

Sunil Kumar Deshmukh
Kandikere Ramaiah Sridhar *Editors*

Entomopathogenic Fungi

Prospects and Challenges

Entomopathogenic Fungi

Sunil Kumar Deshmukh •
Kandikere Ramaiah Sridhar
Editors

Entomopathogenic Fungi

Prospects and Challenges

 Springer

Editors

Sunil Kumar Deshmukh
Research and Development
Greenvention Biotech Pvt. Ltd
Pune, Maharashtra, India

Kandikere Ramaiah Sridhar
Department of Biosciences
Mangalore University
Mangalore, India

ISBN 978-981-97-5990-3 ISBN 978-981-97-5991-0 (eBook)
<https://doi.org/10.1007/978-981-97-5991-0>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2024

This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

If disposing of this product, please recycle the paper.

Preface

In addition to plants and animals, fungi established a highly diverse assemblage with beneficial as well as harmful impacts on plants and animals, including humans. A global conservative estimate of 2.2–3.8 million fungal species speak about their importance in basic, applied, and interdisciplinary studies towards biotechnological applications. Most of the current applications in mycology depend on only about 5% of the global estimate, which opens up new avenues and applications by exposing cryptic or unknown fungal worlds. Fungi are very important, as they are involved in the degradation and recycling of organic matter. Many fungi are capable of associating mutualistically or symbiotically with plants and animals to carry out several essential environmental functions. They are stores for several metabolites such as enzymes, antibiotics, hormones, secondary metabolites, prebiotics, additives, pigments, organic acids, vitamins, biofuels, pharmaceuticals, bioplastics, and a variety of environmentally compatible products.

It is understood that fungi are useful in the green and circular economy to combat food security, industrial security, climate change, and low-carbon economies. Mycorrhizal mutualistic fungi associated with forest tree plant species are accountable for sequestering up to 36% of fossil fuel emissions per year. In addition, fungi protect plants from insect herbivory by various means (e.g., as endophytes in plants and attacking insects in adult or young stages).

The most well-known fungal genera used as biopesticides include *Beauveria*, *Cordyceps*, *Metarhizium*, and *Trichoderma*. Owing to the detrimental impact of synthetic pesticides on humans and the environment, up to 170 fungi have been designated as generally regarded as safe for commercial applications as mycopesticides.

In view of the applied aspects, this book anticipates the significance of entomopathogenic allied fungi in the fields of agriculture and industry. Up to 21 chapters obtained from 78 authors belong to 9 countries (Algeria, Brazil, Hungary, India, Italy, Malaysia, Oman, Pakistan, and the United States). The contents of the investigation on entomopathogens have been classified into four parts: (1) Prospects and challenges; (2) potential of *Beauveria* and *Metarhizium*; (3) role of secondary metabolites; and (4) molecular basis of insect pest control. Part I projects the life

cycle, virulence, pathogenicity, ecology, and endophytic entomopathogenic fungi, including biopesticide formulations, towards plant protection, sustainable agriculture, and pest management. Contributions in Part II focus on the significance of two important genera of entomopathogenic fungi, *Beauveria* and *Metarhizium*. The subject matter includes history, biochemistry, pathogenesis, parasitism, virulence, stress tolerance, and interactions. A special emphasis has been exercised in two chapters on the control of insect pests detrimental to tea plantations in Northeast India. Part III discloses metabolites, enzymes, toxins, and other bioactive compounds of entomopathogens in agriculture and plant health. Part IV discusses the advantages of molecular studies, the importance of nano-biopesticides, and the bio-fabricated technology of entomopathogens in insect pest control. The components of this book are expected to meet the requirements of biological sciences, applied entomology, mycology, zoology, forestry, biochemistry, molecular biology, and nanotechnology.

The editors are grateful to the contributors for offering the chapters on time, and a careful review was performed by the reviewers. Technical assistance was provided by Dr. S. Mahadevakumar, Scientist, Botanical Survey of India, Port Blair. We are thankful to Springer Nature for their persistence in publishing this book within the stipulated time frame.

Pune, Maharashtra India
Mangalore, India

Sunil Kumar Deshmukh
Kandikere Ramaiah Sridhar

Contents

Part I Prospects and Challenges

The Science Behind Entomopathogenic Fungi: Mechanisms and Applications	3
Maunata Ghorui, Shouvik Chowdhury, and Sashidhar Burla	
Life Cycle, Virulence, and Mechanism of Action of Entomopathogenic Fungi	37
Abhishek Rana, Vikas Tandon, Pooja Kapoor, and Abhishek Katoch	
Entomopathogenic Fungi: Prospects and Challenges	57
Mirza Abdul Qayyum, Shafqat Saeed, Waqas Wakil, Umer Sharif, Hasan Taha, Zeeshan Shahid, Aiman Khalid, Owais Hameed, Muhammad Yasin, Ahmad Nawaz, Habib Ali, and Asim Abbasi	
Entomopathogenic Fungi as Biopesticides for Sustainable Agriculture	81
Murugan Arun Thirumeni, Kamarshi Yashkamal, R. Rafi Mohamed, and M. S. Shivakumar	
Exploration of Entomopathogenic Fungi for Insect Pest Management	101
Rajendran Dhanapal, R. Naveenkumar, E. Adlin Pricilla Vasanthi, S. Sumaiya Parveen, B. Sangeetha, and Govindaraju Ramkumar	
Biocontrol Potential and Specificity of Entomopathogenic Fungi	127
Vikas Tandon, Abhishek Rana, Pooja Kapoor, and Abhishek Katoch	
Endophytic Entomopathogenic Fungi: Biology and Applications	141
S. Aouali	
The Application of Endophytic Entomopathogenic Fungi as Biostimulants and for Plant Protection	189
Ana Carolina Loreti Silva, Raymyson Rhuryo de Sousa Queiroz, Gerson Adriano Silva, and Ian Richard Samuels	

Part II Potential of *Beauveria*, *Metarhizium* and *Paecilomyces*

Two Centuries of Knowledge on *Beauveria bassiana* 209

R. Kheddar and S. Aouali

Biochemistry, Pathogenesis, and Parasitism of *Beauveria* 227

Liseth Bibiana Puentes Figueroa, Juliana Marques Ferreira,
Ruth Celestina Condori Mamani, and Filippe Elias de Freitas Soares

Unveiling the Virulence and Stress Tolerance of *Beauveria bassiana* Against Major Insect Pests in Tea with an Emphasis on Bibliometrics Using Data Mining Tools 247

Pranaba Nanda Bhattacharyya, Bhanushree Baishya,
Dhruba Jyoti Borgohain, Bhaskar Sarma, Liza H. Bhattacharyya,
Francesca Garganese, and Tarun Sen Barman

Biochemistry of Entomopathogens and Mycoparasites: *Metarhizium* 299

Ruth Celestina Condori Mamani, Juliana Marques Ferreira,
Liseth Bibiana Puentes Figueroa, and Filippe Elias de Freitas Soares

Interaction of *Metarhizium anisopliae* Against Emergent Insect Pest Problems in the North-Eastern Tea Industry 319

Pranaba Nanda Bhattacharyya, Bharat Chandra Nath, Bhaskar Sarma,
Laith Khalil Tawfeeq Al-Ani, Dhruba Jyoti Borgohain,
Francesca Garganese, Sayasta Ahmed, Sidhatha Batsya,
Abhilisa Mudoj, and Rajani Kumari

***Paecilomyces*: An Eco-Friendly Approach to Control Agricultural Pests and Pathogens 359**

Hemraj Chhipa, Hanuman Singh, and Sunil Kumar Deshmukh

Part III Role of Secondary Metabolites

The Role of Entomopathogenic Fungal Metabolites in Pest Management 381

Indrani Sarkar, Maunata Ghorui, Shouvik Chowdhury,
and Sashidhar Burla

Metabolites, Enzymes, and Toxins in Entomopathogenic Fungi 409

Uzma Azeem, Gurpaul Singh Dhingra, and Richa Shri

Entomopathogenic Fungal Metabolites Useful in Agriculture and Healthcare 433

Rajendran Dhanapal, Kurru Charitha, Pallavi Sonaniya,
and Chitteti Divya

Applications of Bioactive Compounds from Fungal Entomopathogens 453

B. L. Raghunandan, Anand Dave, Pragnesh R. Baria, and Manjari

Part IV Molecular Basis of Insect Pest Control

Molecular Basis of Entomopathogenesis and the Way Forward 481
Ipsita Samal, Tanmaya Kumar Bhoi, Deepak Kumar Mahanta,
and J. Komal

Potential of Fungal Nanobiopesticides on Entomopathogens. 507
Subhoshmita Mondal

Insect Pest Control Through Biofabricated Technology 523
Natasha Kudesia, A. Najitha Banu, A. M. Raut, Neha Rana,
Ritu Bihal, Rudradeb Sarkar, and Johnson Wahengbam

Appendix 555

Index. 557

Editors and Contributors

About the Editors

Sunil Kumar Deshmukh is a Scientific Advisor to Greenvention Biotech, Uruli Kanchan, Pune, India, and AGPharmBioinnovations LLP, Patiala, Punjab, India. He is a veteran industrial mycologist who spent a substantial part of his career in drug discovery at Hoechst Marion Roussel Limited [now Sanofi India Ltd.], Mumbai, and Piramal Enterprises Limited, Mumbai. He has also served TERI-Deakin Nanobiotechnology Centre, TERI, New Delhi, and as an Adjunct Associate Professor at Deakin University, Australia. He has to his credit 8 patents, 152 publications, and 23 books on various aspects of fungi and natural products of microbial origin. He is a president of the Association of Fungal Biologists (AFB) and a past president of the Mycological Society of India (MSI). He is a fellow of the Mycological Society of India, the Association of Biotechnology and Pharmacy, the Society for Applied Biotechnology, and the Maharashtra Academy of Sciences. He has approximately four decades of research experience in getting bioactive from fungi and various aspects of keratinophilic fungi.

Kandikere Ramaiah Sridhar is an Adjunct Professor in the Department of Biosciences, Mangalore University, India. His main areas of research are 'diversity and ecology of fungi of the Western Ghats, mangroves, and marine habitats'. He was an NSERC postdoctoral fellow and visiting professor at Mount Allison University, Canada; the Helmholtz Centre for Environmental Research at UFZ and Martin Luther University, Germany; and the Centre of Biology, University of Minho, Portugal. He was a recipient of the Shome Memorial Award (2004), Vice President (2013), President (2018), and Lifetime Achievement Awardee (2019) by the Mycological Society of India, Chennai. He was a recipient of the Fellow of the Indian Mycological Society, Kolkata (2014), Distinguished Asian Mycologist (2015), and Outstanding Leader in Education and Research, Association of Agricultural Technology of Southeast Asia (2016). He was awarded a UGC-BSR Faculty Fellowship (2014–2017). He is one of the top 2% of scientists in the field of

mycology (2020–2021). He has over 500 publications and has edited 14 books in the field of biology, including mycology.

Contributors

Asim Abbasi Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Sayasta Ahmed Department of Zoology, Tripura University (A Central University), Suryamaninagar, West Tripura, India

Laith Khalil Tawfeeq Al-Ani School of Biology Science, Universiti Sains Malaysia, Minden, Pulau Pinang, Malaysia

Habib Ali Department of Entomology, Khawaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan

Souhila Aouali Forest Pathology and Mycology Laboratory, Forest Protection Division, Forest Research National Institute, Algiers, Algeria

Uzma Azeem Government College Malerkotla, Malerkotla, Punjab, India

Bhanushree Baishya Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya, Tulungia, Bongaigaon, Assam, India

A. Najitha Banu Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Pragnesh R. Baria AICRP on Biological Control of Crop Pests, Anand Agricultural University, Anand, Gujarat, India

Tarun Sen Barman Ex-Head and Scientist, Department of Plant Physiology and Breeding, Tocklai Tea Research Institute, TRA, Jorhat, Assam, India

Sidhatha Batsya DIET, Sonitpur, Assam, India

Pranaba Nanda Bhattacharyya Department of Botany, Nanda Nath Saikia College, Jorhat, Assam, India

DBT-IBT-HUB, Nanda Nath Saikia College, Titabar, Assam, India

Tanmaya Kumar Bhoi Forest Protection Division, ICFRE-Arid Forest Research Institute (ICFRE-AFRI), Jodhpur, Rajasthan, India

Ritu Bihal Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Dhruba Jyoti Borgohain C. S. Central Library, Nanda Nath Saikia College, Titabar, Assam, India

Sashidhar Burla Techno-Commercial, ATGC Biotech Pvt. Ltd., Hyderabad, India

Kurru Charitha Department of Entomology, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India

Hemraj Chhipa College of Horticulture and Forestry, Jhalawar (Agriculture University, Kota), Kota, Rajasthan, India

Shouvik Chowdhury Symbiotic Sciences Pvt. Ltd., Gurugram, Haryana, India

Anand Dave Department of Microbiology, Seventh Day Adventist Arts and Science College, Ahmedabad, Gujarat, India

Sunil Kumar Deshmukh Research and Development, Greenvention Biotech Pvt. Ltd, Pune, Maharashtra, India

Rajendran Dhanapal Department of Entomology, Adhiparasakthi Horticultural College, Tamil Nadu Agricultural University, Ranipet, Tamil Nadu, India

Gurpaul Singh Dhingra Department of Botany, Punjabi University, Patiala, Punjab, India

Chitteti Divya Department of Entomology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Juliana Marques Ferreira Instituto de Patologia Tropical e Saúde, Universidade Federal de Goiás, Goiânia, Brazil

Liseth Bibiana Puentes Figueroa Departamento de Química, Universidade Federal de Lavras, Lavras, Brazil

Filippe Elias de Freitas Soares Departamento de Química, Universidade Federal de Lavras, Lavras, Brazil

Francesca Garganese Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, University of Bari Aldo Moro, Bari, Italy

Maunata Ghorui Symbiotic Sciences Pvt. Ltd., Gurugram, Haryana, India

Ramkumar Govindaraju Department of Entomology, College of Agricultural Environmental Science, University of Georgia, Griffin, GA, USA

Owais Hameed Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

Liza Handique Department of Botany, Jagannath Barooah College (Autonomous), Jorhat, Assam, India

Pooja Kapoor University Institute of Agricultural Sciences, Chandigarh University, Gharuan, Punjab, India

Abhishek Katoch University Institute of Agricultural Sciences, Chandigarh University, Gharuan, Punjab, India

Aiman Khalid Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

R. Kheddar Faculty of Natural and Life Sciences, Agro-Ecology and Biotechnology Department, University of Blida 1, Blida, Algeria

J. Komal Basic Seed Multiplication and Training Centre, Central Silk Board, Kharsawan, Jharkhand, India

Natasha Kudesia Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Rajani Kumari Department of Microbiology, Kaziranga University, Jorhat, Assam, India

Deepak Kumar Mahanta Forest Entomology Discipline, Forest Protection Division, Indian Council of Forestry Research and Education (ICFRE)-Forest Research Institute (ICFRE-FRI), Dehradun, Uttarakhand, India

Ruth Celestina Condori Mamani Departamento de Química, Universidade Federal de Lavras, Lavras, Brazil

Manjari Department of Plant Pathology, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

R. Rafi Mohamed Department of Zoology, C. Abdul Hakeem College (Autonomous), Ranipet, Tamil Nadu, India

Subhoshmita Mondal Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Milan, Italy

Abhilisa Mudoi AICRP on Seed (Crops), Assam Agricultural University, Jorhat, Assam, India

Bharat Chandra Nath Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam, India

AICRP on Seed (Crops), Assam Agricultural University, Jorhat, Assam, India

R. Naveenkumar Division of Plant Pathology, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Ahmad Nawaz Department of Plant Science, Sultan Qaboos University, Muscat, Oman

S. Sumaiya Parveen Division of Entomology, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Mirza Abdul Qayyum Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

B. L. Raghunandan AICRP on Biological Control of Crop Pests, Anand Agricultural University, Anand, Gujarat, India

Abhishek Rana Plant Protection-Entomology, KVK Gopalganj, Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar, India

Neha Rana Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

A. M. Raut Department of Entomology, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

Ian Richard Samuels Department of Entomology and Plant Pathology, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil

Shafqat Saeed Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

Ipsita Samal National Research Centre on Litchi, Muzaffarpur, Bihar, India

B. Sangeetha Division of Plant Pathology, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Indrani Sarkar Amity Institute of Biotechnology, Amity University Noida, Noida, Uttar Pradesh, India

Rudradeb Sarkar Department of Zoology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Bhaskar Sarma Department of Botany, Dhemaji College, Dhemaji, Assam, India

Zeeshan Shahid Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

Umer Sharif Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

M. S. Shivakumar Molecular Entomology Lab, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India

Richa Shri Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India

Ana Carolina Loreti Silva Department of Entomology and Plant Pathology, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil

Gerson Adriano Silva Department of Entomology and Plant Pathology, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil

Hanuman Singh College of Agriculture, Hindoli, Bundi, Rajasthan, India

Pallavi Sonaniya Department of Entomology, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India

Raymyson Rhuryo de Sousa Queiroz Department of Entomology and Plant Pathology, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil

Hasan Taha Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

Vikas Tandon Department of Entomology, CSK HPKV Palampur, Palampur, India

Murugan Arun Thirumeni Molecular Entomology Lab, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India

E. Adlin Pricilla Vasanthi Division of Entomology, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Johansan Wahengbam Department of Entomology, Szent Istvan University, Budapest, Hungary

Waqas Wakil Department of Continuing Education, University of Agriculture, Faisalabad, Pakistan

Kamarshi Yashkamal Department of Biotechnology, K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Tamil Nadu, India

Muhammad Yasin Department of Entomology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Part I
Prospects and Challenges

The Science Behind Entomopathogenic Fungi: Mechanisms and Applications



Maunata Ghorui, Shouvik Chowdhury, and Sashidhar Burla

Abstract Entomopathogenic fungi (EPF) offer a promising solution to mitigate crop losses caused by insect pests, addressing challenges in agricultural sustainability. EPF belong to diverse taxonomic groups and exhibit different modes of action, making them effective against a wide range of insect pests. EPF production involves optimizing growth conditions and selecting suitable substrates, including cost-effective agricultural and industrial by-products. Formulation includes enhancing shelf-life, viability, and application efficacy while minimizing environmental impacts. Various application methods, such as foliar spraying and seed treatment, facilitate the introduction of EPF into the agriculture ecosystem for effective pest management. EPF colonize the plants and not only defends against insect pests but also induces systemic resistance and antagonizes other plant pathogens. Despite their potential, EPF face several challenges, such as susceptibility to adverse field conditions, contamination by saprophytic fungi, and limited efficacy under suboptimal environmental conditions. Additionally, high production costs and the risk of environmental contamination with mycotoxins pose significant hurdles. Careful management and integration into Integrated Pest Management (IPM) programs are essential to maximize the benefits of EPF while minimizing potential contaminants. Addressing these challenges is crucial to realizing the full potential of EPF in sustainable crop protection strategies.

Keywords Crop protection · Cultivation · Formulations · Integrated pest management · Mechanism of action · Toxic substances

M. Ghorui · S. Chowdhury
Symbiotic Sciences Pvt. Ltd., Gurugram, Haryana, India

S. Burla (✉)
Techno-Commercial, ATGC Biotech Pvt. Ltd., Hyderabad, India

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2024

S. K. Deshmukh, K. R. Sridhar (eds.), *Entomopathogenic Fungi*,
https://doi.org/10.1007/978-981-97-5991-0_1

1 Introduction

Agriculture faces significant challenges in mitigating crop yield losses worldwide attributable to various biotic and abiotic stressors, prominently including insect pests. Insects, constituting a diverse taxonomic group with an estimated million recognized species, exhibit a wide host range encompassing crops, weeds, trees, and medicinal plants. While most of these insect species fulfil beneficial ecological roles, a fraction exerts detrimental effects on agricultural productivity, human health, and socioeconomic welfare. Notably, herbivorous insects are implicated in causing approximately 18% of the overall agricultural damage worldwide (Jankielsohn 2018).

The impact of insect pests extends beyond field crops, as they infest stored food commodities, leading to substantial losses and compromising food quality. Every year, between 20% and 40% of the global crop production is lost due to pest infestations. Invasive insects cause around US\$ 70 billion in damage (Researchers Helping Protect Crops from Pests 2023). Taxonomically, insect pests inflicting damage exceeding 10% are categorized as major pests, while those causing harm in the range of 5–10% are designated minor pests (Sharma et al. 2017).

To confront these challenges, extensive efforts are directed toward pest management strategies. Annual global pesticide usage surpasses 2 billion tons, encompassing a repertoire of chemical compounds such as bactericides, fungicides, herbicides, and insecticides (Baron et al. 2018). Noteworthy among these are chlorantraniliprole, cyantraniliprole, novaluron, neonicotinoids, fipronil, farnesyl acetate, emamectin benzoate, phoxim, and pyrethroids, along with juvenile hormone analogs including methoprene, fenoxycarb, and pyriproxyfen. However, the pervasive utilization of these chemical agents incurs deleterious consequences, ranging from ecological perturbations to the development of pest resistance (Rust et al. 2016; Lawler 2017). Synthetic pesticides, notably insecticides, pose inherent risks to the environment, manifesting in water and soil contamination and exerting selection pressures favoring resistance evolution among target pest populations. Furthermore, the indiscriminate application of broad-spectrum insecticides compromises beneficial organisms, including natural enemies of crop pests (Omkar 2016). In response to these challenges, concerted scientific endeavors are directed toward devising sustainable and eco-friendly pest management strategies. Biological control represents a promising avenue for harnessing natural enemies such as predators, parasitoids, and pathogens for pest suppression. As key players in focused pest management, entomopathogens that include bacteria, fungi, viruses, protists, and nematodes cause their host insects to die (Silva et al. 2020). Myco-biocontrol has attracted a lot of attention since it uses fungi to reduce insect populations and minimize agricultural losses. For biocontrol, more than 800 fungal species from various genera have been isolated from insects (Shin et al. 2020; Sinha et al. 2016). Entomopathogenic fungi (EPF) are found in terrestrial ecosystems worldwide, with tropical forests showing the highest diversity. Certain species have even been shown to adapt to harsh environments, such as the Arctic tundra (Hughes et al. 2004).

EPF constitute a distinct category of microorganisms found in soil that invade and eliminate insect pests and other arthropods by penetrating their cuticles (Mantzoukas et al. 2022). These microorganisms parasitize and eliminate arthropods (Litwin et al. 2020), eventually leading to their death (Sharma et al. 2023). Though primarily found in the remains of arthropods, EPF primarily inhabit soil (Behie and Bidochka 2014). According to Araújo and Hughes (2016), these fungi are divided into six groups because they do not belong to a single monophyletic group: Oomycetes (12 species), Chytridiomycota (65 species), Microsporidia (339 species), Basidiomycota (238 species), Ascomycota (476 species), and Entomophthoromycota (474 species).

The most common species from genera within Ascomycota, such as *Beauveria* (e.g., *B. bassiana* and *B. brongniartii*), *Metarhizium* (e.g., *M. anisopliae*, *M. robertsii*, *M. brunneum*, *M. lepidiotae*, *M. globosum*, *M. acridum*, *M. majus*, *M. flavoviride*, *M. rileyi*, *M. pingshaense*, *M. lepidiotae*, and *M. guizhouense*), *Isaria* (e.g., *I. fumosorosea*, *I. farinosa*, and *I. tenuipes*), *Ophiocordyceps* (e.g., *O. sinensis* and *O. unilateralis*), *Cordyceps* (e.g., *C. militaris*), *Torubiella* (including *T. ratticaudata*), *Pochonia* (e.g., *P. chlamydosporia*), *Lecanicillium* (e.g., *L. lecani* and *L. longisporum*), *Hirsutella* (e.g., *H. thompsonii*, *H. nodulosa*, and *H. aphidis*), and *Paecilomyces variotii* and *Purpureocillium lilacinum*, have been recorded in scientific literature for being the EPF (Khan et al. 2012; Tkaczuk et al. 2015; Jaihan et al. 2016). The application of EPF presents a viable economic and ecologically sustainable approach to Integrated Pest Management (IPM). This chapter addresses the mechanism of action of EPF, the production of EPF, crop protection, IPM, and the production of toxic substances.

2 Mechanisms of Action of Entomopathogenic Fungi

A variety of methods, including starvation and toxin synthesis, are employed by EPF to kill insects. EPF generate a variety of toxins as well as extracellular enzymes such as chitinases and proteases that help breach the host's physical barriers. As the primary pathway for fungal penetration, the cuticle serves as the primary barrier against infection in insects. Therefore, it requires a physical or enzymatic method to break through the tough cuticle.

As heterotrophic animals, fungi get their main energy by absorbing the organic molecules that other organisms make. Exogenous carbon sources, such as chitin in the case of *B. bassiana* conidia, are necessary for spore or conidia germination. Additionally, insect epicuticular lipids may help attach the fungus to the host cuticle (Ferron 1978). According to Lecuona et al. (1997), insect epicuticular lipids may have two functions: first, they may give rare conidia readily available energy sources for germination; second, they may have antifungal properties that prevent hyphal development. Proteases, lipases, and chitinases are examples of hydrolytic enzymes that are synthesized and break down the cuticle to release nutrients for the fungus. Lipid degradation in the outer layer of the cuticle also occurs prior to fungal

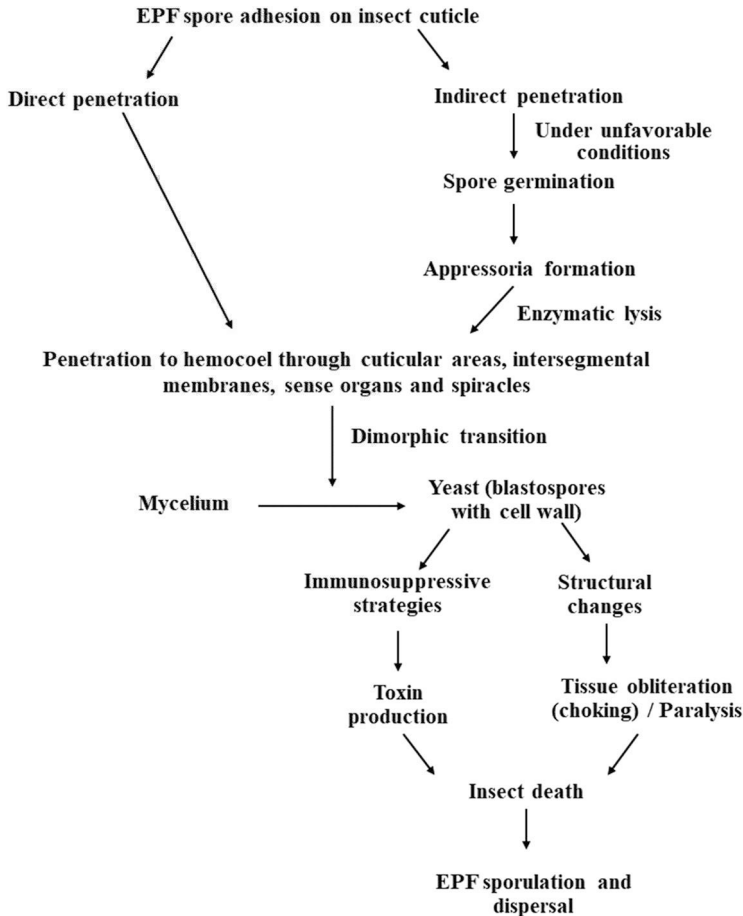


Fig. 1 Mechanism of action by entomopathogenic fungi on insects

penetration. The mechanism of EPF infection in insects or other arthropods is shown in Fig. 1.

2.1 Spore Adhesion and Spore Germination

In most fungi, the infective unit is typically a spore, namely a conidium. Pre-germination conidia swell and stick to the cuticle or exude sticky mucus. There are multiple steps involved in the spore's attachment to the host's cuticle and eventual germination. One can become infected directly by encountering infectious cadavers and vulnerable hosts, or indirectly by encountering spores in the air or spores that are deposited on plants or soil particles (Hesketh et al. 2009).

2.1.1 Adhesion Mechanisms

Fungal propagules adhere to the host cuticle at the first stage of infection, which is made possible by adhesion processes that are influenced by the characteristics of the conidial cell wall (Boucias et al. 1988). This mechanism involves the interaction of proteins found in the conidia with the hydrophobic surface of the exoskeleton of the susceptible insect (Fang et al. 2005). Adhesins are chemicals produced by the fungus that facilitate the adherence between spores and the insect cuticle. For instance, adhesion and virulence in *Metarhizium anisopliae* are significantly influenced by a kind of adhesin termed MAD1, which is found on the surface of conidia (Wang and St Leger 2007a).

2.1.2 Spore Germination

The penetration phase is started when the conidium germinates into a short germ tube and forms tiny swellings called appressoria under favorable conditions, including humidity, temperature, and appropriate nutrients on the cuticle (Télliez-Jurado et al. 2009). Certain fungi, such as some Entomophthorales, have the ability to break through the cuticle right out of the germ tube without developing appressoria (Hajek and Delalibera 2009). In order to enable strong attachment and physical penetration into the host, the appressorium clings to the cuticle and extends an infection peg (Shah and Pell 2003). According to Hajek and Delalibera (2009), certain Entomophthorales fungi break through the cuticle straight from the germ tube without developing appressoria.

2.1.3 Recognition of Receptors

Pre-germinated spores adhere to the epicuticle on the surface of some Hypocreales taxa, including *Beauveria*, *Metarhizium*, and *Isaria*, by recognizing glycoprotein receptors in the insect. Studies have shown that different types of spores exhibit varied adhesion preferences to hydrophobic and hydrophilic surfaces (Holder and Keyhani 2005). For example, *Beauveria* exhibits a hydrophobic nature in its conidia, attributed to the presence of cysteine-rich proteins called hydrophobins in the cell wall. However, hydrophilic conidia are displayed by *Verticillium lecanii* (Inglis et al. 2001).

2.1.4 Penetration into the Hemocoel

Hemocoel penetration refers to the process by which the EPF invade the insect's hemocoel. Subsequently, the hyphae penetrate the integument layers through the enzymatic dissolution of chitin and protein, initially ramifying in the cuticle before reaching the haemocoel and internal organs. Insect cuticle comprises a network of

polysaccharide polymers embedded in a protein matrix (up to 70%) (Vega et al. 2009). The cuticle is divided into three layers: the procuticle, which is a matrix of proteins and chitin; the epicuticle, and the outermost envelope, also known as the cuticulin layer (Locke 2001; Klowden 2013). The procuticle consists of an outside layer called the exocuticle and an inner layer called the endocuticle. Different insect anatomical locations and developmental stages have different cuticular protein compositions (Gilbert et al. 2005). Epidermal cells are situated at the base of the procuticle, and the hemocoel is located beneath them.

2.1.5 Enzymatic Cuticle Degradation

The cuticle needs to be broken through mechanical pressure and the release of enzymes that break down the cuticle in order for fungi to feed on and colonize (Vega et al. 2009). Cuticle characteristics such as thickness, sclerotization, and the presence of nutritional and antifungal chemicals influence the fungal penetration mechanism (Charnley 2003). Cuticle disintegration is dependent on enzymes such as lipases, proteases, and chitinases (Monzón 2002). This process frequently proceeds in a lipase–protease–chitinase sequence (Prior et al. 1988), which may be aided by the production of organic acids such as oxalic acid. PR1 protease in *M. anisopliae* and overexpression of the gene encoding *B. bassiana* chitinase are significant virulence factors, hastening insect death (St Leger et al. 1996; Fan et al. 2007). The secretion of these hydrolytic enzymes underscores their role in fungal virulence, offering potential for selecting strains for biological insecticides.

2.1.6 Alternate Modes

In addition to penetration through cuticular areas and intersegmental membranes, EPF can invade insects via sense organs and spiracles (St Leger 1996). Despite the higher humidity in the digestive tract facilitating rapid spore germination, digestive fluids may degrade spores or hyphae, causing toxicity rather than mycosis in some instances (Charnley 1992).

2.2 Replication of EPF in Hemocoel

2.2.1 Fungal Dimorphic Transition in Hemocoel

Many fungi, especially in Entomophthorales, undergo a dimorphic transition from mycelium to yeast form after infiltrating the hemocoel. In this phase, they develop as protoplasts (blastospores) devoid of cell walls, which helps them evade detection by circulating hemocytes (Vinson 1993). Many EPF prefer spores to grow within the hemocoel of infected insects because they are hydrophilic and vegetative fungal

propagules (Boucias and Pendland 1991; Humber 2008). Some species, such as *Nomuraea rileyi*, have cryptic surface residues that prevent insect hemocytes from phagocytosing their spores, making them invisible to humoral lectins (Boucias et al. 1988). Benefits of this cellular form include increased rates of nutrient uptake and immune system evasion (the insect's immune system normally identifies fungal cells via cell wall epitopes).

2.2.2 Fungal Strategies Against Insect Immune Response

Toxin synthesis and cell wall structural alterations are examples of defensive and immunosuppressive tactics used by fungal infections to subdue insect immune system response mechanisms. These include employing cyclic depsipeptides like destruxins, which cause paralysis in insects by activating calcium channels, and synthesizing extra proteases to weaken the humoral immune system. Along with some protein-based macromolecules, such as melanizing proteins from *B. bassiana*, a glycoprotein from *B. sulphureus*, and hirsutellin A from *H. thompsonii*, several low-molecular-weight secondary metabolites isolated from insect pathogens have shown insecticidal activity (Gillespie and Claydon 1989; Mollier et al. 1994; Fuguet and Vey 2004). These insecticidal compounds work in a variety of ways, but they frequently kill insects directly by concentrating on immune system cells that are specifically designed to stop invasive fungal structures from being attacked (Télliez-Jurado et al. 2009). Among EPF, the development of toxins is a common trait. The mechanism of action of destruxins, which block the synthesis of DNA, RNA, and proteins in insect cells, has been thoroughly explored. Toxins may also harm the insect's Malpighian tubules and muscular system, which would impair excretion and make it difficult for it to eat and move (Pal et al. 2007).

2.2.3 EPF Colonization Inside Insect

The invasion by the fungal mycelium persists until the insect is extensively colonized by the fungus, resulting in a firm texture upon touch. Finally, under favorable temperature and humidity conditions, hyphae can breach the insect's integument, leading to fungal emergence. Emergence typically occurs in less sclerotic regions, such as intersegmental membranes or spiracles, depending on the host and developmental stage. Since *B. bassiana* and *B. brongniartii* synthesize antibiotics such as oosporein to inhibit the growth of opportunistic organisms, hyphae crossing the integument may stay in the vegetative phase and begin sporulation within 24–48 h (Srivastava et al. 2009). Eventually, conidiophores develop into asexual spores that act as infectious agents for spreading. The development of epizootics is ultimately influenced by environmental conditions that are critical to conidia generation, survival, and germination (Fuxa and Tañada 1987). Although sporulation mostly happens in cadavers, it can also happen in live insects. Depending on the properties of

the spore and sporangium, spore dispersal can be either active or passive (Vega et al. 2009).

2.2.4 Insect Immune Response to Fungal Infection

Subsequently, septicemia occurs once the fungus evades the insect's immune defenses (Eilenberg and Michelsen 1999). The insect can react by utilizing cellular processes such as phagocytosis and encapsulation, humoral mechanisms including phenol oxidases, lectins, proteins, and defense peptides, or a mix of the two.

2.2.5 Reversion to Mycelial Growth Upon Nutrient Depletion

Upon depletion of nutrients, particularly nitrogen sources, yeast phases revert to mycelial growth, as observed in *Entomophthora thripidum* (Freimoser et al. 2003).

2.2.6 Physiological Effects on Insects

The host's death is caused by tissue obliteration, often due to choking, and the toxins produced by the fungus. Mycosis induced by fungal infection manifests in physiological symptoms such as seizures, lack of coordination, altered behavior, and paralysis in insects. Death ensues from a combination of factors, including physical tissue damage, toxicity, cell dehydration due to fluid loss, and nutrient consumption.

3 Production of Entomopathogenic Fungi

Nutritional components such as carbohydrates, proteins, lipids, and nucleic acids are essential for microbial growth, with elements such as carbon, hydrogen, nitrogen, sulfur, and phosphorus playing vital roles in host-pathogen interactions and self-defense mechanisms (Raimbault 1998). Growth characteristics, along with growth substances, are essential in tolerance selection studies. The media used for the growth, storage, and transport of microorganisms can be in solid or liquid form. Nutritional studies have been conducted on the production and sporulation of filamentous fungi such as *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* (Kumar and Mukerji 1996). *B. bassiana* spores for coffee berry borer biocontrol are mainly produced using a simple sterilization technique with cooked rice in bottles used for field spray applications. Rice media was washed with a 1% oil-water suspension to harvest the spores (Antía et al. 1992). However, the aqueous spore suspension must be used immediately to prevent germination, as spore viability diminishes rapidly due to the high moisture content in bottles. Similar studies have been conducted on

EPF for controlling date palm pests, particularly with *B. bassiana* and *M. anisoplia* (Latifian et al. 2013).

Jaronski and Mascarin (2017) outlined broad strategies for the production and formulation of hypocrealean fungal propagules, specifically aimed at insect control. First, maintaining genetically uniform cultures is essential to establishing a primary “mother culture” from which subsequent production is derived. Single-spore or single-colony isolation methods are employed to ensure genetic uniformity, with caution taken to limit successive passages on artificial media to prevent morphological and virulence alterations (Jackson et al. 1997; Butt et al. 2007; Shah et al. 2007; Wang et al. 2002; Wang and St Leger 2005; Ansari and Butt 2011). Preservation methods, such as low-temperature storage or desiccation, are employed to maintain viability and inhibit genetic variation (Humber 2008). Process sterility is crucial to prevent contamination, achieved through sterilization of fermentation medium, air, and equipment. Nutrient optimization is key for maximizing propagule yield and quality, with dissolved oxygen being a critical factor in aerobic fermentation. Response surface methodology aids in efficiently determining optimal parameters (Jackson et al. 1997; Garza-López et al. 2011; Tlécuil-Beristain et al. 2009; Prakash et al. 2008). Strain selection is vital, as different strains may respond differently to fermentation conditions, impacting propagule yield (Jaronski and Mascarin 2017).

The selection process for fungal isolates must consider not only their virulence but also their ability to form stable propagules that can be economically mass-produced. These propagules should have long-term stability, be compatible with existing application technologies, possess acceptable environmental and toxicology profiles, and consistently perform well under typical environmental conditions for the target insect(s) (Jackson et al. 2009). However, complications can arise when aiming for commercial success with a single product targeting multiple insect species or crops. The high cost of registration in North America or the European Union may require using a single strain against multiple targets, leading to compromises between efficacy and other criteria, particularly mass production (Jaronski and Mascarin 2017). Selecting the right propagule involves assessing its intended application, effectiveness, tolerance to desiccation and heat, germination and infection speed, environmental stability, reproductive capacity, and resistance to UV radiation. This evaluation considers the fungus’s natural ability to produce the chosen propagule (Jackson et al. 1997; Jackson and Jaronski 2009; Fernandes et al. 2015).

Grain, vegetable waste, seeds, rice husk, sawdust, and liquid media (coconut water, rice wash water, and rice cooked water) were among the products and by-products of agriculture that were evaluated for their potential to produce large quantities of three EPF: *B. bassiana*, *P. fumosoroseus*, and *V. lecanii*. *P. fumosoroseus* and *V. lecanii* produced more spores in sorghum than in wheat, while *B. bassiana* produced the most spores in wheat. Furthermore, okra, carrots, and jack seeds encouraged the three fungi’s vigorous development and sporulation. It was found that coconut water encouraged the fungi to proliferate and sporulate as much as possible (Sahayaraj and Namasivayam 2008).

Cowpea, maize, sorghum, and oat were the four grain media tested to find the best for growth and multiplication in order to mass multiply *B. brongniartii*. With

the highest vertical growth of fungus (6.60–6.97 cm) in test tubes, cowpea was shown to be the ideal substrate. Among cowpeas, the KH I isolate had the highest spore count (2.30×10^7 conidia/mL). Field trials demonstrated the effectiveness of double application of *B. brongniartii* (KH I) at a rate of 10^{14} spores/ha. This application resulted in a 44.85% reduction in tuber damage based on weight at the time of harvesting, compared to the control. Additionally, a 33.99% reduction in grub population was observed in the treated plots compared to the untreated check plots (Soni et al. 2017).

Another study mass-produced *M. anisopliae* using various grains as substrates. Testing was done on a variety of grains and liquid media, including Sabouraud's Dextrose Broth (SDB) and Potato Dextrose Broth (PDB). The findings indicated that, out of 10^3 dilutions, green gram and sorghum had the greatest conidial count (67.6×10^3 spores/mL). Furthermore, it was shown that SDB produced considerably more spores than PDB. On SDB media, the greatest conidial count (63.7×10^3 spores/mL) was recorded, with PDB in 10^3 dilutions following (Agale et al. 2018).

A study provided a protocol employing appropriate media to achieve optimal production of *B. bassiana* biomass, conidial count, and germination. Results indicated that broken rice emerged as the most effective substrate, yielding the highest biomass, conidial count, and germination rates (0.62 g, 10.92×10^7 conidia/mL, and 86.94%, respectively), followed by sorghum (0.54 g, 7.35×10^7 conidia/mL, and 77.43%) and maize (0.37 g, 6.05×10^7 conidia/mL, and 72.44%) (Rai et al. 2021).

A recent study commercially produced large quantities of resilient infective propagules from South African strains of *M. robertsii* and *M. pinghaense*. As solid fermentation substrates, three grain products from agriculture-flaked oats, flaked barley, and rice were examined. Conidial suspensions and liquid fungal cultures of blastospores were the two inoculation techniques employed. Conidial suspension inoculation showed higher contamination levels compared to blastospore inoculation. Flaked oats were not suitable for the growth of either fungus, while flaked barley preferred *M. robertsii* over *M. pinghaense*. Rice grains were effective for conidial production of both strains, with *M. pinghaense* yielding an average of $8.2 \text{ g} \pm 4.38 \text{ g}$ and *M. robertsii* yielding $6 \text{ g} \pm 2 \text{ g}$ of dry conidia harvested from the substrate (Mathulwe et al. 2022).

Chandwani et al. (2022) provided a summary of the large-scale cultivation of *B. bassiana* using readily available agricultural and industrial waste to reduce production costs and enhance the cost-effectiveness of producing potent spores, exploring both solid and liquid media options to expedite the commercialization of *B. bassiana*.

Another study developed a cost-effective solid state fermentation (SSF) method for large-scale production of *Purpureocillium lilacinum* PL1 conidia to control *Aphis devastans* infestations in okra cultivation. Rice and maize were identified as highly suitable substrates, yielding conidia densities exceeding 2×10^{10} conidia/g. The impact of agricultural phytosanitary agents on *P. lilacinum* PL1 growth rates was assessed, with certain pesticides showing no effect and fungicides causing complete inhibition. Laboratory tests demonstrated that 1×10^7 conidia/mL of *P. lilacinum* PL1 reduced *A. devastans* nymph populations by 88.66%. Field trials in okra

plantations revealed a significant 72.87% reduction in pest nymph populations after two applications of *P. lilacinum* PL1 at a concentration of 1×10^7 conidia/mL (Thi et al. 2023).

When *B. bassiana* was inoculated onto samples of white rice and allowed to incubate at 100% moisture content, the highest conidial output was produced by several isolates, such as ARSEF 3462. Significantly, isolates of *B. bassiana*, *M. anisopliae*, and *A. album* showed a high level of resistance to heat and UV-B radiation, but conidia of *S. lanosoniveum* and *L. aphanocladii* did not germinate following heat treatment (Rangel et al. 2023).

Another study tested eight isolates of six EPF on white or brown rice under varying moisture conditions. Conidial production was generally higher on white rice compared to brown rice, except for one fungal species. The 100% moisture condition favored higher conidial production for certain isolates, while the addition of peanut oil enhanced yield for another isolate. With 100% water added to white rice, one isolate of *B. bassiana* produced the highest amount of conidia (1.3×10^{10} conidia/g substrate) (Rangel et al. 2023).

4 Applications and Formulations for Entomopathogenic Fungi

More than 170 strains have been developed into mycopesticides and are currently accessible for commercial purposes (Bamisile et al. 2021). Various mycopesticides have been developed from fungal species such as *B. bassiana*, *B. brongniartii*, *M. anisopliae*, and *I. fumosorosea*, which are commonly used for pest control. *M. anisopliae* strains have been commercialized for combating various pests and disease vectors (Akutse et al. 2020), and products based on *M. anisopliae* and *B. bassiana* have been registered in several countries (Zimmermann 2007a, b; Wraight et al. 2000; Faria and Wraight 2007). *B. bassiana* and *B. brongniartii* spores have been effectively formulated into mycopesticides in numerous countries and are widely used for controlling pest insects (Wraight et al. 2000; Faria and Wraight 2007). *L. lecanii* has been studied and formulated as a mycoinsecticide for controlling aphids and scale insects, with products such as Vertalec and Mycotal registered in various European countries and beyond (Shah and Pell 2003; Yeo et al. 2003). Mycotrol, derived from *B. bassiana*, was registered in 1999 for controlling aphids, grasshoppers, thrips, and whiteflies, among other pests (Bradley et al. 1992). Green Muscle, another mycoinsecticide, was developed to combat desert locust outbreaks and consists of dried conidia of *M. anisopliae* var. *acridum* mixed with kerosene or diesel oil (Lomer et al. 2001). In Russia, Boverin, a *B. bassiana*-based mycopesticide, was extensively utilized to control the Colorado potato beetle and the codling moth. Other similar products, such as BotaniGard and Mycotrol-O, are available for use in glasshouses and by organic farmers in the United States and elsewhere, providing viable alternatives to synthetic insecticides.

Currently available biopesticide formulations on the market come in wettable powders, granules, tablets, oil-based suspensions, and biopolymers for different uses, with stability during storage being critical (Mascarin et al. 2013). Granular formulations are preferred for soil insects, with inert or nutritive granular materials coated with conidia. Microsclerotia, a stable storage form of some *Metarhizium* species, show promise for soil applications and have been used in controlling arbo-real pests such as the Asian long-horned beetle (Jackson et al. 2009). The development of effective formulations is crucial for the successful utilization of commercial biopesticides. Factors influencing the development of commercial formulations encompass various considerations, including:

1. The shelf life of the product is crucial. Long shelf life is highly desirable, enabling formulations to maintain efficacy over multiple cropping seasons, which is beneficial for both manufacturers and end users.
2. Fungal propagules must be stored in a dormant yet viable state, preferably at room temperature rather than under refrigeration. Optimal storage conditions are achieved by incorporating desiccants and oxygen scavengers into packaging. Recent advancements include the development of wax-based carriers for aerial conidia and oil-based formulations for blastospores (Meikle et al. 2008).
3. It is imperative for formulations to be easily suspended in carriers, typically aqueous, for spray applications while also remaining viable on treated substrates despite exposure to UV radiation, rainfall, and other environmental stressors. Incorporating fungal propagules with various additives such as wetting agents, suspension agents, and dispersants is necessary, with nonionic wetting agents often required for proper suspension (Bateman et al. 1996). While aerial conidia are hydrophobic and challenging to suspend in aqueous carriers, spray-dried blastospores are more hydrophilic but may need wetting agents for dispersion (Inglis et al. 1996). Oil formulations are suitable for suspending hydrophobic aerial conidia, enhancing their application in low-volume scenarios.

The drawbacks include:

1. High contamination by saprophytic fungi
2. Low viability of microbes, leading to reduced efficiency.
3. Limited tolerance to adverse field conditions, such as UV exposure, high temperature, drought, and surface application (Immediato et al. 2015; Mascarin and Jaronski 2016).
4. Powder formulations are prone to moisture absorption, which contaminates and diminishes the viability of the active ingredient.
5. Oil formulations suffer from poor spraying characteristics, and powder formulations leave residual talc on plant surfaces after spraying (Jaronski and Mascarin 2017).

These conditions reduce the effectiveness of EPF and necessitate frequent applications, leading to increased costs. To overcome these challenges, researchers have turned to biopolymer-based formulations (Kulinets 2015; Nicodemus and Bryant 2008). Biopolymers, derived from renewable sources such as plants, animals, and

microorganisms, offer an eco-friendly and biodegradable solution. They enhance the stability, adherence, and persistence of EPF in field conditions by shielding them from environmental stressors and creating a favorable microenvironment for growth and insecticidal action (Friuli et al. 2022). Biopolymer-based formulations present a sustainable alternative to synthetic insecticides, reducing harm to nontarget organisms and decreasing reliance on nonrenewable resources (Kumar et al. 2022). By reducing the initial amount of bioinsecticide required and the frequency of application, these formulations make EPF more cost-effective. Furthermore, the protective effect of biopolymers may extend the shelf life of EPF-based products, addressing another limitation in their use as biopesticides (Friuli et al. 2021).

Various methods are used to introduce EPF into plants, such as leaf spraying, stem injection, seed treatment, foliar application, flower treatment, seed soaking, and soil irrigation (Quesada-Moraga et al. 2006; López et al. 2015; Muvea et al. 2014; Greenfield et al. 2016; Bamisile et al. 2018a, b; Rondot and Reineke 2018; González et al. 2020). The specific plant portion targeted for endophytic colonization or the kind of insect to be controlled root eaters, stem borers, or leaf-chewing insects, for example, may influence the choice of injection technique (Bamisile et al. 2018b).

Foliar spraying of EPF such as *M. brunneum* and *B. bassiana* can transiently establish an endophytic presence in plants such as alfalfa, tomatoes, sweet peppers, and melons (Jaber and Araj 2018). Insect pests on the leaf surface (phylloplane) can be effectively controlled by foliar spraying of spore solutions (Vega et al. 2009). Insects that burrow into and eat inside leaves, roots, stems, seeds, and rhizomes are the target of endophytic application of EPF (Resquín-Romero et al. 2016). Artificial inoculation of EPF into tomato plants has demonstrated effective control of *Tuta absoluta* (leaf miner) (Klieber and Reineke 2015; Resquín-Romero et al. 2016). The application frequencies and timings of these mycoinsecticides are comparable to those of traditional insecticides (Wraight et al. 2000; Shah and Pell 2003).

Most EPF formulations available on the market have a shelf life of three to 6 months and contain spore concentrations ranging from 10^9 to 10^{10} spores/g. The formulation, extent of infestation, insect species, and ambient conditions impact the application dosage. The label for mycoinsecticide contains comprehensive usage instructions.

5 Major Applications of Entomopathogenic Fungi

5.1 General Benefits

The colonization of plants by EPF offers various benefits, including promoting plant growth, defending against insect pests, inducing systemic resistance, antagonizing plant pathogens, and mitigating the effects of abiotic stress. Alongside these advantages, fungal endophytes have gained attention for their ability to produce secondary metabolites, potentially surpassing chemical pesticides. Additionally,

they exhibit potential in pharmaceutical and medical settings as immune-suppressing, anticancer, antidiabetic, and antibacterial medicines (Kabaluk and Ericsson 2007; Kim et al. 2008; Vega et al. 2009; Elena et al. 2011; Sasan and Bidochka 2012; Gouda et al. 2016; Vega 2018; Fadiji and Babalola 2020a, b; Prajapati et al. 2024).

5.2 *Integrated Pest Management*

EPF serve as crucial elements in IPM strategies, functioning as biological control agents against arthropods and insect pests. They also constitute essential components of myco-insecticides used in horticulture, forestry, and agriculture. The application of EPF in pest management provides a practical and environmentally friendly substitute for chemical control techniques. Over 800 species of EPF have been described and identified as promising biocontrol agents. The studies on insect pathogenic fungi in the 1980s aimed to find methods for managing silkworm diseases. Bassi's discovery in 1835 of the white muscardine fungus's germ theory, later named *Beauveria bassiana* in his honor, laid the groundwork for using insect-infecting fungi in pest management (Gilbert and Gill 2010). EPF have been actively employed to manage numerous economically significant crop pests for approximately two centuries. *B. bassiana* was initially isolated and identified around 170 years ago, while *B. brongniartii* and *M. anisopliae* have been in use for over 110 and 130 years, respectively (Zimmermann 2007a, b). These fungal species, alongside other hypocrealean fungi such as *I. fumosorosea*, *M. brunneum*, *M. robertsii*, and *H. thompsonii*, are commonly deployed against a wide range of arthropod pests (Dara 2019). EPF primarily act through inundative approaches and has demonstrated efficacy against various insects, including Homoptera (particularly aphids, whiteflies, cicadas, scale insects, locusts, thrips, grubs, moths, and mites), Lepidoptera (particularly larvae), Hymenoptera (bees), Coleoptera (beetles), Diptera (flies and mosquitoes), whiteflies, and tephritid fruit flies (Gulzar et al. 2021). Additionally, these fungi exhibit pathogenicity against phytopathogenic nematodes and other soilborne pests (Pocasangre et al. 2000). Their mode of action varies, from causing starvation to toxin production, with mechanisms including enzymatic dissolution of the insect cuticle and production of toxins that induce host paralysis and eventual death (Table 1).

Nanopesticides for plant protection and pest control introduce novel strategies for developing active compounds at the nanoscale. Research indicates that metal nanoparticles show efficacy against various insects and pests, with silver nanoparticles emerging as particularly potent insecticidal agents. Biologically synthesized silver nanoparticles are becoming increasingly popular due to their ecological safety. These nanoparticles offer a sustainable approach to managing insect pest populations. EPF-based nanosilver exhibits high efficiency against diverse pest species even at very low concentrations (Bihal et al. 2023).