodfellow, S. coelicolor, and T. harzianum (Fang et al. 2005). The epicuticular layer of the insect is made up of non-polar lipids which would be barriers to entomopathogenic fungi without the action of lipases and lipoxygenases (Khachatourians and Qazi, 2008). The growth of EPF is also inhibited by the presence of saturated fatty acid chains. The cuticular lipids affect the conidial germination of B. bassiana and P. fumosoroseus. Also, the nymphs of silver leaf whitefly, B. argentifolii, produce a thick coating of long-chain wax esters affecting spore germination (Lord et al. 2002).

The destructive effects of these virulence enzymes on cuticle can be attributed to the structure and enzyme accessibility of protein polymers in the cuticle. The manipulation of pathogen enzymes helps to understand the best cuticle structure and its natural degradation. The characterization of enzyme regulation will enable manipulation of enzyme levels with the help of chemical and biotechnological procedure for insect control (Kramer et al. 1988).

**Epizootiology of fungal diseases in insects**

An epizootic of insect fungal diseases is a large number of cases of a disease in a host population. The diseased hosts are usually very abundant during epizootics. Epizootiology of fungal diseases involves the natural history of the disease, phenology of both pathogen and host, impact of the pathogen on host populations, and association of epizootics with weather conditions. The disease development and spread are affected by the host and pathogen populations, the environment, and the impact of human activities. The pathogen having different characteristics includes virulence, dispersal, survival, and inoculum density. The disease incidence depends on the insect age and position in the tree canopy. Abiotic factors such as moisture, temperature, and sunlight may determine whether infection can occur. The moisture helps in the germination and sporulation, and temperature is a limiting factor for disease development. The primary interactions within host-pathogen systems increase, appreciation of community-level influences may aid in understanding, and predicting the development of epizootics. The low-density host populations have less infection, resulting in a host increase (preepizootic phase). Host populations reach high densities, and disease epizootics cause population decline (epizootic phase). The reduced host populations have a high infection, owing to abundant fungi in the environment (post-epizootic phase) (Goettel et al. 2005).

The epizootiology of insect pathogenic fungi on insects includes Entomophthora muscae (Cohn) (Entomophthorales:
Entomophthoraceae) infection on the onion fly, *Delia antiqua* Meigen (Diptera: Anthomyiidae), and *N. rileyi* on *Anticarsia gematalis* Hubner (Lepidoptera: Noctuidae) in soybean (Carruthers and Haynes 1986). The EPF *Erynia radicans* and *Entomophaga aulicae* Batko (Entomophthorales: Entomophthoraceae) produce the highest mortality in spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), which is a major defoliator in balsam and spruce trees (Perry and Whitfield, 1984). *E. grylli* causes high mortality in *C. pellucida* and *Melanoplus bivittatus* Say (Orthoptera: Acrididae) (Pickford and Riegert 1963). Soil is a complex habitat that harbors flora and fauna. *M. anisopliae* is the most frequent mycopathogens of soil insects, particularly of beetles (Keller and Zimmermann 1989). *M. anisopliae* and *B. bassiana* are isolated from temperate soils and have a broad host range. *B. brongniartii* is primarily a pathogen of cockchafer, *Melolontha* spp., and other Scarabidae (Zimmermann 1992). Epizootics have been found on wireworms, *Agrotis* spp., and larvae of *Amphimallon solstitialis* Linnaeus (Coleoptera: Scarabaeidae). On the other hand, under in vitro conditions, various native parasitoids such as *Leptopilina heterotoma* Thomson (Hymenoptera: Figitidae), *Pachycrepodeus vindemiae* Rondani (Hymenoptera: Pteromalidae), and *Trichopria drosophilae* Perkins (Hymenoptera: Diapriidae), have been reported to distinguish and parasitize invasive pest, *Drosophila suzuki* (Matsumura) (Diptera: Drosophilidae) at varying degrees of adequacy (Iboub et al. 2019).

Soil is a complex habitat that harbors flora and fauna. *M. anisopliae* is the most frequent mycopathogens of soil insects, particularly of beetles (Keller and Zimmermann 1989). *M. anisopliae* and *B. bassiana* are isolated from temperate soils and have a broad host range. *B. brongniartii* is primarily a pathogen of cockchafer, *Melolontha* spp., and other Scarabidae (Zimmermann 1992). Epizootics have been found on wireworms, *Agrotis* spp., and larvae of *Amphimallon solstitialis* Linnaeus (Coleoptera: Scarabaeidae). Soil is a reservoir for fungi that infect insects present on aerial parts of plants. EPF persist in the soil as mycelium or pseudosclerotia. The pathogen is reduced by the production of various metabolites, such as antibiotics, bioactive volatile compounds (e.g., ammonia, hydrogen cyanide, alkyl pyrones, alcohols, acids, esters, ketones and lipids) and enzymes. Some other mechanisms are also involved like competition, antibiosis, hypovirulence, parasitism, and induced systemic resistance (Ownley and Windham 2007). The EPF like *B. bassiana*, *Lecanicillium*, and *Metarhizium* are isolated from soil. The soil not only protects EPF from damaging solar radiation but also from the extremes of temperature (Inglis et al. 2001). The plant rhizosphere has free carbon in abundant amount and exploited by saprotrophic microorganisms (Whipps 2001). EPF interact with plant roots for growth or survival (St Leger and Wang, 2010). The ability of EPF has impaired through the antimicrobial metabolites secreted by microbes present in the soil. The *B. bassiana* applied to control Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), but there is low mortality due to increased soil fungistasis levels (Groden and Lockwood 1991). *M. anisopliae* applied as conidia to corn seeds before planting to reduce damage from wireworms, and increased stand density and fresh weight of field corn (Kabaluk and Ericsson 2007). The fungus which infect internal tissues of above ground plant parts without causing symptoms are known as fungal endophytes (Arnold and Lutzoni 2007). The sum of

### Role of EPF in nature

EPF play important role as plant disease antagonists, rhizosphere colonizers, biocontrol agent of insect-pests, plant growth promoting fungi, and fungal endophytes. The use of natural or modified fungi or bacteria that are antagonists of plant pathogens is considered as biological control. The survival or disease-causing activity of a pathogen is reduced by the production of various metabolites, such as antibiotics, bioactive volatile compounds (e.g., ammonia, hydrogen cyanide, alkyl pyrones, alcohols, acids, esters, ketones and lipids) and enzymes. Some other mechanisms are also involved like competition, antibiosis, hypovirulence, parasitism, and induced systemic resistance (Ownley and Windham 2007). The EPF like *B. bassiana*, *Lecanicillium*, and *Metarhizium* are isolated from soil. The soil not only protects EPF from damaging solar radiation but also from the extremes of temperature (Inglis et al. 2001). The plant rhizosphere has free carbon in abundant amount and exploited by saprotrophic microorganisms (Whipps 2001). EPF interact with plant roots for growth or survival (St Leger and Wang, 2010). The ability of EPF has impaired through the antimicrobial metabolites secreted by microbes present in the soil. The *B. bassiana* applied to control Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), but there is low mortality due to increased soil fungistasis levels (Groden and Lockwood 1991). *M. anisopliae* applied as conidia to corn seeds before planting to reduce damage from wireworms, and increased stand density and fresh weight of field corn (Kabaluk and Ericsson 2007). The fungus which infect internal tissues of above ground plant parts without causing symptoms are known as fungal endophytes (Arnold and Lutzoni 2007). The sum of
fungi and fungal endophytes protect host plants against insect pests (Rudgers et al. 2007). Acremonium, Beauveria, Cladosporium, Clonostachys, and Isaria are some of the EPF (Vega et al. 2008).

EPF are also employed in integrated pest management programs as one of the natural enemies against arthropods including insect pests. Nowadays, the usage of fungal pathogens is drawing special attention as a biological control agent of many insect pests and this approach is reliable, cost-effective, and environmentally safe (Wraight et al. 2001). EPF possesses several attributes that make them a potential candidate to be utilized in the IPM program. Some of the fungi including Beauveria, Metarhizium, Isaria, Lecanicillium, Hirsutella, and Entomophthorales are used as entomopathogens. The B. bassiana is effective against lepidopteran, coleopteran, and hemipteran pests. B. bassiana has also been found to be pathogenic in opposition to the larvae of Cappnodis tenebrionis (Linnaeus) (Coleoptera: Buprestidae) in laboratory assays as reported by El Khoury et al. (2020) wherein the mortality/transience of C. tenebrionis ran between 26 and 76%.

Metarhizium anisopliae is effective against scarab beetle grubs, weevils, termites, and cutworms. L. lecanii used against insects belong to order Hemiptera and Thysanoptera. Paecilomyces lilacinus is used against nematodes (Root-knot, cyst, lesion burrowing) (Skinner et al. 2014). These fungal-based products are harmful to specific insect pests (host-specific) and relatively safe to beneficial insects, non-target organisms, and ecosystems. There is no problem of toxic residues on crops as agrochemicals (Laich et al. 1990). The EPF has been developed or is being evaluated as an integral and essential component of IPM or IDM. Parasitoids have likewise been accounted for to go about as vectors of EPF to the hosts during instances of foraging, as reported by Oreste et al. (2016), wherein Encarsia formosa acted as a passive vector to transfer the fungal propagules from tainted to uninfected populations due to the virtue of its oviposition and host handling.

Mycoinsecticides: present scenario
The market of chemical pesticides represents as much as 98% of the worldwide market of crop protection and consequently, the portion of biopesticides is just 2%. (Anon 2005). A more profound investigation of the share of the overall industry of biopesticides has uncovered that mycoinsecticides have contributed for an exceptionally little division of the market of biopesticide, on the grounds that a large share has been accounted by formulations which are based on the bacterium Bacillus thuringiensis. The development of biopesticides for pest control was at first upheld and contributed by multinational agrochemical organizations yet a large portion of them have opted out since 1980s (Charnley 2003). Thus, the market is presently directed by small to medium-sized organizations.

Another explanation behind the small share of the overall industry of the fungi as mycoinsecticides is its moderately slow killing rate and an expansion in share of the market value legitimately corresponds to the killing speed (St Leger and Wang 2010). In spite of the fact that these products have the upside of a confined host range, this particularity is likewise one of the restricting elements for their commercial use (Owney et al. 2004). In this manner, a mycopesticide with a more extensive host extend however with next to zero impact on other natural enemies of useful organisms may have an important commercial advantage in the event that it all the while controls different insect pests and additionally plant infections (Wraight and Carruthers 1999).

However, since the advent of worries over the effect of synthetic compounds, cost of enlisting synthetic concocions for high value crop plants, development of insecticide resistance, and the developing enthusiasm for “organic/natural” food has now guaranteed a business opportunity for biological agents of pest control, thereby incorporating formulations dependent on EPF. EPF have generally been utilized more broadly on forest pests than on pests of crops (Feng 2003).

Mycopesticide formulations depend on a confined number of fungal species, fundamentally Beauveria bassiana, B. brongniartii, Lecanicillium muscariurn, L. longisporum, M. anisopliae, Paecilomyces fumosoroseus, and Verticillium lecanii. About 33.9% of the mycoinsecticidal formulations are based on B. bassiana, trailed by M. anisopliae (33.9%), I. fumosorosea (5.8%), and B. brongniartii (4.1%) (Faria and Wraight 2007). However, to build the share of the overall industry of EPF, the killing speed which is the significant deterrent restricting their utilization as mycoinsecticides ought to be enhanced (St Leger and Wang 2010).

As the natural strains of these EPF are deficient in terms of adequate levels of virulence (Rangel et al. 2005), therefore, manipulation at genetic level is important to improve their viability and ecological wellness (Fang et al. 2005). Also, dual infection by different entomopathogens does not affect the mortality of insects. For instance, according to Tarasco et al. (2011) when contrasted with the values which were noted when the two entomopathogens (entomopathogenic nematode Steinernema ichnusae (Nematoda: Steinernematidae) and EPF B. bassiana) were inoculated individually against Galleria mellonella Fabri-cius (Lepidoptera: Pyralidae), concurrent infection showed neither synergistic nor additive effects.

Future prospects
Future investigations on EPF should zero in on attempting to comprehend the ecosystem of the fungal growths
in a setting that centers on their role as antagonists of plant diseases. Most intriguing future prospect in terms of EPF is the possibility to devise stratagems for strain improvement. Attributes which could be tended to include expanded killing power (reduced LD₉₀), capacity to start disease at low humidity conditions, upgraded timeframe of realistic usability, and environmental steadiness (for example, temperature tolerance, and resistance against UV), augmented kill (reduced LT₅₀), improved sporulation during large-scale manufacturing and extension of the host. Lane et al. (1991) suggested that culture conditions impact the attributes of contagious fungal spores and can be controlled to increment mycoinsecticide effectiveness. For instance, Blastospores of *B. bassiana* from carbon-restricted cultures had lower groupings of starch and lipid and were fundamentally less harmful toward the rice green leafhopper than were the blastospores from nitrogen-restricted cultures.

Direct manipulation at genetic level would give upgraded targeting for single genes or clusters of genes, for example, epizootic capability of *Beauveria* spp. and *Metarhizium* spp. was enhanced by genetic manipulation, thereby enhancing their saprophytic potential by Wang and St Leger (2005). ESTs and cDNA microarrays were utilized to investigate gene expression during development on a plant root exudate. Genetic manipulation needs the foundation of cloning and transformation frameworks, which have been accomplished for *P. fumosoroseus*, *B. bassiana*, and *M. anisopliae* (Lima et al. 2006).

Keeping the agro-ecosystems aside, EPF are also finding the exploit of their activity against the human and animal pests, for example, work on mosquitoes (Blanford et al. 2005) and tsetse flies (Maniania et al. 2003). Investigations on Brassica root flies (*Delia florallis* (Fallen)) (Diptera: Anthomyiidae) and *Delia radicum* Linnaeus (Diptera: Anthomyiidae)) (Eilenberg and Meadow 2003), fire ants (Brinkman and Gardner 2004), and mound building termites (Milner 2003) show the assortment of expected targets for mycoinsecticides. Other potential targets researched for utilization of mycoinsecticides incorporate bee parasite *Varroa destructor* Anderson & Trueman (Arachnida: Varroidae) (Shaw et al. 2002), blowflies (Wright et al. 2004), parasitic mites (Smith et al. 2000), reduviid bugs such as *Triatoma* (Lazzarini et al. 2006) and ticks (Samish et al. 2004).

On the other hand, EPF have potential for control of a certain number of insect pests, and it is imperative to distinguish potential targets for mycoinsecticide development and advancement. For instance, beetles and larval pests are every now and again host to fungal diseases however seem to have similarly not many viral and bacterial pathogens. Subsequently, fungi are frequently the microbes of choice for beetle and bug pests (Samson et al. 1988) and the staggering interest has been investigating the potential for *B. bassiana* and *M. anisopliae*. A few instances of late examinations are the investigations on the rice water weevil, a pest of rice in North and South America (Chen et al. 2005), the Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae), a major insect pest of wheat and barley in West and Central Asia (Parker et al. 2003), and thrips (Ekesi and Maniania 2003).

Investigations into the strain improvement and potential targets for mycoinsecticides will give insights into enhanced disposition of mycoinsecticides for the best pest control and improved development of formulations, detailing to upgrade their adequacy so utilization of the EPF can turn into an essential share of agricultural frameworks as well as the integrated pest management stratagems.

**Conclusion**

In the present review, an attempt is made to sum up the utilization of fungal organisms as biopesticides, the authors endeavor and gather the information about the EPF as biocontrol operators. We gather knowledge on the past and present researches about EPF to investigate approaches to improve their capacities. The action mechanism of infection by the EPF is not only complex but specialized as well. Therefore, there is a need to acquire the knowledge of insect-fungus interaction as the host-pathogen interactions are basic determinants of pathogenicity and epizootic turn of events. Molecular and biochemical examinations of host-pathogen interactions are characterizing those properties yielding expanded pathogenicity and are focused on the management of explicit fungal processes. Examinations at the level of an organism include investigations of the turn of events and activity of different phases of host and pathogen, which are regularly in association with variations in natural conditions of the ecosystem. The utilization of EPF in agro-ecosystems has expanded as of late because of the extraordinary potential they have in the field of pest management, speaking to a productive option in contrast to the utilization of synthetic chemical compounds, which are viewed as exceptionally hurtful to the soundness of man and environment alike. Understanding the components engaged with the process of infection, will permit the development of new organic/biological formulations that are compelling for field use and securing the beneficial species of insects. Be that as it may, there are various limitations on the utilization of EPF as an insecticide, for instance, the potential outcomes of contamination with mycotoxins such as, citrinin, zearalenone, aflatoxins, fumonisins, and trichothecenes delivered by various saprophytic fungi as environmental toxins cannot be precluded. Other
limitations include the facts that not only the application of EPF must coincide with high relative humidity conditions but also there is a need for a period where no chemical fungicide application has taken place. Moreover, the inoculum has short timeframe of realistic usability and the formulation requires 2–3 weeks to kill the insects. Along these lines, novel thoughts and speculation need to rise which will further assist in building up the fungi’s abilities as biocontrol specialists.

Abbreviations
mtDNA: Mitochondrial deoxyribonucleic acid; rRNA: Ribosomal ribonucleic acid; PR1: Pathogenesis related protein-1; IPM: Integrated pest management; IDM: Integrated disease management; LD: Lethal dose; LT: Lethal time; EST: Expressed sequence tag; cDNA: Complementary deoxyribonucleic acid

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Authors’ contributions
Both RS and PS researched and reviewed the existing literature on the topic at hand. RS worked on the “Background,” “Classification of Entomopathogenic Fungi,” “Mycoinsecticides: Present Scenarios,” “Future prospects,” and “Conclusion” sections of the manuscript. PS worked on sections titled “Biological Control of Insects,” “Pest Control by EPF (Approaches),” “Infection process,” “Virulence enzymes associated with EPF,” “Epizootiology of fungal diseases in insects,” and “Role of EPF in Nature.” RS contributed to the critical review and editing of the manuscript. Both authors read and approved the final manuscript.

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