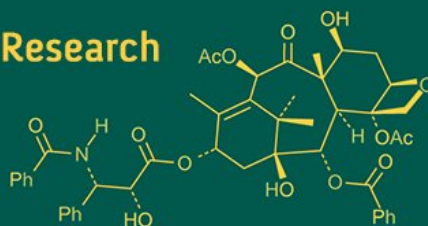


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GP Shetty
 Multiplex Group of Companies,
 Bengaluru, Karnataka, India

A Meghana
 Multiplex Group of Companies,
 Bengaluru, Karnataka, India

Shivaprasad Maranabasari
 College of Agriculture,
 Bengaluru, Karnataka, India

Mahesh G Shetty
 Multiplex Group of Companies,
 Bengaluru, Karnataka, India

Entomopathogenic and beneficial roles of metarhizium: A review

GP Shetty, A Meghana, Shivaprasad Maranabasari and Mahesh G Shetty

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Abstract

Chemical pesticides are widely used for insect pest management, but their overuse has led to resistance, environmental contamination, and health hazards. This has created a need for effective biological alternatives, among which the genus *Metarhizium* (Ascomycota: Hypocreales) is notable. First described in 1879 as a cause of "green muscardine disease," *Metarhizium* includes over 110 species that act as insect pathogens, soil saprobes, and plant endophytes. This review summarizes the taxonomy, biology, and infection process of *Metarhizium*, including adhesion, germination, and cuticle penetration, while examining the role of secondary metabolites like destruxins in virulence and safety. It highlights the genus's applications beyond pest control, such as promoting plant growth and suppressing soil pathogens. The review also discusses formulation technologies, application methods, and challenges like shelf-life and production costs that hinder adoption. Finally, it outlines future research directions like genome editing and nano formulation to enhance the efficacy of *Metarhizium*-based biopesticides, showcasing its potential as a multifunctional organism in sustainable agriculture.

Keywords: *Metarhizium*; entomopathogenic fungi; biological control; plant growth promotion; en sustainable agriculture

Introduction

Synthetic chemical pesticides have long been the primary means of controlling insect pests in agriculture, but their intensive and repeated use has generated serious problems. (Sharma *et al.*, 2019) [57]. Continuous exposure has driven the evolution of resistance in numerous economically important pest species, progressively reducing the efficacy of many conventional insecticides. Chemical residues persist in soil, water and food produce, contributing to environmental pollution and posing risks to human health, non-target organisms and beneficial insects such as pollinators and natural enemies. These drawbacks, together with rising regulatory restrictions on hazardous molecules and growing consumer demand for residue-free produce, have intensified the search for effective, environmentally compatible biological alternatives to chemical pest control. One of the best alternatives to this is the use of microorganisms, especially entomopathogenic fungi popularly known as mycobiococontrol. Belonging to the order Hypocreales (phylum Ascomycota) encompassing over 2,600 species distributed among 14 families, it is one of the most ecologically diverse, with its members functioning as saprophytes, endophytes, and parasites (Hyde *et al.* 2020) [31]. Entomopathogenic fungi are among the most promising biological control agents because, unlike bacterial or viral pathogens, they infect their hosts directly through the cuticle rather than requiring ingestion, allowing them to act against both chewing and sap-sucking insects. Among the entomopathogenic fungi, the genus *Metarhizium* (phylum Ascomycota, order Hypocreales) is one of the most extensively researched and commercially important. *Metarhizium*-based biopesticides are now marketed worldwide for the control of pests as diverse as locusts, termites, mosquitoes, weevils, whiteflies and soil grubs, reflecting the genus's broad host range and adaptability. Its growing commercial footprint mirrors an expanding global market for microbial biopesticides, driven by regulatory pressure on synthetic chemicals and the demand for integrated pest management (IPM)-compatible products. Despite significant advances in understanding the diverse ecological roles of Hypocrealean fungi, the complex, multifunctional behaviors of the genus *Metarhizium*

Corresponding Author:
GP Shetty
 Multiplex Group of Companies,
 Bengaluru, Karnataka, India

require further exploration. As a key endophytic insect pathogenic fungus (EIPF), *Metarhizium* engages in dynamic interactions with both insect hosts and plant tissues that remain insufficiently characterized. While foundational research has highlighted these unique symbiotic and pathogenic relationships, comprehensive studies detailing the underlying molecular mechanisms and ecological impacts are still lacking. Ultimately, resolving these research gaps will deepen our comprehension of *Metarhizium*'s ecological significance and facilitate its application in sustainable agriculture and innovative biological pest control programs.

History and popularity of *Metarhizium*

The genus was first documented in 1879 by Metchnikoff, who observed its asexual morph infecting the wheat cockchafer (*Anisoplia austriaca*) in Russia and associated it with "green muscardine disease" (Zimmermann *et al.*, 1995)^[75]. The species was originally described as *Entomophthora anisopliae*; it was reclassified as *Isaria destructor* in 1880, before Sorokin established the genus *Metarhizium* three years later. *Metarhizium* has since become one of the most popular entomopathogenic fungi for biological control because of its wide host range Latch (1965)^[41] recorded natural infection in 166 insect species its ease of mass production on inexpensive substrates such as broken rice, its tolerance of diverse environmental conditions, and its additional beneficial activities as a plant growth promoter and endophyte. These combined attributes have supported the development of a substantial global market for *Metarhizium*-based mycoinsecticides and have made the genus a model system for studying fungal-insect-plant interactions.

Encompassing taxonomic diversity of the order Hypocreales

Taxonomy and classification

Origin, distribution and species diversity

Metarhizium exhibits considerable variation in life cycle and asexual reproductive structures (Kepler *et al.*, 2012)^[38]. Phylogenetically, the genus groups with *Pochonia chlamydosporia* in a monophyletic clade; evolutionary estimates suggest this lineage arose from plant-root symbionts such as *Claviceps* and *Epichloë* around 300 million years ago, before diverging to acquire pathogenic capability against insects and nematodes approximately 180 million years ago (Sheng *et al.*, 2022)^[59]. *Metarhizium* has been isolated from infected insects and soils on every continent except Antarctica, with isolations recorded even near the Antarctic Circle (Roddam and Rath, 1997)^[54]. Neotypes for *M. anisopliae* (CBS H-14432 and culture ex-neotype CBS 130.71) were designated by Mongkolsamrit *et al.* (2020)^[50]. More than 110 species epithets are currently recognized within the genus (Index Fungorum, 2024). Species inhabit soil as saprobes and rhizosphere colonizers (Hu and St. Leger, 2002), as endophytes (Behie *et al.*, 2012)^[8], and as pathogens of insects (Vega *et al.*, 2009)^[65], and antagonists of fungal plant pathogens (Sasan and Bidochka, 2012)^[56].

Taxonomic history

Tulloch (1976)^[64] reviewed the genus and reduced it to only two species *M. anisopliae* and *M. flavoviride* while recognizing two varieties of the former, *M. anisopliae* var.

anisopliae (small conidia) and var. *majus* (large conidia). Prior to this taxonomic revision and the subsequent work of Rombach, Humber and Evans (1987)^[55], several species, including *M. album*, *M. brunneum* and *M. chrysorrhoeae*, had already been described but were largely synonymized. Early classifications relied on morphological characters, particularly conidial size and shape.

Advances in molecular techniques, including isozyme, RAPD and RFLP analyses, subsequently uncovered numerous cryptic species and varieties (Bidochka *et al.*, 1994)^[11]. Driver, Milner and Trueman (2000)^[20] combined conidial measurements, RAPD data and ribosomal DNA (ITS and 28S D3) phylogenetics on 123 isolates to identify ten monophyletic clades, three of which retained the specific names *M. album*, *M. flavoviride* and *M. anisopliae*, with the latter two further subdivided into clades assigned variety-level names. However, limited resolution in the ITS region left relationships within *M. anisopliae* and *M. flavoviride* largely unresolved. Bischoff, Rehner and Humber described *M. frigidum* in 2006 and, in 2009^[12], applied multi-locus phylogenetic analysis (EF-1 α , RPB1, RPB2 and β -tubulin) to resolve the *M. anisopliae* species complex, identifying nine distinct monophyletic lineages and clarifying that most pre-2007 literature refers to *M. anisopliae sensu lato*, whereas post-2007 studies typically refer to the narrower *sensu stricto* group.

Multilocus analyses have since expanded the genus to include around 30 species, incorporating former members of *Metacordyceps* and *Nomuraea* as well as species from the newly erected genus *Chamaeleomyces* (Kepler *et al.*, 2014)^[37]. New species continue to be discovered, such as *M. dendrolimatis* (Chen *et al.*, 2017)^[18], and previously unrecognized lineages (Mani1-Mani3) have been detected among Mexican and Brazilian isolates of the *M. anisopliae sensu lato* group (Brunner-Mendoza *et al.*, 2017)^[15].

Nomenclature

Metarhizium Sorokin, Veg. Parasitenk. Mensch Tieren: 268, 1879.

Synonyms: *Chamaeleomyces* Sigler (2010); *Metacordyceps* G.H. Sung *et al.* (2007); *Nomuraea* Maubl. (1903).

Type species: *Metarhizium anisopliae* (Metschn.) Sorokin (1883). The *M. anisopliae* species complex currently comprises 21 species, and its taxonomic foundation has itself been revised: the neotype designated by Tulloch (1976)^[64] from an Ethiopian locust has been rejected due to geographical and host disparities, and the Ukrainian strain CBS 130.71 is now proposed as the more accurate neotype, aligning with Metchnikoff's original 1879 description.

Micromorphology of key *metarhizium* species

Species within the genus are traditionally distinguished on SDAY/4 medium by phialide shape, conidial shape and dimensions, and colony colour. Representative examples include: *M. anisopliae* (cylindrical phialides, 8-11.5 $\times$ 2-3 μ m; cylindrical to ellipsoid conidia, 5-7 $\times$ 2-3.5 μ m; greyish-green colony); *M. acridum* (cylindrical or ellipsoid phialides; ellipsoid to globose conidia; greyish-yellow colony); *M. album* (cylindrical phialides, ellipsoid to cylindrical conidia; pale brown colony); *M. brunneum* (cylindrical phialides; cylindrical, ellipsoid conidia; white-cream colony); *M. flavoviride* (long cylindrical phialides, 9-26 $\times$ 1.5-2 μ m; pyriform, reniform or ovoid conidia; yellowish-white colony); *M. robertsii* (cylindrical phialides;

cylindrical, ellipsoid conidia; white to pale-yellow colony); and *M. pingshaense* (cylindrical phialides; ellipsoid conidia; olive colony), among many others. These morphological traits, while historically foundational for identification,

show overlap among closely related species and are now used in conjunction with molecular markers for reliable species delimitation.

Table 1: Morphological characteristics, conidial and phialide dimensions, colony characteristics, and geographical distribution of *Metarhizium* species

Species	Countries found	Phialides(μm)	Conidia(μm)	Colony description
<i>M. anisopliae</i>	Brazil, Colombia, Ethiopia, France, India, Indonesia, Japan, Moldova, Myanmar, New Zealand, Papua New Guinea, Philippines, USA, Western Samoa	Cylindrical, 8-11.5×2-3	Cylindrical to ellipsoid, 5-7×2-3.5	Greyish green
<i>Metarhizium acridum</i>	Australia, Benin, Guinea-Bissau, Madagascar, Mali, Mexico, Niger, Senegal, Tanzania, Thailand, Tschad, USA	Cylindrical or ellipsoid, 4.5-12.5×2.5-4.5	Ellipsoid, globose, 4-5.5×3-4	Greyish yellow
<i>M. album</i>	Indonesia, Philippines	Cylindrical, 5-14×1.5-2	Ellipsoid, cylindrical, 5-6×1.5-2	Pale brown
<i>M. brunneum</i>	Argentina, Australia, Austria, Canada, Denmark, Finland, France, Germany, Indonesia, Italy, Japan, Mexico, Myanmar, New Zealand, Norway, Philippines, Portugal, Republic of Georgia, Switzerland, Turkey, UK, USA	Cylindrical, 7-22×1.5-2	Cylindrical, ellipsoid, 4.5-9×2-3	White cream
<i>M. alvesii</i>	Brazil	Ovoid to broadly ellipsoid, 7.55-14.46×1.6-2.8	Cylindrical, 3.88-6.55×2.16-3.25	Yellow to greenish
<i>M. blattodeae</i>	Brazil, Thailand	Ellipsoid, cylindrical, 5-15×2-2.5	Ellipsoid to cylindrical, 6-8×2-3	Pale yellow
<i>M. cicadae</i>	Thailand	Cylindrical, 4-7×2-3.5	Two types of conidia; ovoid, ellipsoid, 2-6×2.5-4; cylindrical, 10-17×3-4	Dark green
<i>M. cylindrosporum</i>	China, Japan, Taiwan	Ovoid, 4-7×2-3	Two types of conidia; ovoid, subglobose, 3-8×2-3; cylindrical, 14-22×3-4	Pale yellow
<i>M. flavoviride</i>	Australia, Czech Republic, France, Germany, Malaysia, Netherlands	Cylindrical, 9-26×1.5-2	Pyriiform, reniform, ovoid 4-10×1.5-3	Yellowish white
<i>M. globosum</i>	India	Clavate, ovoid 5-12×3-4	Globose, 4-5×4-5	Greyish green
<i>M. gryllidicola</i>	Thailand	Cylindrical, utriform, 6-11×2-3	Cylindrical to ovoid, obclavate, 4-7×2-3	Sulphur yellow
<i>M. guizhouense</i>	China	7-12×2-3	7-10×2-3	Olive
<i>M. majus</i>	Australia, France, Indonesia, Japan, Malaysia, Philippines, Poland, Western Samoa	Cylindrical to ellipsoid, 11-22×2-3	Oblong-elliptical, 10-14×2-4	Yellowish orange to green
<i>M. pingshaense</i>	Australia, Brazil, China, Colombia, India, Indonesia, Japan, Myanmar, New Zealand, Oman, Pakistan, Papua New Guinea, Philippines, Solomon Islands, Thailand	Cylindrical, 7-17×2.5-3.5	Ellipsoid, 6-8×2.5-3.5	Olive
<i>M. purpureum</i>	Thailand	-	-	Pale cream
<i>M. robertsii</i>	Argentina, Australia, Brazil, Canada, Colombia, Germany, Italy, Japan, Mexico, Myanmar, Norway, Portugal, Republic of Georgia, Romania, USA	Cylindrical, 7-13×2-2.5	Cylindrical, ellipsoid, 5-9×2-3	White to pale yellow
<i>M. humeri</i>	Brazil, Mexico	Ovoid to broadly ellipsoid, 6.6-12.85×1.77-2.45	Cylindrical, 4.14-6.05×1.69-2.59	Grey green
<i>M. minus</i>	Benin, Ecuador, Philippines, Solomon Islands, Thailand	Cylindrical, 6-14×2.5-4	Cylindrical, ellipsoid, 4-6×2.5-3	White
<i>M. ovoidosporum</i>	Thailand	Obpyriform, ovoid, 3-7×2-4	Ovoid, ellipsoid, subglobose, 3-5×2-4	Olive yellow
<i>M. reniforme</i>	Indonesia, Ghana, Philippines, USA	Spherical to broadly ellipsoid, 2.5±0.4×2.3±0.2	Reniform, 4.5±0.7×2.2±0.4	Greyish green, olive, dull green
<i>M. viridulum</i>	Taiwan, Thailand	Oval-cylindrical to ellipsoid, 5-9×3-5	Ellipsoid, cylindrical, 7-13×3-4	Pale yellow

Molecular identification

Advances in molecular techniques such as isozyme, RAPD, and RFLP analyses along with physiological data, helped uncover cryptic species and varieties (Bidochka *et al.*, 1994)^[11]. A major step forward came with Driver, Milner, and Trueman (2000)^[20], who combined conidial measurements, RAPD data, and phylogenetic analysis of ribosomal DNA regions (ITS and 28S D3), identifying ten distinct monophyletic clades. However, due to limited variation in the ITS region, relationships within *M. anisopliae* and *M. flavoviride* remained largely unresolved.

In 2006, Bischoff, Rehner, and Humber described *M. frigidum*, and by 2009, they applied multi-locus phylogenetic analysis using EF-1 α , RPB1, RPB2, and β -tubulin genes to clarify the taxonomy of the *M. anisopliae* complex. They found that sequence variation in the 5' end of EF-1 α offered greater resolution for distinguishing terminal clades within the *M. anisopliae* sensu lato group, which includes *M. anisopliae* sensu stricto. Notably, most studies published before 2007 refer to the broader *M. anisopliae* sensu lato, whereas those after 2007 typically refer to the more narrowly defined sensu stricto. These findings, supported by conidial measurements, provided evidence for nine distinct monophyletic lineages within the *M. anisopliae* complex.

To date, multilocus analyses have expanded the genus *Metarhizium* to include around 30 species, encompassing both asexual and sexual forms, and integrating former members of *Metacordyceps* and *Nomuraea*, as well as species from the newly established genus *Chamaeleomyces* (Kepler *et al.*, 2014)^[37].

Biology and life cycle

The life cycle of *Metarhizium* begins with attachment of hydrophobic conidia to the insect cuticle, followed by germination, germ-tube growth, appressorium formation, cuticle penetration, hemocoel colonisation as yeast-like blastospores, host death, and finally saprophytic re-growth and sporulation on the cadaver to produce new conidia for dispersal. Conidial production is a critical stage in this cycle, and sporulation is strongly influenced by the nutritional substrate: cereal grains such as broken rice support significantly higher conidial yields than other substrates, sorghum, wheat bran and pearl millet, indicating that nutrient-rich media enhance fungal growth, reproduction and subsequent infectivity (Kumar *et al.*, 2022)^[40]. Growth of *M. anisopliae* is also markedly temperature-dependent; most isolates show optimum colony growth between 25 and 30 °C, although isolates from cold-adapted hosts can show optimal growth as low as 20 °C, reflecting adaptation of different strains to their native environments (Latch, 1965)^[41].

Conidial storage conditions strongly affect viability and virulence. Conidia stored at 4 °C retain significantly higher viability than those stored at 20 °C, and dry, clay-based dust formulations retain higher viability than liquid formulations, particularly under low humidity (Ansari and Butt, 2011^[4]; Daoust *et al.*, 1983)^[19]. At room temperature, conidia remain highly viable for around eight months before germination declines rapidly, with negligible viability after 11 months; prolonged laboratory subculturing likewise reduces virulence, underscoring the importance of maintaining fresh, low-passage cultures for effective biocontrol (Ansari and Butt, 2011)^[4].

Infection mechanism

Metarhizium infects insects through a sequential, multi-step process:

Adhesion: Conidia attach to the insect cuticle through hydrophobins and adhesin proteins that mediate the initial, largely non-specific contact between spore and cuticle surface.

Germination: Germination is strongly influenced by ambient moisture and temperature. Rangel *et al.* (2005)^[53] reported considerable variability in conidial thermotolerance among *M. anisopliae* isolates, with acridid-derived isolates showing the greatest heat tolerance; elevated temperatures reduce germination and biocontrol efficacy, making thermotolerance an important selection criterion for field-adapted isolates. Heat exposure also delays germination more under wet-heat than dry-heat conditions, and thermotolerance is not necessarily correlated with UV-B tolerance, indicating that different environmental stresses act through distinct physiological mechanisms (Rangel *et al.*, 2005)^[53].

Appressorium formation and penetration

Following germination, the germ tube differentiates into an appressorium that generates mechanical pressure to breach the cuticle, aided by a coordinated battery of extracellular hydrolytic enzymes, including proteases, chitinases, lipases and collagenases. Kang *et al.* (1999)^[36] purified a novel 60-kDa chitinase with both endo- and exochitinase activity, optimally active at pH 5.0 and inducible by chitin, underscoring its role in cuticle degradation and virulence. St. Leger *et al.* (1993) further demonstrated that *M. anisopliae* produces multiple extracellular chitinase isozymes that are induced only in chitin-containing media, consistent with an inducer-repressor regulatory mechanism, and that chitinase-deficient mutants show markedly reduced pathogenicity.

Hemocoel colonization and immune evasion

After penetration, the fungus proliferates within the hemocoel as walled, yeast-like blastospores, which evade and suppress host cellular immune responses. Beys-da-Silva *et al.* (2014)^[10] identified 71 secreted proteins including lipolytic enzymes, Pr1 proteases, oxidoreductases and ROS-related and signalling proteins associated with degradation of host barriers and modulation of host physiology during cotton-pest infection, highlighting the complexity of the host-pathogen molecular interface. In mosquito larvae, extracellular proteases (particularly Pr1 and Pr2) rather than destruxins were identified as the principal virulence factors, inducing stress-mediated apoptosis via caspase activation in the face of a comparatively weak host immune response (Butt *et al.*, 2013)^[16].

Host recognition and transcriptional response

M. anisopliae var. *acridum* recognizes suitable hosts through host-specific chemical and surface cues: only cuticle from the locust *Schistocerca gregaria* induced complete germination and appressorium formation, while cuticles of non-host insects inhibited or reduced differentiation, with transcriptomic upregulation of genes for metabolism, cuticle degradation, detoxification, signal transduction and cell division in response to host-derived cues (Wang and St. Leger, 2007)^[69]. Freimoser *et al.* (2005)^[23] similarly showed host-specific transcriptional responses,

including differential regulation of subtilisin genes, reflecting transcriptional flexibility that supports host adaptation.

Toxin production, host death and sporulation

Death of the host results from a combination of nutrient depletion, toxin action and septicemia. Following death, the fungus sporulates externally, producing the characteristic green conidial layer that gives “green muscardine disease” its name, enabling transmission to new hosts.

Secondary metabolites and toxins

Metarhizium produces a range of bioactive secondary metabolites, of which the **destruxins** are the best characterized and most important virulence factors. Destruxins (Dxs) are cyclic hexadepsipeptides composed of an α -hydroxy acid and five amino-acid residues; more than 38 destruxin analogues have been described, grouped into five chemical classes (A-E). Destruxin A, B and E predominate among natural isolates, although not all isolates produce destruxins, and production varies with strain, growth conditions and nutritional status. Destruxins disrupt insect cell membranes, inhibit mitochondrial ATPase activity, suppress cellular (encapsulation) immune responses, and impair phagocytosis, thereby facilitating infection, although highly virulent isolates can occur with low or negligible destruxin output, indicating that destruxins are not the sole determinant of pathogenicity (Zimmermann, 2007; Skrobek *et al.*, 2008) [60, 74]. Destruxins undergo rapid, temperature-dependent degradation following host death, limiting their environmental persistence and supporting the overall safety of *Metarhizium*-based biocontrol products for non-target organisms (Zimmermann, 2007) [74]. Wang *et al.* (2005) [68], further showed that culture degeneration under prolonged subculturing is associated with reduced destruxin production and diminished virulence.

Other notable metabolites include swainsonine, an indolizidine alkaloid first reported from *M. anisopliae* isolate F-3622, which inhibits lysosomal α -mannosidase and mannosidase II and has attracted interest for antitumor and immunostimulatory applications; and various novel low-molecular-weight compounds such as viridoxins (toxic to Colorado potato beetle larvae), cytochalasins, and immunosuppressive substances such as metacytofilin, several of which show potential pharmacological rather than insecticidal significance. Collectively, toxicological assessment of *Metarhizium* metabolites indicates that the fungus does not secrete copious toxic metabolites into the environment under field conditions, supporting its continued use as a biocontrol agent.

Applications in insect pest management

The broad host range of *Metarhizium* Latch (1965) [41], recorded 166 naturally infected insect species underlies its status as one of the most versatile entomopathogenic fungi for biological control across cropping systems.

Maize and corn

In maize, *M. anisopliae* (Ex: Multiplex *Metarhizium*) has been used to control termites and increase grain yield by reducing lodging, while seed treatment with strain F52 increased corn stand density and biomass while suppressing the wireworm *Agriotes obscurus*, with fungus-infected

wireworm cadavers recovered from treated plots confirming fungal-mediated suppression (Kabaluk and Ericsson, 2007) [35].

Cotton

Secretome analysis of *M. anisopliae* infecting the cotton pest *Dysdercus peruvianus* identified 71 secreted proteins associated with host-barrier degradation, illustrating the molecular basis of pathogenicity relevant to cotton pest management (Beys-da-Silva *et al.*, 2014) [10].

Sugarcane

Metarhizium (Multiplex *Metarhizium*) has been used against sugarcane spittlebugs (*Aeneolamia* spp.), with phylogenetic surveys of Mexican isolates identifying 29 isolates representing eight haplotypes with strain-specific temperature-growth responses relevant to selecting locally adapted biocontrol strains (Hernández-Domínguez *et al.*, 2016 [26]; López-Rodríguez *et al.*, 2023) [43, 44]. Against the sugarcane white grub *Lepidiota stigma*, isolates JT-2 and BL-20 caused 80-90% larval mortality at 1.5×10^9 conidia mL⁻¹ (Asbani and Indrayani, 2024) [5], while soil temperature (16-31 °C), moisture and inoculum concentration were shown to govern pathogenicity against *Ligyris subtropicus* (Raid and Cherry, 1988) [52]. Termite infestation by *Heterotermes tenuis* in Brazilian sugarcane can be managed through combined application of *Beauveria bassiana* (Multiplex Baba) and *M. anisopliae* (Multiplex *Metarhizium*) (Moino Jr. *et al.*, 2002) [49].

Cocoa

A commercial *M. anisopliae* formulation (Biomagic WP) achieved up to 100% reduction in pod infestation by cocoa mirid (*Sahlbergella singularis*) in small-scale trials and 89% reduction in large-scale trials, with strong antifeedant activity, no phytotoxicity, complete biodegradability and no detectable pesticide residues in cocoa beans properties that support its integration into organic cocoa production (Awe *et al.*, 2026) [6].

Vegetables, tomato and cowpea

In tomato, *M. carneum* suppressed the root-knot nematode *Meloidogyne enterolobii*, reducing root galling and improving plant growth, although chemical nematicides remained more effective (López-Lima *et al.*, 2023) [43, 44], combining *M. anisopliae* (Multiplex *Metarhizium*) with oak-debris soil amendment reduced *M. javanica* gall formation by approximately 76% and reproduction factor by up to 86-90% (Abdollahi, 2018) [1]. In cowpea, *M. anisopliae* (Multiplex *Metarhizium*) reduced *M. incognita* juvenile populations, egg masses and root galls while improving nodulation and yield, with the greatest effect at 1×10^8 spores mL⁻¹ (Youssef *et al.*, 2020) [71].

Plantation and horticultural crops

In strawberry, *M. anisopliae* (Multiplex *Metarhizium*) combined with the nematode *Steinernema kraussei* improved suppression of black vine weevil compared with either agent alone (Ansari *et al.*, 2010), while in soilless potting media the fungus remained viable for up to 342 days, colonized the rhizosphere effectively, and produced 93.5-97.5% larval mortality of black vine weevil through soil incorporation (Bruck, 2005) [14].

Locusts and grasshoppers

Metarhizium is particularly well established against acridids. The Australian isolate FI985 (*M. flavoviride* group 3, ARSEF 324) was mass-produced on rice, formulated as an oil-based mycoinsecticide, and achieved substantial field mortality of five major acridid pests within 10-30 days, albeit more slowly than the chemical insecticide fenitrothion (Milner, 1997) [47]. Oil formulation enhanced infectivity of *M. flavoviride* against desert locusts even under low humidity by improving conidial adhesion and protecting spores from desiccation (Bateman *et al.*, 1993) [7]. Field application of *M. anisopliae* var. *acridum* strain FI-985 at $1-5 \times 10^{12}$ conidia/ha achieved >90% mortality of the Australian plague locust within 12-14 days, with persistent activity against subsequently emerging nymphs (Hunter *et al.*, 2001) [30], while transcriptomic studies during hemolymph colonization of *Locusta migratoria* revealed extensive metabolic and stress-response gene expression (Zhang and Xia, 2009) [72].

Pasture pests

Against the black field cricket *Teleogryllus commodus*, an oil-based formulation of isolate FI1099 achieved 30-70% population reduction within 21 days under field conditions, demonstrating feasibility for pasture IPM despite the faster action of chemical malathion (Milner *et al.*, 1998) [48].

Mosquitoes and disease vectors

Unlike infection of terrestrial insects, *M. anisopliae* (Multiplex *Metarhizium*) does not penetrate the cuticle of *Aedes aegypti* larvae; conidia are instead ingested and mortality is mediated by extracellular proteases rather than tissue invasion or destruxin production (Butt *et al.*, 2013) [16]. Against the malaria vector *Anopheles arabiensis*, an extra-domiciliary, odour-baited delivery system successfully transmitted fungal conidia to adult mosquitoes and reduced survival, offering a complementary approach where insecticide resistance limits conventional vector control (Lwetolijera *et al.*, 2010) [45]. In mosquitoes co-infected with dengue virus, *M. anisopliae* (Multiplex *Metarhizium*) increased mortality and reduced the proportion of survivors carrying virus in the head while decreasing fecundity and ovarian development, suggesting potential for integrated dengue vector management (Garza-Hernández *et al.*, 2013) [24].

Termites

M. anisopliae (Multiplex *Metarhizium*) treated mulch caused termite mortality increasing from 16.8% to 100% as conidial concentration rose from 10^3 to 10^8 conidia mL⁻¹, with tree-based mulches enhancing persistence by providing moisture and UV protection (Sun *et al.*, 2008) [62]. Indigenous isolates against *Coptotermes heimi* showed faster action on filter paper (LT₅₀ 50-83 h) than in soil (LT₅₀ 65-106 h) (Ahmed *et al.*, 2009) [3], while termites such as *Macrotermes michaelseni* can detect and behaviourally avoid highly virulent isolates, indicating a co-evolutionary arms race between host and pathogen (Mburu *et al.*, 2009) [46].

Ticks and mites

M. anisopliae reduced female body mass and egg production in *Ixodes scapularis* (Benjamin *et al.*, 2002) [9], and caused 76-85% mortality, 85-99% reduction in

fecundity and 94-100% reduction in egg hatchability in *Rhipicephalus appendiculatus* and *Amblyomma variegatum* under field conditions, while remaining compatible with organophosphate acaricides (Kaaya *et al.*, 1996) [34].

Other pests

Against western flower thrips (*Frankliniella occidentalis*), the most virulent isolate caused 94-100% adult mortality within 7 days at an LC₅₀ of $\sim 3 \times 10^5$ conidia mL⁻¹ (Vestergaard *et al.*, 1995) [66]. Against the cabbage stem flea beetle, isolates V208 and V245 showed LT₅₀ values of 3.8-10 days depending on concentration and were also pathogenic to several other crucifer pests (Butt *et al.*, 1994) [17]. In rice, *M. album* causes natural epizootics in leaf- and planthoppers (Rombach *et al.*, 1987) [55], while *M. anisopliae* strain CQMa421 was shown to alter feeding behaviour of the whitebacked planthopper, a vector of southern rice black-streaked dwarf virus, via changes in olfactory gene expression (Wang *et al.*, 2022) [70]. Against *Helicoverpa armigera*, mortality of first-instar larvae reached 100% at 1.8×10^9 spores mL⁻¹ (Taliyan *et al.*, 2020) [63]. *Metarhizium brunneum* volatile organic compounds (1-octen-3-ol, 3-octanone and 1-octene) additionally show activity as repellents and molluscicides against garden snails and grey field slugs (Khoja *et al.*, 2019) [39], and *M. anisopliae* controls plant-parasitic nematodes including the reniform nematode on sugarcane and cyst nematodes, often in combination with other biocontrol fungi such as *Beauveria bassiana* and *Paecilomyces lilacinus* (Youssef *et al.*, 2020) [71].

Plant growth promotion

Beyond its role as an entomopathogen, *Metarhizium* (Multiplex *Metarhizium*) is increasingly recognized as a plant growth-promoting fungus through direct root colonization and endophytic association. Proposed mechanisms include enhanced nutrient uptake, nitrogen transfer from insect cadavers to plant tissue, iron solubilization via siderophore production, phosphate mobilization, and production of phytohormones such as indole-3-acetic acid (IAA), alongside ACC deaminase activity that alleviates plant stress ethylene levels. This dual endophytic-entomopathogenic capability positions *Metarhizium* as a multifunctional root symbiont capable of simultaneously improving plant nutrition and health while suppressing insect pests. (Siqueira, A.C.O., *et al.*, 2020) [58].

Induced systemic resistance

Root colonization by *Metarhizium* (Multiplex *Metarhizium*) can trigger induced systemic resistance (ISR) and, in some contexts, systemic acquired resistance (Sal.al.like responses in host plants, mediated through jasmonic acid (JA), salicylic acid (SA) and ethylene signalling pathways. Downstream activation of defence enzymes including phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase and accumulation of phenolic compounds contribute to enhanced resistance against subsequent pest and pathogen attack, extending the fungus's protective role beyond direct pathogenicity toward priming of the plant immune system (Gupta, R., *et al.* 2022) [25].

Role in soil health

As a soil-inhabiting saprobe and rhizosphere colonizer, *Metarhizium* (Multiplex *Metarhizium*) contributes to soil

microbial diversity, carbon cycling and organic matter decomposition, and interacts with resident soil microbial communities in ways that can improve rhizosphere function. Isolate-level variation in thermotolerance and other physiological traits should be considered during strain selection, since isolates combining high virulence with broad environmental tolerance are more likely to provide consistent biocontrol and soil-health benefits across diverse climatic conditions (Li and Feng, 2009).

Disease suppression

Although less extensively studied than its insect-pathogenic activity, *Metarhizium* also contributes to suppression of soil-borne plant pathogens such as *Rhizoctonia*, *Fusarium*, *Pythium* and *Sclerotium* through mechanisms including competitive exclusion, mycoparasitism, rhizosphere colonization and induction of systemic resistance, positioning the fungus as a candidate component of integrated disease management alongside its pest-control functions.

Compatibility with other biocontrol agents and agrochemicals

Successful integration of *Metarhizium* into IPM programmes depends on its compatibility with other biological control agents and agrochemicals. Combining *M. anisopliae* with entomopathogenic nematodes can be synergistic or antagonistic depending on fungal virulence and application timing: a moderately virulent isolate (LPP39) combined with *Heterorhabditis bacteriophora* accelerated mortality of the sugarcane borer *Diatraea saccharalis*, whereas a highly virulent isolate (LPP45) produced antagonistic interactions and reduced both fungal sporulation and nematode reproduction, reflecting within-host competition (Acevedo *et al.*, 2007) [2]. These findings emphasize that careful selection of compatible strains and optimized application schedules are essential when combining *Metarhizium* with other biocontrol agents, neem-based products, other entomopathogens such as *Beauveria bassiana*, and chemical tank-mix partners.

Formulation technologies

Commercial deployment of *Metarhizium* relies on formulation strategies that preserve conidial viability and enhance field performance, including oil dispersions, wettable powders (WP), water-dispersible granules (WG), suspension concentrates (SC), granules, microencapsulation, and emerging nanoformulation approaches. Formulation choice strongly affects shelf life and storage stability: dry, clay-based dust formulations retain higher conidial viability than liquid formulations, particularly under cool, low-humidity storage conditions (Daoust *et al.*, 1983; Ansari and Butt, 2011) [4, 19], and substrate selection during mass production (e.g., broken rice) improves both conidial yield and Pr1 protease activity, enhancing inoculum quality (Kumar *et al.*, 2022; Ansari and Butt, 2011) [4, 40].

Application methods

Metarhizium-based products can be applied through multiple delivery routes, including seed treatment, seedling dip, soil application, drip irrigation, foliar spray, root dip, granule application and farmyard manure enrichment, with the optimal method depending on the target pest's habitat and behaviour. Behavioural interactions between insects and

the fungus also influence application outcomes: Japanese beetle grubs actively avoided soil containing actively growing *M. anisopliae* mycelium (though not conidia alone), reducing pathogen contact, whereas adult females preferentially oviposited in mycelium-containing soil, illustrating those behavioural responses together with environmental conditions and delivery method shape the field success of fungal biocontrol programmes (Villani *et al.*, 1994) [67].

Factors affecting field performance

Field efficacy of *Metarhizium* is governed by an interacting set of environmental and biological factors. The fungus generally grows within 15–35 °C, with optimum germination and growth at 25–30 °C; high relative humidity favours germination while low humidity delays it, and exposure to UV-A and UV-B radiation rapidly reduces conidial viability, limiting field persistence (Zimmermann, 2007) [74]. Johari *et al.* (2022) [33], confirmed that fungal isolates show optimum germination, radial growth and conidial production, together with peak virulence against *Bemisia tabaci*, at 25–30 °C, with markedly reduced performance at 15–20 °C. Soil-based factors temperature (16–31 °C), moisture and inoculum concentration similarly govern pathogenicity against soil pests such as the sugarcane white grub (Raid and Cherry, 1988) [52]. Substrate composition during mass production also affects downstream field performance, since nutrient-rich substrates such as broken rice enhance conidial yield and quality (Kumar *et al.*, 2022) [40], while *M. flavoviride* combines high efficacy against target locusts with limited pathogenicity toward non-target arthropods, supporting a favourable safety profile for field deployment (Peveling and Demba, 2003) [51].

Challenges

Despite its considerable promise, wider adoption of *Metarhizium*-based biopesticides continues to face several practical constraints: lengthy and costly regulatory registration processes; limited shelf life of conidial formulations; low farmer awareness and adoption; variable field efficacy driven by environmental sensitivity (temperature, humidity, UV); compatibility issues with certain agrochemicals; technical and economic challenges in mass multiplication; and the need for robust quality-control standards to ensure consistent conidial viability and virulence across production batches.

Conclusion

Since its original description in 1879, *Metarhizium* has evolved from being viewed narrowly as the causal agent of green muscardine disease into a recognised multifunctional beneficial microorganism with roles spanning insect pathogenicity, plant growth promotion, induced systemic resistance, soil health improvement and suppression of soil-borne plant pathogens. Its broad host range, tractable mass-production biology, and compatibility with integrated pest management make it one of the most commercially important entomopathogenic fungi worldwide, with demonstrated efficacy against locusts, termites, mosquitoes, ticks, mites, nematodes, molluscs and a wide array of crop pests across cereals, cotton, sugarcane, cocoa, vegetables and plantation crops. Nevertheless, realising the full potential of *Metarhizium*-based biopesticides will require continued research into strain improvement, formulation

stability, and environmental resilience, alongside efforts to reduce production costs, streamline regulatory pathways, and improve farmer awareness. Addressing these challenges through emerging tools such as genome editing, Nano formulation and precision application will be central to establishing *Metarhizium* as a mainstream component of sustainable, environmentally responsible pest and disease management.

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